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

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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 angiosperm assemblages 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 impli-
cations 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 speci-
mens 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 stami-
nate flowers of
Spanomera mauldiniensis
Drinnan, P. R. Crane, E. M. Friis & K. R. Pedersen [13], which are related to extant Bux-
ales. 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 sCMOS 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 alu-
minium 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 mauldiniensis
sp. nov.

Generic diagnosis. Flower small, sessile, with an associ-
ated 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,
broader distally from a narrower base, keeled, with a thin
lamina and median rib; petal aestivation open below, quincun-
cial 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, tetraspor-
angiate 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–Ad H.
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 1–4; electronic supplementary material, 1), the anthetic flower (figure 4a) and one of the post-anthetic flower fragments have both carpels and

Figure 2. SRXTM volume renderings of flower bud of Caliciflora mauldinensis gen. et sp. nov. from the Late Cretaceous (earliest Cenomanian) Mauldin Mountain locality, MD, USA; holotype (PP53985; sample Mauldin Mountain 116). (a) Volume rendering of apical view of entire floral bud showing subtending bract (br), prophylls (pp) and five revolute-valvate sepals (s). (b–g) Transverse views of flower bud at different levels from apex (b) to middle of floral cup (g) showing subtending bract (br), two prophylls (pp), five revolute-valvate sepals (s), five petals (p), three antesepalous stamens (arrowheads), five antepetalous stamens (asterisks) and three carpels; note three closely appressed stigmas (c) and the free ventral margins of carpels below the stigmatic region (fg) (a, not cut; b, section at level of orthoslice xy0766; c, section at level of orthoslice xy1756; d, section at level of orthoslice xy1916; e, section at level of orthoslice xy2044; f, section at level of orthoslice xy2233; g, section at level of orthoslice xy2427). (h,i) Longitudinal views of flower bud in median section showing floral cup surrounding the carpels and with antesepalous stamens (arrowheads) and antepetalous stamens (asterisks) on the rim of the floral cup (b, section at level of orthoslice xz1112; i, section at level of orthoslice yz0983).
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 and 2a–i). 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.
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 tetrasporangiate, 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 tricolporate.

The tectum is psilate-imperforate with weakly rugulate ornamentation. Tiny, rounded orbicules, about 1 μm in diameter, 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,i) 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 placenta. However, in the more mature specimens (figure 4b,c) placentaent 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 reticulate 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 sclereids. The outer epidermis of the fruit wall consists of three long colpi, each with a small bridge in the middle of the colpi (figure 4i,k,l) indicating that the grains are tricolporate.

The tectum is psilate-imperforate with weakly rugulate ornamentation. Tiny, rounded orbicules, about 1 μm in diameter, 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 androecial features of Caliciflora are distinctive. Similar hairs occur on the outer surface of sepals and petals in Rhizophora–Ceratopetalum (Cunoniaceae, Oxalidales: COM) [25]. These difficulties, coupled with uncertainties over deep relationships in the rosids, complicate characterization of major clades using flower morphology [22,26–28] and there are no recent comprehensive attempts to evaluate the evolution of floral characters in the group as a whole. We therefore compare Caliciflora to extant rosids only in general terms based on the extensive literature, as well as the few discussions of floral structure that have 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 pentameres with a well-differentiated calyx and corolla as in Caliciflora. The revolute-valvate sepal aestivation of Caliciflora is also a feature of certain rosids and occurs scattered in the Cucurbitales (Anisophylleaceae, [31]), Malpighiales (Rhizophoraceae and Erythroxylaceae, [36]) and also in Oxalidales (Cunoniaceae and Tremaandraceae, [30]).

A characteristic feature of many rosids is flowers with the same number of floral organs in all whorls (isomeres) including the gynoecial whorl. However, monocarpellate, bicarpellate and tricarpellate forms often occur in typically isomeric groups, and some groups of rosids, for example, many Malpighiales [46] are characterized by heteromorous 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 Amygdaloideae [50].

The androecial features of Caliciflora are less informative systematically, but are also consistent with the androecial structure of some members of the N-fixing 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, tricolporate 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-fixing 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 sepals and petals in Rhizophoraceae, and in the closely related Ctenolophonaceae [36], as well as on the outer surface of the sepals in some Cunoniaceae and 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 between taxa that are widely separated phylogenetically (e.g. flowers of Anisophyllea (Anisophylleaceae, Cucurbitales: fabids) and Centopetalum (Cunoniaceae, Oxalidales: COM)) [25].

4. Discussion
(a) Systematic assessment
The combination of characters in Caliciflora, including the actinomorphic organization with an open floral cup, pentameres whorls of free sepals and petals, two whorls of free stamens, tricolporate and tectate-imperforate pollen, one whorl of three free carpels and follicular fruitlets, unequivocally 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 combination 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 extant species, [17]), many of which have not been studied in detail, combined with the absence of clear morphological synapomorphies that correspond to the higher level groupings, orders and families recognized based largely on
Tremandraceae [30,59]. In flower buds of Rhizophoraceae and Ctenolophonaceae, the perianth parts are congenitally connected by the hairs in the overlapping regions [36], which also appears to be the case for Caliciflora. Congenital connection of sepals by hairs is also reported for Cunoniaceae [30]. Stellate hairs similar to those of Caliciflora are not widespread in Rosaceae, but occur in a few taxa (e.g. Sorbaria) [60].

(b) Implications for the origin of core eudicots
Caliciflora mauldinensis from the earliest Cenomanian, very close to the Early–Late Cretaceous boundary, establishes a minimum age of about 100 Myr for the origin of core eudicots. Setting aside the report of a rosid flower from Burmese amber [61], the age of which is uncertain (possibly Cenomanian), the only other mid-Cretaceous core eudicot flower currently known from around the Early–Late Cretaceous boundary is the Rose Creek flower reported from the Dakota Formation [10], which is of broadly similar age, or perhaps very slightly older [62]. While Caliciflora and the Rose Creek flower differ dramatically in size, their floral architecture is fundamentally similar. Both are regular actinomorphic and bisexual flowers with whorled, pentalamous organization and free floral parts (figure 5a,b), a prerequisite for the development of more integrated patterns of floral architecture [63] that accompanied the rapid diversification of core eudicots through the Late Cretaceous and Cenozoic [1,64].

Subsequent to the earliest Cenomanian there are only few fossil floras of Late Cenomanian or Turonian age. Currently no core eudicot flowers have been described from the Late Cenomanian flora of the Bohemian Basin [65–67] or from the Cenomanian–Turonian Sarbay flora [68,69]. The mesofossil flora from Old Crossman Clay Pit of possible Turonian age (e.g. [8,70]) does contain abundant flowers of core eudicots. However, the Old Crossman flora is very similar in many respects to younger mesofossil floras from the Santonian–Campanian of North America [1]. The interval between the first appearance of early core eudicot flowers (Caliciflora, Rose Creek flower) and later flowers with more pronounced synorganization (e.g. Raritaniiflora tonentosa Crepet, Nixon & Daghlian) is therefore a minimum of about 10, or possibly as much 15–20 Myr.

The Late Cretaceous record of both mesofossils and palynofloras both suggest an extensive post-Cenomanian radiation of core eudicot angiosperms [1,71,72]. However, at the level of Caliciflora and the Rose Creek flower, and also in older sediments, all other angiosperms that are known so far appear to be related to early diverging lineages of angiosperms [1], including early diverging lineages of eudicots (taxa related to Lardizabalaceae and other members of Ranunculales, Buxales, Platanaceae and Nelumbonaceae [1,3,73]). Similarly, among the 21 species of fossil leaves reported from the Rose Creek locality, 70% of the leaf species and 90% of the leaf specimens appear to be related to magnoliids or magnoliid grade angiosperms [74]. Four species were unassigned. Only two species were assigned to core eudicots [74].

5. Conclusion
The characters of Caliciflora unequivocally place this new fossil angiosperm flower among core eudicots, most likely among the rosids rather than asterids, perhaps near the base of the N-fixing clade. Flowers with a floral cup, heteromerous organization, tricolporate, rugulate-tectate pollen and an apocarpous gynoecium are common at this level of angiosperm evolution, including in certain Rosaceae, and also in the fabalean family Surianaceae. More precise systematic placement is precluded by incomplete knowledge of floral structure over a very large number of extant species as well as problems of defining the currently recognized rosid lineages based on floral features alone. Caliciflora and the Rose Creek flower establish a minimum age for core eudicots that is broadly in agreement with 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.

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