The emergence of core eudicots: new floral evidence from the earliest Late Cretaceous

Else Marie Friis1, Kaj Raunsgaard Pedersen2 and Peter R. Crane3,4

1Department of Palaeobiology, Swedish Museum of Natural History, Stockholm, Sweden
2Department of Geoscience, University of Aarhus, Aarhus, Denmark
3Yale School of Forestry and Environmental Studies, New Haven, CT, USA
4Oak Spring Garden Foundation, Upperville, VA, USA

Eudicots, the most diverse of the three major clades of living angiosperms, are first recognized in the latest Barremian–earliest Aptian. All Early Cretaceous forms appear to be related to species-poor lineages that diverged before the rise of core eudicots, which today comprise more than 70% of angiosperm species. Here, we report the discovery of a well-preserved flower, Caliciflora mauldinensis, from the earliest Late Cretaceous, with unequivocal core eudicot features, including five sepals, five petals and two whorls of stamens borne on the rim of a floral cup containing three free carpels. Pollen is tricolporate. Carpels mature into follicular fruitlets. This character combination suggests a phylogenetic position among rosids, but more specific assignment is precluded by complex patterns of character evolution among the very large number of potentially relevant extant taxa. The whorled floral organization is consistent with ideas that this stable pattern evolved early and was a prerequisite for more integrated patterns of floral architecture that evolved later. However, limited floral synorganization in Caliciflora and all earlier eudicot flowers recognized so far, calls into question hypotheses that substantial diversification of core eudicots had already occurred by the end of the Early Cretaceous.

1. Introduction

Hypotheses of relationships among living angiosperms recognize a species-poor basal grade, within which are embedded three major clades; eumagnoliids, monocots and eudicots. Soon after their first appearance in the fossil record about 135 Ma, Early Cretaceous angiosperms include diverse extinct taxa related to basal grade angiosperms (Austrobaileyales, Chloranthaceae and Nymphaeales), certain eumagnoliids (Laurales, Magnoliales and Piperales), early monocots (Alismatales) and basal grade eudicots (e.g. [1–4]). These Early Cretaceous assemblages contrast markedly with Late Cretaceous angiosperms that are dominated by fossils related to lineages of core eudicots. Core eudicots comprise more than 70% of living angiosperm species and both major clades within the group are well represented in the Late Cretaceous. Late Cretaceous rosids include a rich record of early Fagales (e.g. [5]) as well as diverse fossils related to other clades [1]. Late Cretaceous asterids include many taxa related to extant Cornales and Ericales (e.g. [1,6–9]), the two earliest diverging lineages of the group. The transition from Early Cretaceous floras, dominated by basal grade lineages of angiosperms, eumagnoliids and early eudicots, to Late Cretaceous floras dominated by core eudicots, occurred sometime between the mid-Albian and the Turonian–Santonian. Here, we describe a new flower from the earliest Late Cretaceous (earliest Cenomanian) of eastern North America with distinctive features of core eudicots. Together with an unnamed fossil flower of approximately
similar age [10], this new discovery provides the earliest direct evidence of floral structure in early core eudicots and has implications for understanding the evolution of floral structure within this hyperdiverse clade of extant angiosperms.

2. Material and methods

The fossil material described here consists of one complete flower bud, one anthetic flower and three fragments of post-anthetic flowers, one of which has the remains of a stamen. All five specimens were recovered from samples of Potomac Group sediments (Mauldin Mountain samples 022, 116, 117) collected from the Elk Neck Beds at the Mauldin Mountain locality on the Elk Neck Peninsula, northeastern Maryland, USA (39°29′15″ N, 75°59′44″ W). The Elk Neck Beds of earliest Cenomanian age have also yielded inflorescences and flowers of *Mauldinia mirabilis* Drinnan, P. R. Crane, E. M. Friis & K. R. Pedersen [11], which is related to extant Lauraceae, fruits of *Couperites mauldinensis* K. R. Pedersen, P. R. Crane & E. M. Friis [12], which are of uncertain affinity among early diverging angiosperms, and pistillate and staminate flowers of *Spanomera mauldinensis* Drinnan, P. R. Crane, E. M. Friis & K. R. Pedersen [13], which are related to extant Buxales. For further details on the geology and age of the Mauldin Mountain assemblage, see [11].

The specimens are coalified and were treated following standard methods for Cretaceous mesofossils [1]. The flower bud was mounted on a brass stub for synchrotron radiation X-ray microtomography (SRXTM) at the Tomcat beamline of the Swiss Light Source, Paul Scherrer Institute, Switzerland [14]. It was measured using a ×20 objective with isotropic pixel size of 0.325 μm at 10 keV using a scCMOS detector and a 20 μm thick LAG:Ce scintillator screen and the specimen was vertically stacked (for more details on the technique, see [15]). Data derived from the SRXTM [16] were reconstructed and imaged using Avizo (v. 6.3, 7.1, 9.0.1, 9.1.1) software for computed tomography.

After SRXTM the flower bud was remounted on an aluminium stub for SEM scanning electron microscopy (SEM), sputter coated with gold, and examined using a Hitachi S-4300 Field Emission Scanning Electron Microscope at 2 kV. The four other specimens were also prepared for SEM in the same way.

3. Results

**Angiospermae**

**Core eudicots**

**Genus.** *Caliciflora* gen. nov.

**Type species designated here.** *Caliciflora mauldinensis* sp. nov.

**Generic diagnosis.** Flower small, sessile, with an associated bract and two prophylls borne on a stout stalk. Staminate and pistillate organs in the same flower. Floral cup distinct. Perianth with five sepals and five petals borne on the rim of the floral cup. Sepals free, thick, with broad base and acute apex; sepal aestivation revolute-valvate. Petals free, broadening distally from a narrower base, keeled, with a thin lamina and median rib; petal aestivation open below, quincuncial above. Indumentum dense on the outer surfaces of the floral cup, sepals and petals composed of interlocking stellate hairs. Stamens in two whorls, minute with dithecate, tetrasporangiate and dorsifixed anthers. Pollen minute, tricolporate, psilate. Orbicules present. Gynoecium trimerous with three free carpels borne on the inside of the floral cup.

**Specific diagnosis.** As for the genus.

**Etymology.** Generic name from *calice* (Latin for cup) and *flos* (Latin for flower) and specific name from the Mauldin Mountain locality where the fossils were collected.

**Holotype designated here.** PP53985 (sample Mauldin Mountain 116); figure 1–4(d).
Paratypes. PP34773 (sample Mauldin Mountain 116), PP54159 (sample Mauldin Mountain 117), PP54160, PP54161 (sample Mauldin Mountain 022).

Type locality. West of Mauldin Mountain, Elk Neck Peninsula, MD, USA, (39°29′15″ N, 75°59′44″ W).

Type horizon and age. Elk Neck Beds, Potomac Group; Late Cretaceous (Early Cenomanian; lowermost palynological Zone III).

Description. The taxon is based on a single flower bud (PP53985), one anthetic flower (PP34773) and three flower fragments preserved post-anthesis (PP54159–PP54161). The specimens are linked mainly by their identical stellate trichomes, similarities in the shape of the sepals, the presence of a floral cup and identical pollen grains in situ in specimens PP34773 and PP54161 and on the surface in PP54160. Sepals and petals are well-developed in the floral bud indicating that it was close to anthesis when fossilized.

The floral bud (figure 1–4d,h; electronic supplementary material, 1), the anthetic flower (figure 4a) and one of the post-anthetic flower fragments have both carpels and
stamens. However, the bud is unusual in having carpels that appear partly open, with no signs of ovule initiation. It is therefore possible that the flower was functionally unisexual, and that only stamens developed to maturity. Alternatively, ovule development may have been interrupted by insect damage. There are burrows in the bud and an insect larva is present between the bract and sepals.

The flower and the associated bract and two prophylls are borne on a short, stout stalk (figures 1a–c, 2a–f and 4a). There is no other information about how the flower was borne on the plant. The flower bud is about 1.32 mm long and 0.75 mm in diameter, and has both staminate and pistillate organs preserved. There is also a distinct floral cup, about 0.17 mm deep and about 0.3 mm in diameter. The anthetic flower (figure 4a) is similar in size, about 1.2 mm in diameter, somewhat compressed and with fully formed staminate and pistillate organs.

The perianth is differentiated into an outer whorl of sepals and an inner whorl of petals. Sepal aestivation is revolute-valvate (figures 1a–c, 2a–f and 4a) and sepals have broad bases and acute apices. The post-anthetic flower fragments show that the sepals are persistent. On their ventral surface, the sepals have a conspicuous hypodermis of thin-walled, empty cells. This hypodermis is two to several cell layers thick in the median-basal region, but only one cell layer thick distally and along the sepal margins (figure 3a,c–e). Hypodermal cells on the dorsal surface of the sepals are distinctly different with amorphous contents that obscure the anatomical details of the sepal lamina, including the number of vascular bundles. The amorphous contents may indicate that these cells were mucilaginous or perhaps tanniferous (figure 3a,c–e).

Petals are present in the floral bud and in the specimen preserved at anthesis, but not in the post-anthetic specimens. Petals do not have a distinct claw, but broaden distally from a narrow base into a broad, keeled lamina with a prominent median rib and a single vascular bundle (figure 4d). Petals are folded longitudinally over the midrib and at least the inner petals appear conduplicate (figure 3a,b). The petals are narrow at the base, and widely separated by large interspaces.

**Figure 3.** SRXTM reconstructions (orthoslices) of flower bud of *Caliciflora mauldinensis* gen. et sp. nov. flower from the Late Cretaceous (earliest Cenomanian) Mauldin Mountain locality, MD, USA; holotype (PPS3985; sample Mauldin Mountain 116). (a) Detail of perianth in transverse section below the apex of the flower bud showing the thicker sepals with a layer of thin-walled hypodermal cells (asterisks), thin lamina of a folded petal with an almost glabrous inner surface and a dense indumentum on the outer surface; note the especially clear stellate hair (arrowhead) on the keel of the outer surface of the petal (orthoslice xy1200). (b) Detail of perianth in transverse section close to the apex of the floral bud showing folded petal with dense indumentum on the outer surface and scattered hairs on the inner surface (arrowhead) (orthoslice xy1050). (c) Longitudinal sections of sepals (s) and petals (p) showing dense indumentum; note sepals with dense, infilled cells towards the outside, and a layer of thin-walled hypodermal cells (asterisks) (orthoslice xz1485). (d) Detail of perianth and androecium in transverse section close to the rim of the floral cup showing sepals (s) with dense indumentum of stellate hairs on the outer surface and sepal margins (arrowhead), narrow base of petal (p, arrow) and an antepetalous anther (an, arrow) (orthoslice xy1900). (e) Detail of sepal (s) showing glabrous inner surface and dense indumentum of stellate hairs on the outer surface and sepal margins; note thin-walled cells on the inner surface of the sepal (asterisk) (orthoslice xy1950).
Petal aestivation is open at the base (figures 2d and 3d), but quincuncial above (figures 2b, c and 3a, b).

The androecium consists of two whorls of stamens. Anthers are almost sessile in the bud and filaments are also short in both of the more mature specimens (PP34773, PP54161). In the floral bud, the three stamens that are opposite the sepals (antesepalous) are borne on the rim of the floral cup. Five stamens opposite the petals (antepetalous) are borne...
slightly below this level (figure 2c–e,h,i). Anthers are tetra- 
sporangiate, dithecal and dorsifixed (figure 4a,h). They are 
very small, up to about 0.15 mm long in the bud and about 
0.4 mm long in the anthetic flower. Pollen grains are not 
obvious in the flower bud. Anthers in two of the more 
mature specimens have abundant minute pollen grains, 
about 10 μm long and 5 μm in equatorial diameter. Grains 
are prolate-rhomboidal in equatorial view (figure 4i,k,l) 
and semi-angular in polar view (figure 4j). Pollen grains 
have three long colpi, each with a small bridge in the middle 
of the colpi (figure 4i,k,l) indicating that the grains are tricolpo- 
rate. The tectum is plicate-imperforate with weakly rugulate 
ornamentation. Tiny, rounded orbicules, about 1 μm in diam- 
eter, often with a small central depression, occur on the inside 
of the theca wall and the surface of the pollen grains 
(figure 4i,h), most probably indicating a secretory tapetum. 
The three free carpels (figures 2c–i and 4a,h) are borne on 
the inside of the floral cup above the free space of the floral 
apex. In the flower bud, the carpels are fully open along 
the ventral suture below the stigmatic region (figure 2c–g). 
In this specimen, no ovules are developed and there is no 
indication of placentation. However, in the more mature 
specimens (figure 4b,c) placentation is ventral and apparently 
concentrated to the middle part of each carpel. 
Ovules/ seeds are exposed in one mature carpel 
(figure 4b,c). They are small, elongated, anatropous, with a reti- 
culate outer surface, and do not fill out the locule. The fruitlets 
are dry follicles that open along their ventral sutures. The inner 
part of the fruit wall consists of transversely oriented fibrous 
scleroids. The outer epidermis of the fruit wall consists of 
equiaxial cells, each with a central papilla (figure 4f); no stoma- 
 mata have been observed on the carpel or fruit wall. 
The outer surface of sepalas and petals, as well as the outer 
surface of the floral cup is covered by a dense indumentum 
of interlocking stellate hairs each composed of up to about 10 
long unicellular elements that radiate from a central point 
(figures 1a–c, 2a–i, 3a–c and 4e). Scattered stellate hairs also 
occur along the margins of the sepalas (figure 3d) and on the 
inner surface of the petals in the apical region (figures 2i and 
3b,c). The inner surface of the sepalas and the median and 
asal parts of the petals are glabrous (figure 3a,c–e).

4. Discussion

(a) Systematic assessment

The combination of characters in Caliciflora, including the 
actinomorphic organization with an open floral cup, penta- 
merous whorls of free sepalas and petals, two whorls of free 
stamens, tricolporate and tectate-imperforate pollen, one 
whorl of three free carpels and follicular fruitlets, unequiv- 
cially place the fossil taxon among core eudicots. In 
addition, while flowers with a floral cup, together with free 
petals (choripetalous) and an apocarpous gynoecium, are 
found in several groups of rosids, to our knowledge this com- 
bination of features is not recorded among asterids or in any 
other group of core eudicots.

Resolving the phylogenetic position of Caliciflora among 
extant rosids is complicated by the vast number of living species 
(around 70 000 extinct species, [17]), many of which have not 
been studied in detail, combined with the absence of clear mor- 
phological synapomorphies that correspond to the higher level 
groupings, orders and families recognized based largely on 
molecular data (e.g. [17–24]). A further problem is that floral 
morphology among rosids exhibits considerable variation, and 
sometimes there are strong similarities in unusual features 
taken into account new molecular models of angiosperm 
relationships (e.g. [25,26,28–41]).

One of the most distinctive characters of Caliciflora is the floral cup, which is also characteristic of many rosids, where 
it occurs scattered in three major clades (N-fixing clade, COM 
clade and malvids), as well as in Myrtales [28,42–45] and 
Crossomostomatales [33]. In the N-fixing clade, a floral cup 
is present in certain Rosales [40,41] and Cucurbitales [31], while 
in the COM clade, it occurs in Celastrales [32], Oxalidales 
[30] and Malpighiales [29,35–37,46]. Among malvids, a floral 
cup occurs in certain Brassicaceae [47], Huerteales [28] and 
Sapindales [48,49]. Actinomorphic flowers with free perianth 
parts are also characteristic of many rosids, and typically 
such flowers are pentamemous with a well-differentiated 
calyx and corolla as in Caliciflora. The revolute-valvate sepal 
estivation of Caliciflora is also a feature of certain rosids and 
occurrs scattered in the Cucurbitales (Anisophylleaceae, [31]), 
Malpighiales (Rhizophoraceae and Erythroxylaceae, [36]) and 
also in Oxalidales (Cunoniaceae and Tremandraceae, [30]).

A characteristic feature of many rosids is flowers with 
the same number of floral organs in all whorls (isomorous) 
including the gynoecial whorl. However, monocarpellate, 
bicarpellate and tricarpellate forms often occur in typically iso- 
merous groups, and some groups of rosids, for example, many 
Malpighialas [46] are characterized by heteromorhs 
flowers with one to three carpels. Also common in several groups of 
rosids is apocarpy and in the N-fixing clade it occurs in certain 
Rosaceae, as well as Surianaceae and Quillajaceae (both fabids). 
In Rosaceae, dry, follicular fruitlets with several seeds borne 
centrally on ventral placentae, as well as open ventral sutures, 
are known in some members of subfamily Amygdalioideae [50].

The androecial features of Caliciflora are less informative 
 systematically, but are also consistent with the androecial 
structure of some members of the N-fxing clade. For example, while 
most flowers of Rosaceae have 15 or more stamens, flowers 
with fewer stamens occur in the North American cushion 
plant, Kelseya uniflora (S. Wats.) Rydb. [51]. Reduction of 
stamen number in one of the stamen whorls, as seen in the 
Caliciflora flower bud, occurs also in Surianaceae (Fabales).

Pollen morphology among rosids is diverse, but small, tri- 
colporate grains similar to those of Caliciflora are common in 
many taxa. Often the tectum is reticulate, microreticulate or 
foveolate, but grains with a finely rugulate tectum as in 
Caliciflora occur in many taxa in the N-fxing clade, including 
in Fabaceae (e.g. [52–55], Surianaceae and Quillajaceae [56], 
and Rosaceae (e.g. [57,58]).

The stellate hairs of Caliciflora are distinctive. Similar hairs 
occur on the outer surface of sepalas and petals in Rhizophora-
ceae, and in the closely related Ctenolophonaceae [36], as well 
as on the outer surface of the sepalas in some Cunoniaceae and
The Late Cretaceous record of both mesofossils and palynomorphs is extensive, suggesting that the origin of core eudicots was already underway in the Early Cretaceous [17,75–77]. However, ideas based on molecular data that suggest a major radiation of core eudicots in the mid-Cretaceous [17,75–77]. However, hypotheses suggesting that a significant diversification of core eudicots was already underway in the Early Cretaceous [78] are more problematic. Direct fossil evidence of core eudicots prior to around the Early Cretaceous–Late Cretaceous is currently lacking.

Data accessibility. The specimens are housed in the palaeobotanical collections of the Field Museum of Natural History, Chicago, USA (PP). Raw data from the SRXTM study are stored at the Swedish Museum of Natural History, Stockholm, Sweden (raw data PP53985a_B1_B2_).

Competing interests. We declare we have no competing interests.

Funding. Financial support was provided by the Swiss Light Source (European Union FP7 projects 20130185), by the Swedish Research Council and by the Edward P. Bass Distinguished Visiting Fellowship at Yale University.

Acknowledgements. We thank Marco Stampolini, Federica Marone and Anna Lindström for help with the SRXTM analyses performed at the Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland, and Pollyanna von Knorring for the line drawings.

Figure 5. Floral diagrams of Caliciflora mauldinensis gen. et sp. nov. from the Late Cretaceous (earliest Cenomanian) Mauldin Mountain locality, MD, USA (a) and the Rose Creek Flower [10] from the latest Early Cretaceous or earliest Late Cretaceous (latest Albian–earliest Cenomanian) Rose Creek locality, NE, USA (b).