



Evolution and phylogenetic significance of pollen in Annonaceae

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The remarkable diversity of pollen morphology in Annonaceae has attracted systematic attention since the 1970s. Optimization of pollen characters on a molecular tree confirms that granular monosulcate pollen, as in *Anaxagorea* and subfamily Ambavioideae, was ancestral in the family. However, granular structure is derived for angiosperms as a whole, and exine characters are highly homoplastic in Annonaceae. Because columellar taxa (*Annickia*, Bocageae) diverged earlier than previously thought, columellae may have re-originated in the common ancestor of Malmeoideae and Annonoideae, which make up most of the family, with several later reversals to granular structure. In Malmeoideae, loss of the sulcus and origin of a verrucate tectum occurred in the tribe Miliuseae, followed by an uncertain number of origins of disulcate pollen. Tetrads arose several times in Annonoideae, but reverted to monads in *Isolona* and probably Uvarieae. Recognition that microspores rotate during development in *Annona* and *Cymbopetalum* suggests that the proximal thin area in these taxa is a modified distal sulcus, but rotation does not occur in all tetrad groups; a broader survey is needed to evaluate this phenomenon. © 2012 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2012, **169**, 190–221.

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INTRODUCTION

Besides being the largest family of early-diverging angiosperms (i.e. groups other than monocots and eudicots), Annonaceae are also noteworthy for having the most diverse pollen morphology. Because Magnoliales, the APG III (2009) order to which Annonaceae belong, were once widely viewed as the most primitive living angiosperms, the discovery of this diversity stimulated much research on annonaceous pollen by botanists interested in the original pollen morphology of angiosperms and its early diversification. As a result of molecular phylogenetic analyses (reviewed by Soltis *et al.*, 2005), which root the phylogenetic tree of angiosperms near *Amborella* Baill., Magnoliales now appear to have less bearing on the ancestral pollen type (Doyle, 2005). However, the diverse pollen of Annonaceae is still significant as a conspicuous

aspect of the evolutionary radiation of the family and a source of systematic characters within it.

PRE-CLADISTIC STUDIES

Although pollen of Annonaceae was briefly surveyed by Canright (1963), the first comprehensive palynological studies of the family were by Walker (1971a, b, 1972), using light microscopy (LM) and scanning electron microscopy (SEM). Walker (1971a; Fig. 1) argued that the ancestral pollen type in Annonaceae was monosulcate, with a single elongate germination furrow, or sulcus, as generally assumed for angiosperms as a whole, and that pollen grains of this type were modified into inaperturates (by loss of the sulcus), disulcates (with two furrows parallel to the equator and perpendicular to the polar axis, which is defined by the centre of the meiotic tetrad and the centre of the microspore), permanent tetrads and polyads. He used pollen characters as the primary basis for a classification of Annonaceae into informal

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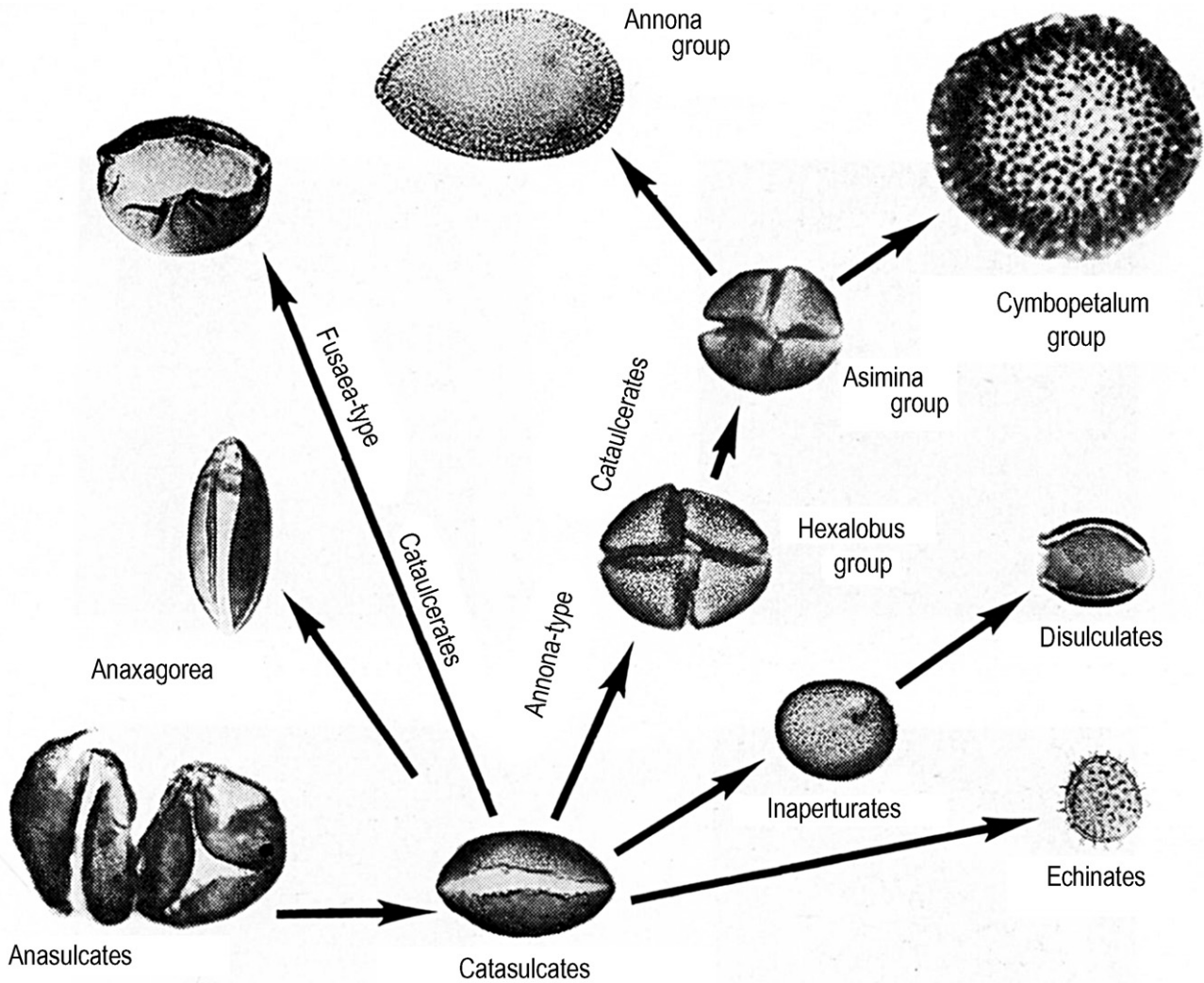


Figure 1. Relationships among major pollen types in Annonaceae proposed by Walker (1971a, pl. 55).

subfamilies and tribes. Because the single grains (monads) making up the tetrads have thinner exine on their proximal surface (toward the centre of the tetrad), a condition that Walker called cataulcerate, he assumed that the sulcus in most monosulcate Annonaceae was also proximal, or catasulcate, rather than distal (away from the centre of the tetrad) in other monosulcate angiosperms. He recognized one exception, in *Pseudoxandra* R.E.Fr., in which he found tetrads of grains with the sulcus at the distal pole. At that time the catasulcate condition was unknown in other angiosperms, but it has been recently reported in the monocot *Tillandsia* L. (Bromeliaceae) by Albert *et al.* (2010).

The monosulcates that Walker (1971a) considered most primitive, in his 'Malmea tribe', have columellar exine structure, with radial rods or columellae connecting the inner and the outer layers of the exine

(nexine and tectum). In these groups, the tectum varies from finely perforate to reticulate, with muri surrounding larger perforations. [The term nexine designates the whole continuous inner layer of the exine, regardless of composition. The exine can also be divided into endexine and ectexine, based on differences in staining; the nexine may consist of endexine, a foot layer of ectexinous composition or both. Endexine and ectexine can be distinguished with transmission electron microscopy (TEM) but not with SEM.] Walker (1971a) interpreted the large columellar tetrads and polyads of his 'Annona subfamily' as derived from columellar monosulcates (Fig. 1). Other monosulcate taxa, such as the Neotropical and Asian genus *Anaxagorea* A.St.-Hil., and tetrad-producing genera such as *Cananga* (DC.) Hook.f. & Thomson and *Xylopi*a L., which Walker grouped in his 'Fusaea subfamily,' have glassy-appearing exines with no

visible structure under LM. Walker called these grains 'microtectate' and interpreted them as derived from the columellar type.

This scenario for exine evolution was challenged by TEM studies of Le Thomas & Lugardon (1974), who showed that Walker's (1971a) microtectate exines have a layer of minute granules under a continuous tectum (Fig. 2A–D), a condition first described by Van Campo & Lugardon (1973) as granular ('structure grenue'). They suggested that granular structure was primitive for Annonaceae, as Van Campo & Lugardon (1973) and Doyle, Van Campo & Lugardon (1975) proposed for angiosperms as a whole. In a TEM survey of exine structure in 'primitive' angiosperms, Walker & Skvarla (1975; Walker, 1976b) came to similar conclusions, but they argued that essentially structureless exines, which they called atectate, as in the magnolialean genera *Degeneria* I.W.Bailey & A.C.Sm. (Degeneriaceae) and *Eupomatia* R.Br. (Eupomatiaceae), were still more primitive than granular ones. Doyle *et al.* (1975) interpreted atectate as an extreme form of granular structure. Le Thomas & Lugardon (1972) also showed that the nexine of many Annonaceae is unusual in consisting of conspicuous foliations, which they originally compared with the laminated endexine of gymnosperms, but later reinterpreted as ectexine, i.e. foot layer, because of its continuity with ectexine elements and similar electron density (Lugardon & Le Thomas, 1974; see discussion in Doyle, 2005).

By studying developing tetrads, Le Thomas & Lugardon (1975) and Le Thomas (1980/1981) also showed that the sulcus in monosulcate Annonaceae is distal, as in other angiosperms, and not proximal, like the cataulcus in the tetrads (Fig. 2E). They therefore argued that the cataulcus is not an aperture but rather corresponds to the proximal thinning of the exine seen in many other angiosperms with permanent tetrads, and as a result they interpreted annonaceous tetrads as inaperturate. Experiments have shown that monads regularly germinate from the proximal surface in *Asimina* Adans., *Cananga* and some species of *Annona* L., but more irregularly in *Annona montana* Macfad. (Periasamy & Swamy, 1959; Le Thomas, Morawetz & Waha, 1986; Hesse, Halbritter & Weber, 2009).

In a synthesis of SEM and TEM data, Le Thomas (1980/1981) hypothesized that Annonaceae originally had granular monosulcate monads, which were modified into inaperturate monads and then tetrads. In exine structure, she proposed a trend from granular and continuous–tectate to columellar and reticulate, occurring in both monosulcate and inaperturate lines, via intermediates with larger granules or irregular columellae and a finely verrucate, 'dislocated' tectum (Figs 2F, G, 3H, I). She also proposed a trend for

origin and then multiplication of nexine foliations. These views appeared to be strengthened by a morphological cladistic analysis of 'basal' angiosperms by Donoghue & Doyle (1989), which identified Magnoliales as the sister group of all other angiosperms. Part of the evidence for this result was the occurrence of granular structure both in Magnoliales and in Gnetales, Bennettitales and *Pentoxylon* Srivastava, which morphological cladistic analyses had identified as the closest outgroups of angiosperms (Crane, 1985; Doyle & Donoghue, 1986). However, this argument has been weakened by molecular evidence that Gnetales are not related to angiosperms (see Donoghue & Doyle, 2000; Doyle, 2008; Mathews, 2009).

MORPHOLOGICAL CLADISTIC ANALYSES

Doyle & Le Thomas (1994, 1995, 1996) tested these ideas with cladistic analyses, using morphological characters from all parts of the plant, with other Magnoliales included as outgroups. In all these studies, Annonaceae were rooted among lines with granular monosulcate pollen, with *Anaxagorea* (Fig. 2A, B) the first branch (the sister group of all other Annonaceae). In Doyle & Le Thomas (1996; Fig. 4), the second line was a small clade called the ambavoids (*Ambavia* Le Thomas, *Cleistopholis* Pierre ex Engl. and *Tetrameranthus* R.E.Fr., which form part of subfamily Ambavioideae of Chatrou *et al.*, 2012), followed by two isolated granular monosulcate lines [*Greenwayodendron* Verdc., segregated from *Polyalthia* Blume by Verdcourt, 1969; '*Polyalthia*' *stuhlmannii* (Engl.) Verdc.] and a large clade consisting of both a small granular monosulcate group, the piptostigmoids (Fig. 2C, D), and more diverse columellar monosulcates, the malmeoids ('*Malmea* tribe' of Walker, 1971a; Fig. 2H). The piptostigmoids correspond in part to tribe Piptostigmateae, whereas most of the malmeoids belong to tribe Malmeeae (Chatrou *et al.*, 2012). The rest of the family split into the miliusoid clade, corresponding to tribe Miliuseae, which contained taxa with round monosulcate, inaperturate and disulcate pollen, and a large clade called the inaperturates, which is largely equivalent to subfamily Annonoideae (Chatrou *et al.*, 2012). At the base of Annonoideae was a 'uvaroid' clade including *Uvaria* L and related lianas, now Uvarieae, which have inaperturate monads (Fig. 3F). Some of these, in the '*Monanthotaxis* group', are distinctive in having spines (apparently remnants of the tectum) set in a reduced granular layer (Fig. 3D, E). The inaperturate clade also included most Annonaceae with tetrad pollen. The earlier-diverging members had granular exine structure (e.g. *Xylopia*: Fig. 3G), which became col-

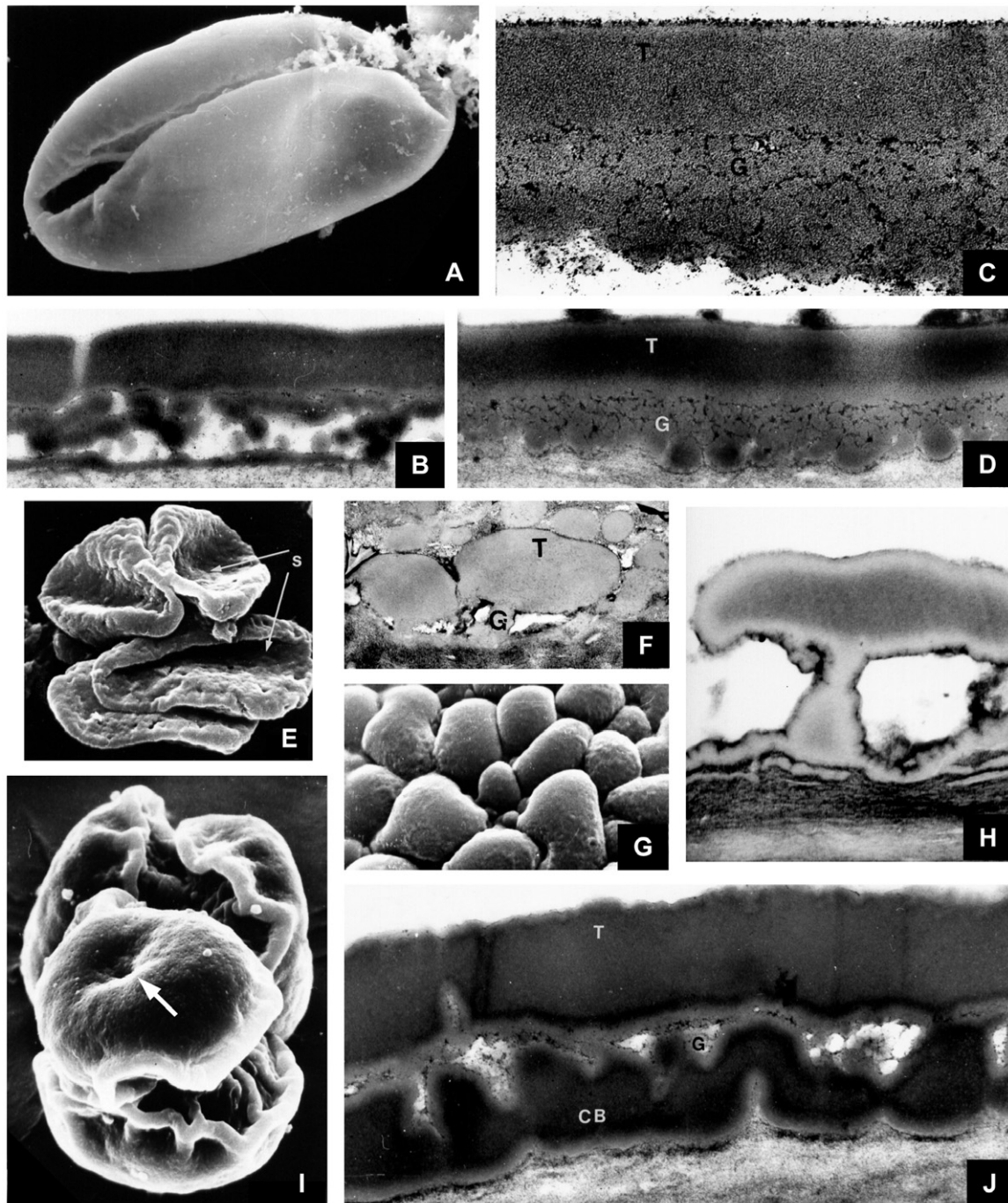


Figure 2. Pollen of *Anaxagorea*, Ambavioideae and Malmeoideae. A, *Anaxagorea dolichocarpa* Sprague & Sandwith, scanning electron microscopic (SEM) image of tectate–granular monosulcate grain, 1200 ×. B, *Anaxagorea brevipes* Benth., transmission electron microscopic (TEM) section of exine showing granular infratectum and one to two nexine foliations, 25 000 ×. C, *Piptostigma calophyllum* Mildbr. & Diels, TEM section of exine showing granular infratectum (G) and lack of foliations, 70 000 ×. D, *Polyceratocarpus pellegrinii* Le Thomas, TEM section of exine showing granular infratectum and fused basal granules, 40 000 ×. E, *Cleistopholis patens* (Benth.) Engl. & Diels, SEM of immature tetrad showing distal position of sulcus (S), 2600 ×. F, *Miliusa indica* Lesch. ex A.DC., TEM section of exine showing intermediate infratectum and ‘dislocated’ tectum (T), 10 000 ×. G, *M. indica*, SEM showing verrucate tectum, 5000 ×. H, *Klarobelia candida*, TEM section of exine showing perforate tectum, columellae and two to three foliations, the outermost of which is thickened, 30 000 ×. I, *Cananga odorata* (Lam.) Hook.f. & Thomson, SEM of tetrad with possible vestigial sulcus (arrow), 750 ×. J, *C. odorata*, TEM section of exine showing nexine apparently consisting of fused granules (CB), 28 000 ×.

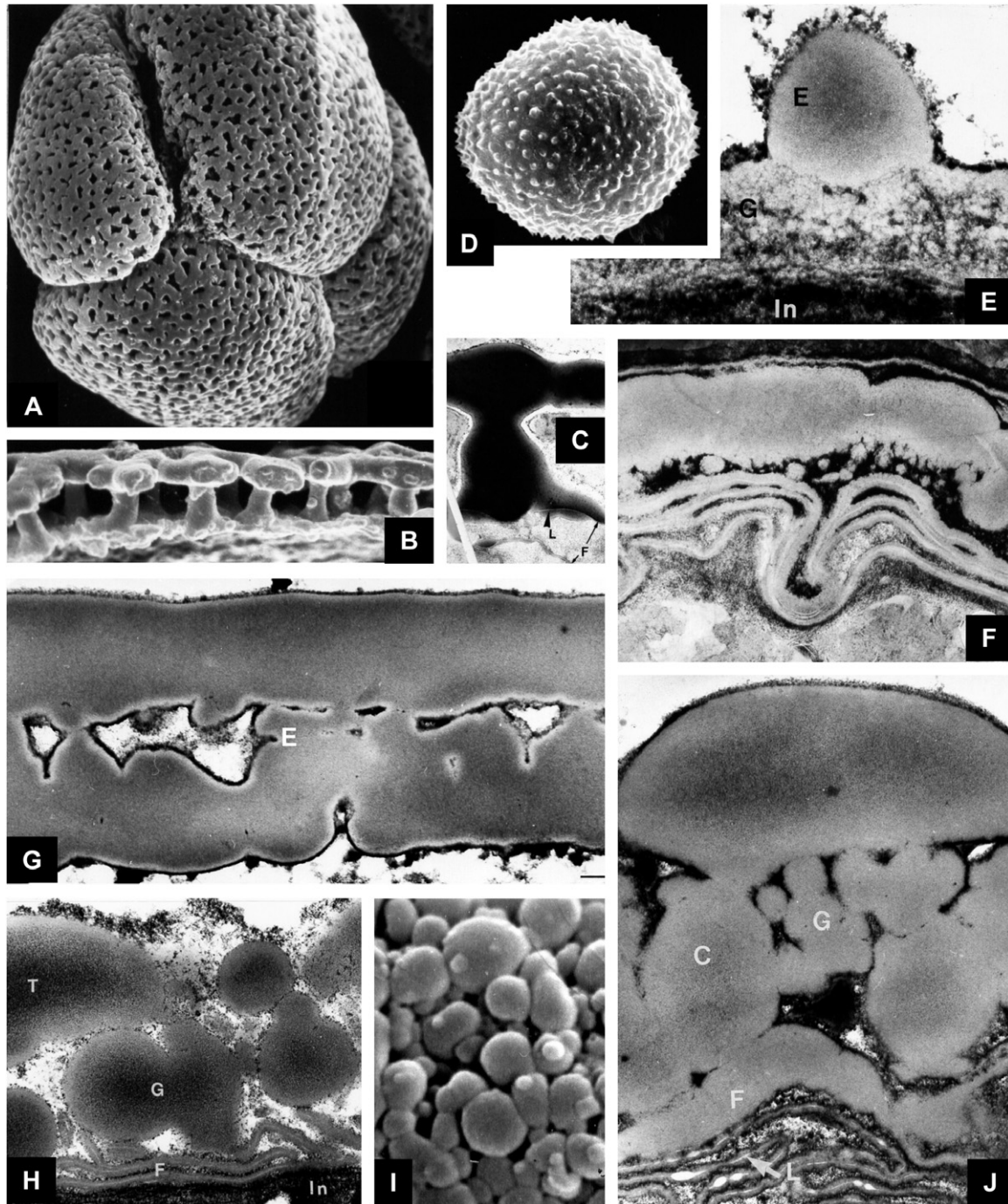


Figure 3. Pollen of Annonoideae. A, *Annona glauca* Schumach. & Thonn., scanning electron microscopic (SEM) image of reticulate-columellar tetrad, 750 \times . B, *A. glauca*, SEM of broken exine showing columellar structure, 3750 \times . C, *A. glauca*, transmission electron microscopic (TEM) section of exine showing one basally widened columella and foliations (F), 7500 \times . D, *Friesodielsia enghiana* (Diels) Verdc. ex Le Thomas, SEM of echinate inaperturate grain, 2500 \times . E, *F. enghiana*, TEM section of exine showing granular layer and spine presumably representing the reduced tectum (E), 50 000 \times . F, *Uvaria klaineana* Engl. & Diels, TEM section of exine showing granular infratectum and multiple foliations, 30 000 \times . G, *Xylopia staudtii* Engl. & Diels, TEM section of exine showing massive irregular nexine, 20 000 \times . H, *Isolona thonneri* (De Wild. & T. Durand) Engl. & Diels, TEM section of exine showing intermediate infratectum, 'dislocated' tectum and numerous foliations, 35 000 \times . I, *I. thonneri*, SEM showing verrucate tectum, 13 000 \times . J, *Uvariastrum pynaertii* De Wild., TEM section of exine showing intermediate infratectum, numerous foliations and thickened outer foliation (F), 25 000 \times .

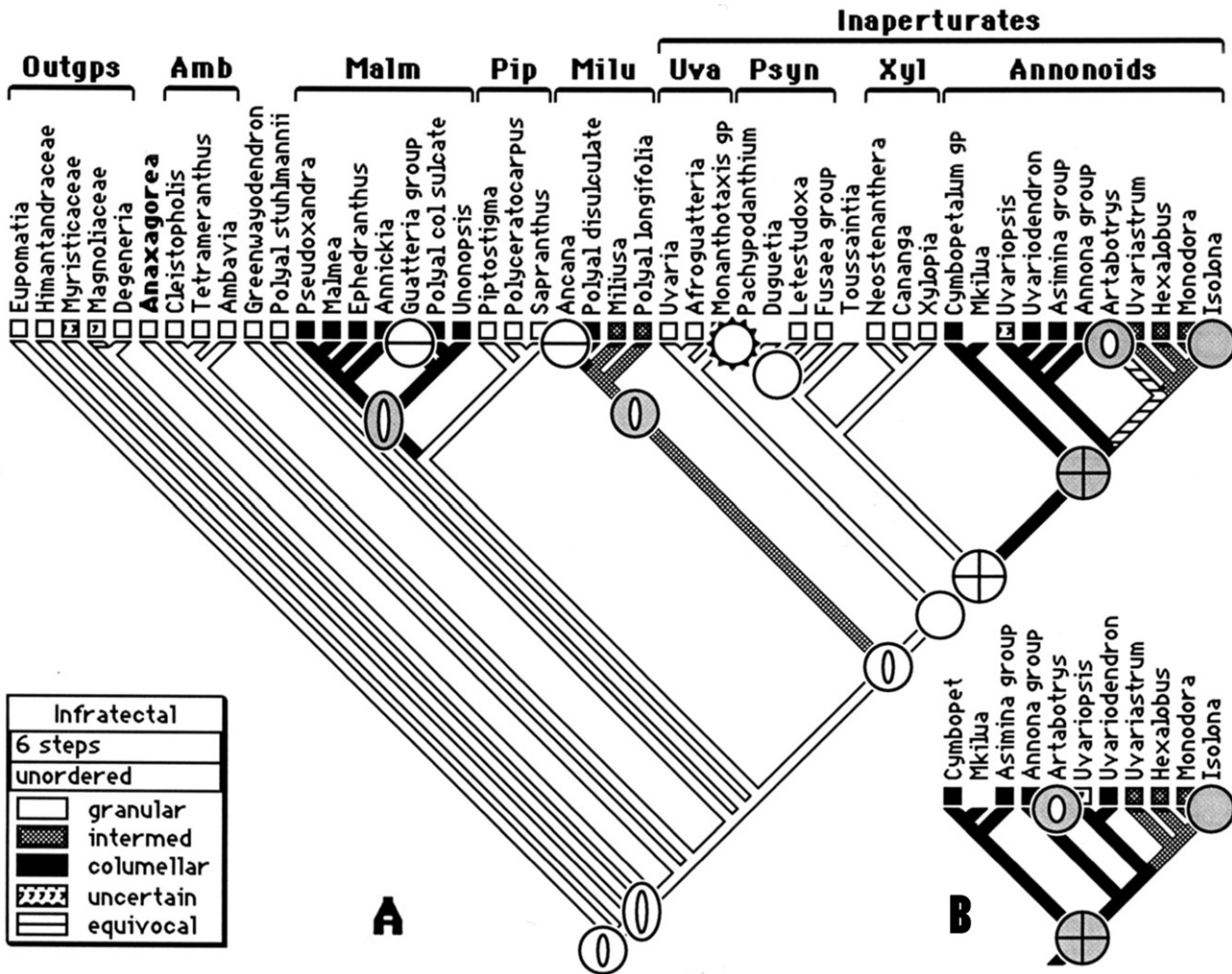


Figure 4. Representative most parsimonious phylogenetic trees of Annonaceae and other Magnoliales based on morphological characters (Doyle & Le Thomas, 1996, Fig. 10), showing the most parsimonious course of evolution of infratectal structure, with sketches of major pollen types placed where they appear on the cladogram. Abbreviations: Outgps, outgroups (other Magnoliales); Amb, ambavioids (Ambavioideae in part); Malm, malmeoid, malmeoids (Malmeae in part); Pip, piptostigmoids (Piptostigmataeae in part); Milu, miliusoids (Miliuseae); Uva, uvarioids (Uvarieae); Psyn, pseudosyncarpis (Duguetieae); Xyl, xylopioids (Xylopieae in part); Polycol sulcate, columellar sulcate *Polyalthia* group (= *Maasia*). Annonoids correspond to Bocageae, Annonaeae and Monodoreae. Here, and in subsequent figures, boxes below taxon names indicate the character state for the taxon; absence of a box means that the character was scored as unknown (?), whereas ‘uncertain’ means it was scored as having either of two states (e.g. 0/1 = granular or intermediate). ‘Equivocal’ indicates that the reconstructed state on a line is uncertain.

umellar in the taxa that Walker (1971a) assigned to his *Annona* subfamily (Fig. 3A–C).

MOLECULAR ANALYSES

Analyses of *trnL-trnF* spacer sequences from 19 genera of Annonaceae by Van Zuilén (1996) confirmed the position of *Anaxagorea* as sister to the rest of the family, inferred from morphology, but disagreed in grouping the members of Malmeae and Miliuseae and nesting *Uvaria* within Annonoideae. To test the

implications of molecular data with a larger number of taxa, Doyle, Bygrave & Le Thomas (2000) combined the morphological data of Doyle & Le Thomas (1996) with *rbcl* sequences of Bygrave. This analysis confirmed the position of *Anaxagorea* and grouped the remaining species into three major clades. One was the Ambavioideae, with the addition of *Cananga* (previously associated with *Xylopia* and *Neostenanthera* Exell). The second, called the MPM clade, which corresponds to Malmeoideae (Chatrou *et al.*, 2012), consisted of Malmeae, *Maasia*, Piptostigmataeae and

Miliuseae, with *Sapranthus* Seem. moved from Piplostigmateae into Miliuseae, a change consistent with its disulculate pollen. The third clade corresponded more precisely to Annonoideae, with the loss of *Cananga* and the addition of the zonasulcate 'Guatteria group.' Uvarieae were nested among taxa with columellar tetrads, implying that their granular monads were secondarily derived rather than primitive. Based on *rbcL* data alone, Ambavioideae were linked with Annonoideae, but when *rbcL* and morphological data were combined, both this arrangement and the morphological position of Ambavioideae as sister to both Annonoideae and Malmeoideae were equally parsimonious.

Molecular analyses (reviewed in Soltis *et al.*, 2005) have called into question the importance of annonaecous pollen for angiosperm evolution by showing that the earliest-diverging angiosperms are not Magnoliales but rather the 'ANITA' lines: *Amborella*, Nymphaeales and Austrobaileyales (*Austrobaileya* C.T.White, *Trimenia* Seem., *Illicium* L and *Schisanthraceae*). As discussed by Doyle (2005, 2009), these results imply that the first angiosperms had columellar exine structure, as this is the type found in the ANITA lines. Based on similar reasoning, the granular structure seen in Annonaceae and some other Magnoliales is derived. However, the closest outgroups of Annonaceae [*Eupomatia*, *Degeneria* and *Galbulimima* F.M.Bailey (Himantandraceae): Sauquet *et al.*, 2003] have granular exines, consistent with the view that granular structure is ancestral in Annonaceae.

RECENT PALYNOLOGICAL ADVANCES

Since 2000 there have been two sorts of advances relating to pollen evolution in Annonaceae. First, molecular analyses based on many more taxa and sequences (Mols *et al.*, 2004, 2008; Richardson *et al.*, 2004; Pirie *et al.*, 2006; Couvreur *et al.*, 2008b, 2011; Surveswaran *et al.*, 2010; Xue *et al.*, 2011; Chatrou *et al.*, 2012) have produced greatly expanded and more strongly supported phylogenetic trees, thereby providing an improved framework for reconstruction of character evolution. These analyses have confirmed the earlier branching position of the enlarged Ambavioideae (thus supporting morphology over *rbcL* alone) and the monophyly of the four main clades now recognized as subfamilies, while clarifying relationships within clades.

Second, there have been new discoveries on pollen morphology in Annonaceae. The most surprising of these came from developmental studies by Tsou & Fu (2002, 2007) and Lora *et al.* (2009) on tetrads of *Annona* and polyads of *Cymbopetalum* Benth. Exine begins to form when the microspores are separated by

callose. Contrary to expectation, the exine is thick and sculptured on the proximal side and thin at the distal pole. Then, as the callose breaks down, it contracts to the centre of the tetrad, pulling the microspores through 180°, so that the thin areas rotate to the centre of the tetrad. Therefore, the thin area corresponds in its initial position to a distal sulcus, not to the proximal thinning in tetrads of other angiosperms.

Other new data have come from surveys of pollen morphology made during broader phylogenetic investigations. In a study of Miliuseae, in which Walker (1971a) described most genera as inaperturate, but Waha & Hesse (1988), Waha & Morawetz (1988) and Le Thomas (1988) reported the common occurrence of disulculate pollen, Mols (2004) concluded that many more species are inaperturate than disulculate. However, this result is problematic, because Mols (2004) defined grains as disulculate only when the sulculi are visible with SEM, whereas Waha & Hesse (1988) included grains with two bands of modified exine that are not visible in surface view. More recently, Chaowasku, Mols & van der Ham (2008) accepted that many supposed inaperturate grains are disulculate and presented new TEM data on exine structure in Miliuseae. Couvreur *et al.* (2008a) provided important new data on exine structure in African members of Annonoideae. TEM data of M. Suarez-Cervera (unpubl. data) (briefly reported in Le Thomas, 1998) clarify exine structure in *Ephedranthus* S.Moore, *Malmea* R.E.Fr. and the three genera segregated from *Malmea* by Chatrou (1998). The goal of the present study is to update and synthesize evidence on pollen evolution in Annonaceae and its systematic significance in the context of the most recent molecular phylogenetic trees.

MATERIAL AND METHODS

Definitions of 13 pollen characters, a list of the five outgroups and 73 taxa of Annonaceae and sources of palynological data are provided in the Appendix. The taxa include those treated in Doyle & Le Thomas (1996), selected for availability of data on exine structure and coverage of diversity in the family, and additional taxa sampled in molecular analyses for which pollen data exist. Problems in determining character states in particular taxa are discussed in the taxon list. Strictly ultrastructural characters (e.g. nexine foliations) are still unknown for genera that have been studied with LM only (mostly by Walker, 1971a, b, 1972), although in many cases tectal structure (continuous, perforate, verrucate) and infratectal structure can be tentatively inferred from LM. Given the difficulties in correlating LM and SEM observa-

tions near the limit of resolution of light, some of these scorings are in need of testing with SEM and/or TEM.

In Doyle & Le Thomas (1994, 1996), *Polyalthia*, which Le Thomas (1980/1981) had shown to be highly variable palynologically, was represented by two single species [*P. longifolia* (Sonn.) Thwaites and *P. stuhlmannii*] and two palynologically defined subgroups: Asian and Malagasy species with reticulate-columellar monosulcate pollen (Rogstad & Le Thomas, 1989) and Asian species with disulcate pollen. The analyses of Doyle & Le Thomas (1994, 1996) confirmed suspicions that *Polyalthia* is polyphyletic, and molecular analyses of Mols *et al.* (2004, 2008), Xue *et al.* (2011) and Chatrou *et al.* (2012) have further rearranged its component groups. Mols *et al.* (2008) assigned the Asian species of the reticulate-columellar sulcate group (the '*Polyalthia hypoleuca* complex' of Rogstad, 1989), which form a clade not directly related to other *Polyalthia* spp., to the new genus *Maasia* Mols, Kessler & Rogstad. Molecular analyses assign members of the disulcate *Polyalthia* group to *Polyalthia s.s.* and *Enicosanthum* Becc. We have retained '*Polyalthia stuhlmannii*' but included '*P. longifolia* in *Enicosanthum*'. The Australian '*Ancana* group' of Morawetz (1988) also appears to be polyphyletic (Mols *et al.*, 2004; Xue *et al.*, 2011): *Ancana* F.Muell. and *Fitzalania* F.Muell. are nested in *Meiogyne* Miq., whereas *Haplostichanthus* F.Muell. belongs in *Polyalthia s.s.* We have used palynological data from species formerly placed in these obsolete genera to score characters of the genera to which they are now assigned. Following Chatrou (1998), we have segregated *Klarobelia* Chatrou, *Mosannonna* Chatrou and *Pseudomalmea* Chatrou from *Malmea*. Another traditional genus now known to be polyphyletic is *Melodorum* Lour. (Zhou *et al.*, 2010; Chatrou *et al.*, 2012); data of Le Thomas (1980/1981) for African material identified as *Melodorum gracile* (Engl. & Diels) Verdc., already transferred to *Sphaerocoryne* Scheff. ex Ridl. by Verdcourt (1986) as *S. gracilis*, are used to score *Sphaerocoryne*. Based on molecular evidence of Erkens *et al.* (2007) that *Guatterrella* R.E.Fr., *Guatterriopsis* R.E.Fr. and *Heteropetalum* Benth. are nested within *Guatteria* Ruiz & Pav., we refer to the former *Guatteria* group simply as *Guatteria*. Following Richardson *et al.* (2004), we have removed *Anonidium* Engl. & Diels from the '*Annona* group.' The *Asimina*, *Cymbopetalum* and *Monanthes* groups all appear to be monophyletic.

The resulting data matrix is presented in Table 1. Cases where a taxon is scored as having either of two states (e.g. 0/1 = either 0 or 1), for example because both states occur in the taxon and the ancestral state is unclear, are termed 'uncertain,' whereas 'unknown' (?) means that no data are available or the character

cannot be interpreted (for binary characters these two situations are effectively equivalent). The matrix includes all pollen characters used by Doyle & Le Thomas (1996), plus two new characters: one incorporating an alternative interpretation of aperture condition in tetrad taxa, as discussed below; the other for the perisaccate to subsaccate exine of four genera of Malmeeae not included in previous studies (*Bocageopsis* R.E.Fr., *Onychopetalum* R.E.Fr., *Oxandra* A.Rich. and *Ruizodendron* R.E.Fr.: Walker, 1971a). We have rescored *Xylopia* and *Neostenanthera* as unknown for fusion of basal infratectal granules and thickening of the outer nexine foliation, for reasons discussed in Doyle *et al.* (2000) and below. We have also treated the nexine foliation character as unordered rather than ordered because of uncertainty concerning processes involved in origin of the non-foliated nexine in *Xylopia* and *Neostenanthera*, as discussed in Doyle *et al.* (2000) and below. Although the character of reduced proximal exine is problematic in light of rotation of the microspores in some tetrad groups (Tsou & Fu, 2002, 2007), we have retained it for purposes of reference. As in previous analyses, some taxa have such reduced exines that we have scored several of their exine characters as unknown (?), as the distinctions among states are inapplicable (notably *Duguetia* A.St.-Hil., *Guatteria*, *Mkilua* Verdc., '*Pachypodanthium*' Engl. & Diels and *Toussaintia* Boutique: Le Thomas, 1980/1981; Waha, 1987a).

The tree used for analysis of character evolution is based on the Bayesian multigene analysis of Chatrou *et al.* (2012). This is largely consistent with the earlier analyses of Richardson *et al.* (2004), Pirie *et al.* (2006), Mols *et al.* (2004) and Couvreur *et al.* (2008b, 2011); exceptions are the position of *Maasia* on the line leading to Miliuseae and arrangements of genera within Malmeeae and Miliuseae. Relationships of outgroups (other Magnoliales) follow the combined molecular and morphological analysis of Doyle & Endress (2000) and the multigene analyses of Sauquet *et al.* (2003), which placed Myristicaceae as sister to the rest of the order and Magnoliaceae one node higher, as the sister group of *Degeneria*, *Galbulimima*, *Eupomatia* and Annonaceae. Character evolution was reconstructed by plotting (optimizing) characters on the tree based on the principle of parsimony, using MacClade (Maddison & Maddison, 2003). MacClade was also used to study the evolution of characters on alternative trees and to calculate measures of homoplasy (consistency indices) for subsets of characters on the tree of Chatrou *et al.*, using the present data set for pollen characters and the data set of Doyle & Le Thomas (1996) for other characters. In comparisons of homoplasy in different character sets, vegetative characters are characters

Table 1. Data matrix for pollen characters in Annonaceae and outgroups. See text for discussion and Appendix for definitions of characters and taxa and documentation of scoring

Taxa	Pollen characters												
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Degeneria</i>	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Eupomatia</i>	0	0	2	2	1	0	0	0	0	0	1	0	0
<i>Galbulimima</i>	0	0	0	0	1	0	0	0	0	0	0	0	0
Magnoliaceae	0	0	0	0	0	0/1	0	0	2	0	1	1	1
Myristicaceae	0	0	0	0	1	2	0	0	2	0	1	1	0
<i>Afroguatteria</i>	0	0	2	2	1	0	0	0	0	0	2	0	0
<i>Alphonsea</i>	0	0	1/2	1/2	1	1	0	0	1	0	1	0	1
<i>Ambavia</i>	0	1	0	0	0	0	0	0	0	0	1	0	0
<i>Anaxagorea</i>	0	0	0	0	0	0	0	0	0	0	1	0	1
<i>Annickia</i>	0	0	0	0	0	2	0	0	2	0	1	1	1
<i>Annona</i> group	1	1	1	0	1	2	0	0	2	0	2	1	2
<i>Anonidium</i>	1	1	1	?	1	2	0	0	2	0	2	1	2
<i>Artabotrys</i>	0	0	0	0	1	1/2	0	0	1/2	0	2	1	1
<i>Asimina</i> group	1	1	1	1	1	2	0	0	2	0	2	1	2
<i>Asteranthe</i>	1	1	1	?	1	2	0	0	2	0	2	1	2
<i>Bocageopsis</i>	0	0	0	0	0	2	1	0	2	0	1	1	0
<i>Cananga</i>	1	1	1	?	1	0	0	0	0	1	0	0	2
<i>Cleistopholis</i>	0	0	0	0	0	0	0	0	0	1	1	0	0
<i>Crematosperma</i>	0	0	0	0	0	2	0	0	2	?	?	?	1
<i>Cyathocalyx</i>	0	0	0	0	1	0/2	0	0	0	1	1	0	1
<i>Cymbopetalum</i> group	1	1	1	0	1	2	0	0	2	0	1	1	2
<i>Dasymaschalon</i>	0	0	1	1	1	?	?	1	?	?	?	?	0
<i>Desmopsis</i>	0	0	1	1	1	1	0	0	2	0	1	?	1
<i>Disepalum</i>	1	1	1	?	1	2	0	0	2	?	?	?	2
<i>Duguetia</i>	0	0	1	1	1	1	0	0	?	?	?	?	1
<i>Enicosanthum</i>	0	0	1/2	1/2	1	1	0	0	1/2	0	1	0	0
<i>Ephedranthus</i>	0	0	0	0	0	2	0	0	2	0	2	1	1
<i>Fusaea</i> group	1	1	1	?	1	0	0	0	0	0	2	0	2
<i>Goniothalamus</i>	1	1	1	?	1	0	0	0	?	?	?	?	2
<i>Greenwayodendron</i>	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Guatteria</i>	0	0	2	2	0/1	?	?	0	?	?	?	?	1
<i>Hexalobus</i>	1	1	1	?	1	1/2	0	0	1	0	2	1	1
<i>Isolona</i>	0	1	1	?	1	1	0	0	1	0	2	0	1
<i>Klarobelia</i>	0	0	0	0	0	2	0	0	2	0	2	1	0
<i>Letestudoxa</i>	0	0	2	2	1	1	0	0	0	0	2	0	1
<i>Lettowianthus</i>	0	0	0	0	1	0	0	0	0	0	1	0	2
<i>Maasia</i>	0	0	0	0	0	2	0	0	2	0	1	1	1
<i>Malmea</i>	0	0	0	0	0	2	0	0	1	0	1	1	1
<i>Marsypopetalum</i>	0	0	2	2	1	1	0	0	2	?	?	?	1
<i>Meiocarpidium</i>	0	0	0	0	1	0	0	0	0	1	0	0	2
<i>Meiogyne</i>	0	0	1/2	1/2	1	1	0	0	2	0	1	0	0
<i>Mezzettia</i>	0	0	?	?	1	1	0	0	?	?	1	0	0
<i>Milusa</i>	0	0	1/2	1/2	1	1	0	0	1	0	1	0	0
<i>Mischogyne</i>	1	1	1	?	1	2	0	0	1	0	2	1	1
<i>Mitrephora</i>	1	0	1	1	1	1	0	0	2	?	?	?	1
<i>Mkilua</i>	1	1	1	?	1	?	?	0	?	?	?	?	2
<i>Monanthotaxis</i> group	0	0	1	1	1	?	?	1	0	?	?	?	0
<i>Monocarpia</i>	0	0	0	0	0	2	0	0	2	0	1	1	1
<i>Monodora</i>	1	1	1	?	1	2	0	0	1	0	2	1	1
<i>Mosannona</i>	0	0	0	0	0	2	0	0	2	0	2	1	1
<i>Neostenanthera</i>	1	1	1	?	1	0	0	0	0	?	0	?	1

Table 1. Data matrix for pollen characters in Annonaceae and outgroups. See text for discussion and Appendix for definitions of characters and taxa and documentation of scoring

Taxa	Pollen characters												
	1	2	3	4	5	6	7	8	9	10	11	12	13
<i>Onychopetalum</i>	0	0	0	0	0	?	1	0	2	?	?	?	0
<i>Ophrypetalum</i>	1	1	1	?	1	2	0	0	2	0	2	1	1
<i>Orophea</i>	0	0	1/2	1/2	1	1	0	0	2	0	1/2	0	0
<i>Oxandra</i>	0	0	0	0	0	?	1	0	2	?	?	?	0
' <i>Pachypodanthium</i> '	0	0	1	1	1	?	?	1	?	?	?	?	0
<i>Phaeanthus</i>	0	0	1	1	1	1	0	0	?	?	?	?	1
<i>Piptostigma</i>	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Platymitra</i>	0	0	1	1	1	1	0	0	2	0	1	0	0
<i>Polyalthia s.s.</i>	0	0	1/2	1/2	1	1	0	0	2	0	1	0	0
' <i>P. stuhlmannii</i> '	0	0	0	0	0	0	0	0	0	0	1	0	0
<i>Polyceratocarpus</i>	0	0	0	0	0	0	0	0	0	1	1	0	1
<i>Popowia</i>	0	0	1	1	1	1	0	0	?	?	?	?	0
<i>Pseudomalmea</i>	0	0	0	0	0	0	0	0	1	0	2	1	?
<i>Pseudoxandra</i>	1	0	0	0	0	2	0	0	2	0	1	1	1
<i>Pseuduvaria</i>	1	1	1	1	1	1	0	0	2	?	2	?	0
<i>Ruizodendron</i>	0	0	0	0	0	?	1	0	2	?	?	?	0
<i>Sapranthus</i>	0	0	2	2	1	0	0	0	0	0	0	0	1
<i>Sphaerocoryne</i>	0	0	1	1	1	1	0	0	1	0	2	?	1
<i>Stenanona</i>	0	0	1	1	1	1	0	0	2	?	?	?	1
<i>Tetrameranthus</i>	0	0	0	0	1	0	0	0	0	0	2	0	0
<i>Toussaintia</i>	1	1	1	?	1	?	?	0	?	?	2	?	1
<i>Unonopsis</i>	0	0	0	0	0	2	0	0	2	0	1	1	1
<i>Uvaria</i>	0	0	1	1	1	0	0	0	0	0	2	0	1
<i>Uvariastrum</i>	1	1	1	?	1	2	0	0	1	0	2	1	1
<i>Uvarioidendron</i>	1	1	1	?	1	2	0	0	2	0	2	1	1
<i>Uvariopsis</i>	1	1	1	?	1	2	0	0	1/2	0	2	0/1	1
<i>Xylopia</i>	1	1	1	?	1	0	0	0	0	?	0	?	1

1–21 of Doyle & Le Thomas (1996), floral characters 22–41 and 53–57 and fruit and seed characters 58–75.

RESULTS

Cladograms with optimized characters are presented in Figures 5–14, with shading of the boxes at the tips of the tree representing observed character states in taxa and shading of the branches representing the reconstructed most parsimonious state on the branch. For all characters except pollen form, the same character optimization in Annonaceae is found if outgroups are removed. Consistency indices for subsets of characters are presented in the Discussion.

DISCUSSION

EVOLUTION OF POLLEN CHARACTERS

Exine structure and sculpture

Analysis of our updated data set confirms earlier inferences that granular monosulcate pollen is ances-

tral in Annonaceae and homologous with similar pollen in other Magnoliales, and that columellae arose within the family. This is illustrated in Figure 5, where infratectal structure, with granular, intermediate and columellar states, is plotted on the tree of Chatrou *et al.* (2012). As previously noted, present data for angiosperms as a whole indicate that the origin of columellae in Annonaceae was not part of a general trend from granular to columellar structure, but rather a reversal to the ancestral condition in angiosperms (Doyle, 2005).

Figure 5 implies that columellar structure originated earlier than was inferred by Doyle & Le Thomas (1996; Fig. 4), in the common ancestor of Malmeoideae and Annonoideae. This is because molecular data place columellar taxa lower in both of these clades. In Malmeoideae, the African columellar monosulcate genus *Annickia* Setten & Maas is linked with the granular monosulcate Piptostigmateae (now including *Greenwayodendron*), rather than nested among the Neotropical Malmeae. In Annonoideae,

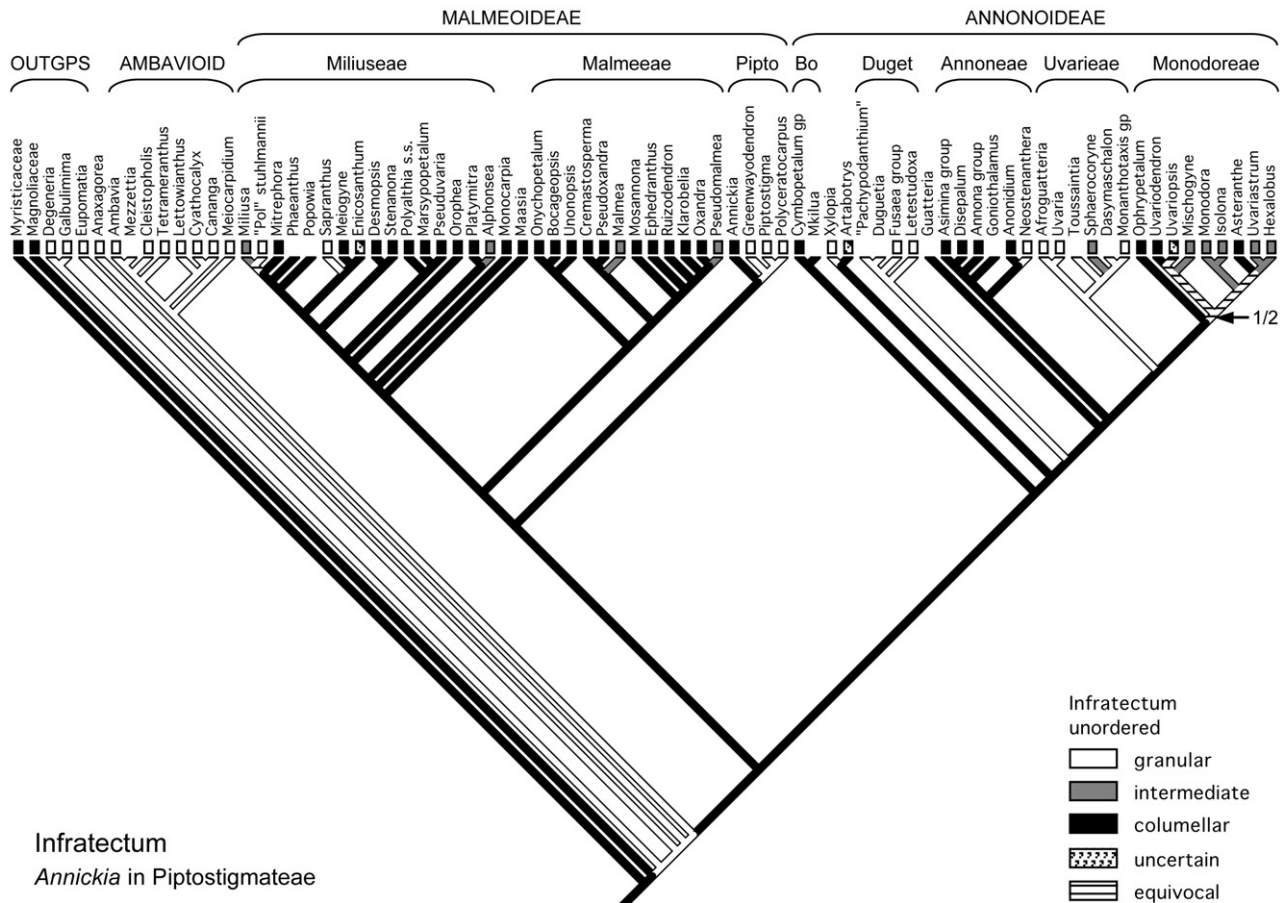


Figure 5. Phylogeny of Annonaceae and other Magnoliales based on molecular data (Chatrou *et al.*, 2012), showing the most parsimonious course of evolution of infratectal structure. Abbreviations: OUTGPS, outgroups; AMBAVIOID, Ambavioideae; Pipto, Piptostigmatae; Bo, Bocageae; Duget, Duguetieae. See Figure 4 for explanation of unknown, uncertain and equivocal.

Bocageae, most of which have columellar polyads, are sister to the rest of the subfamily, rather than nested among taxa with columellar tetrads.

Because the columellar genus *Annickia* is sister to the remaining Piptostigmatae, Figure 5 implies that the granular structure of the latter (Fig. 2C, D) is a reversal, rather than plesiomorphic, as previously inferred (Doyle & Le Thomas, 1996; Doyle *et al.*, 2000). However, the molecular support for this connection is weak. If *Annickia* is moved to the branch leading to Miliuseae, *Monocarpia*, *Maasia* and Malmeae (Fig. 6), a more parsimonious position in terms of morphology, the inferred state in the common ancestor of Malmeoideae and Annonoideae becomes equivocal, either columellar or granular, and it becomes equally parsimonious to assume that granular structure persisted up to granular members of Piptostigmatae and Annonoideae.

Different results were also found by Couvreur *et al.* (2008b), using Bayesian stochastic methods, which

are thought to counteract artefacts attributable to differing rates of evolution among lines. In their analysis of exine evolution, with *Annickia* linked with other Piptostigmatae, Couvreur *et al.* (2008b) concluded that the ancestral state in Annonoideae was most likely granular (posterior probability, PP = 0.64; PP = 0.34 for columellar). However, it is not clear that this result is entirely a function of Bayesian methods, as Couvreur *et al.* scored infratectal structure in *Artabotrys* R.Br. and *Uvariopsis* Engl. as unknown (?), rather than uncertain (1/2, either intermediate or columellar), as here. Known members of these taxa are columellar or intermediate, but scoring them as unknown assumes that they could just as likely be granular. In terms of parsimony, this difference in scoring would have an effect on the results. If *Annickia* is linked with other Piptostigmatae and *Artabotrys* is rescored as unknown, the most parsimonious ancestral state in both Annonoideae and the combined Annonoideae–Malmeoideae shifts from columellar to either granular or columellar (rescoring

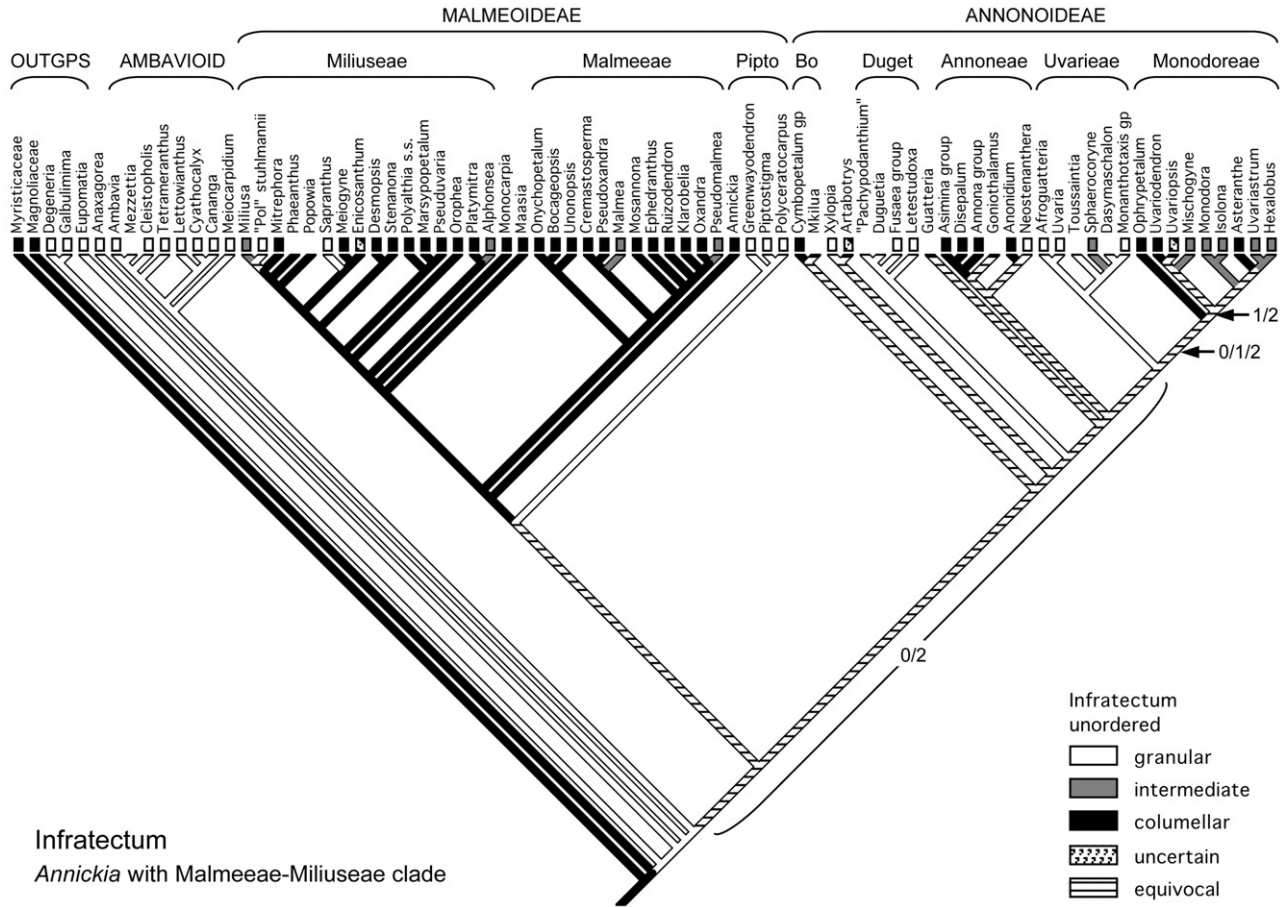


Figure 6. Alternative phylogeny of Annonaceae and other Magnoliales, with *Annickia* moved to the branch leading to the Malmeeae–*Maasia*–*Monocarpia*–Miliuseae clade, showing the most parsimonious course of evolution of infratectal structure.

Uvariopsis has no effect). Assuming that it is more appropriate to treat *Artabotrys* as uncertain, these considerations may weaken the inference of Couvreur *et al.* (2008b) that granular structure was ancestral in Annonoideae.

A critical taxon for understanding exine evolution in Annonaceae is *Xylopia*, which has tetrads of smooth grains, as opposed to tetrads with columellar and intermediate structure in most members of the clades that include *Annona* (Fig. 3A–C) and *Hexalobus* A.DC., the Annoneae and Monodoreae, respectively. Le Thomas (1980/1981) and Doyle & Le Thomas (1996) interpreted *Xylopia* as having a granular infratectum and a nexine formed by fusion of large basal granules (Fig. 3G). This interpretation was based on comparison with *Polyceratocarpus* Engl. & Diels (Fig. 2D), *Cleistopholis* and *Cananga* (Fig. 2I, J), the sister group of *Xylopia* in Doyle & Le Thomas (1996; Fig. 4), in which the innermost layer is clearly made up of enlarged granules. However, as discussed by Doyle *et al.* (2000), it now appears that none of

these genera is closely related to *Xylopia*. The morphological analyses of Doyle & Le Thomas (1994, 1996; Fig. 4) placed *Polyceratocarpus* in Piptostigmateae and *Cleistopholis* in Ambavioideae (positions confirmed by molecular data), whereas molecular analyses also place *Cananga* in Ambavioideae, along with *Cyathocalyx* Champ. ex Hook.f. & Thomson. This raises the possibility that the supposed large granules in *Xylopia* are actually modified columellae (Doyle & Le Thomas, 1995, 1996; Doyle *et al.*, 2000). Their shape is often suggestive of columellae with widened bases, which are common in Annonoideae with columellar tetrads (compare Fig. 3B and C with 3G). It is also suggestive that the exine is thinner in *Xylopia* than in most columellar tetrads, consistent with the hypothesis that its ancestors had columellae that lost their typical form as a consequence of general exine reduction (Doyle & Le Thomas, 1995). Similar considerations apply to *Neostenanthera*, nested still higher among columellar Annonoideae. Developmental studies could test these hypotheses. Evidence that

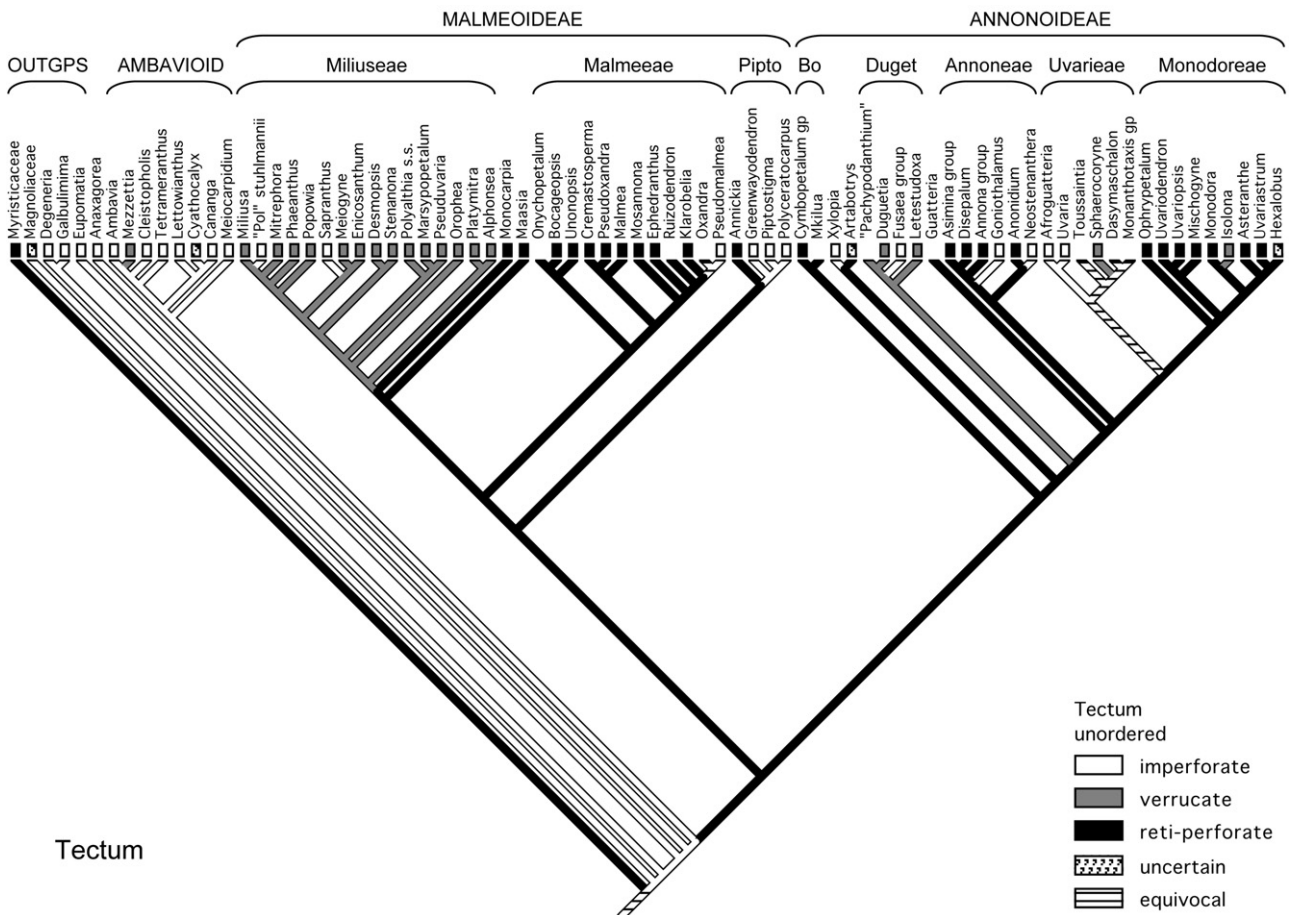


Figure 7. Phylogeny of Annonaceae and other Magnoliales, showing the most parsimonious course of evolution of tectal structure.

Xylopia is basically columellar would increase support for an earlier origin of columellar structure.

These uncertainties also affect interpretation of exine evolution in Uvarieae, in which most taxa have monads with granular structure (Fig. 3E, F), becoming intermediate in *Sphaerocoryne*. If columellae originated in the common ancestor of Malmeoideae and Annonoideae (Fig. 5), the granular structure of Uvarieae would represent a reversal, but it could be a retention of the ancestral state if the common ancestor was granular (Fig. 6). Most Uvarieae have thin exines (cf. Waha, 1987a), which would be consistent with the hypothesis that columellae were lost as a result of exine reduction. In *Toussaintia*, the exine is reduced to a few nexine foliations (Le Thomas, 1980/1981). The Bayesian analysis of Couvreur *et al.* (2008a) suggested a third scenario: that granular structure in Uvarieae was a reversal from intermediate structure, reconstructed as the most likely state (PP = 0.70) in the common ancestor of Uvarieae and Monodoreae.

Most genera in Monodoreae (= African long branch clade of Couvreur *et al.*, 2008b; roughly equivalent to the 'Hexalobus tribe' of Walker, 1971a, 1972) have intermediate infratectal structure (Fig. 3H, J), which Le Thomas (1980/1981) interpreted as a transitional stage in the trend from granular to columellar. However, the morphological analysis of Doyle & Le Thomas (1996; Fig. 4) indicated that intermediate structure in this clade (represented by *Uvariastrum* Engl., *Hexalobus*, *Monodora* Dunal and *Isolona* Engl.) was derived from columellar. With the addition of *Ophrypetalum* Diels, *Mischogyne* Exell and *Asteranthe* Engl. & Diels, and changes in inferred relationships, this situation has become more uncertain. In Figure 5, parsimony optimization implies that intermediate structure in Monodoreae was derived from columellar, either once, with two or three reversals to columellar in *Uvariadendron* (Engl. & Diels) R.E.Fr., some *Uvariopsis* spp. and *Asteranthe*, or up to four times, in *Mischogyne*, within *Uvariopsis* and in the *Monodora–Isolona* and

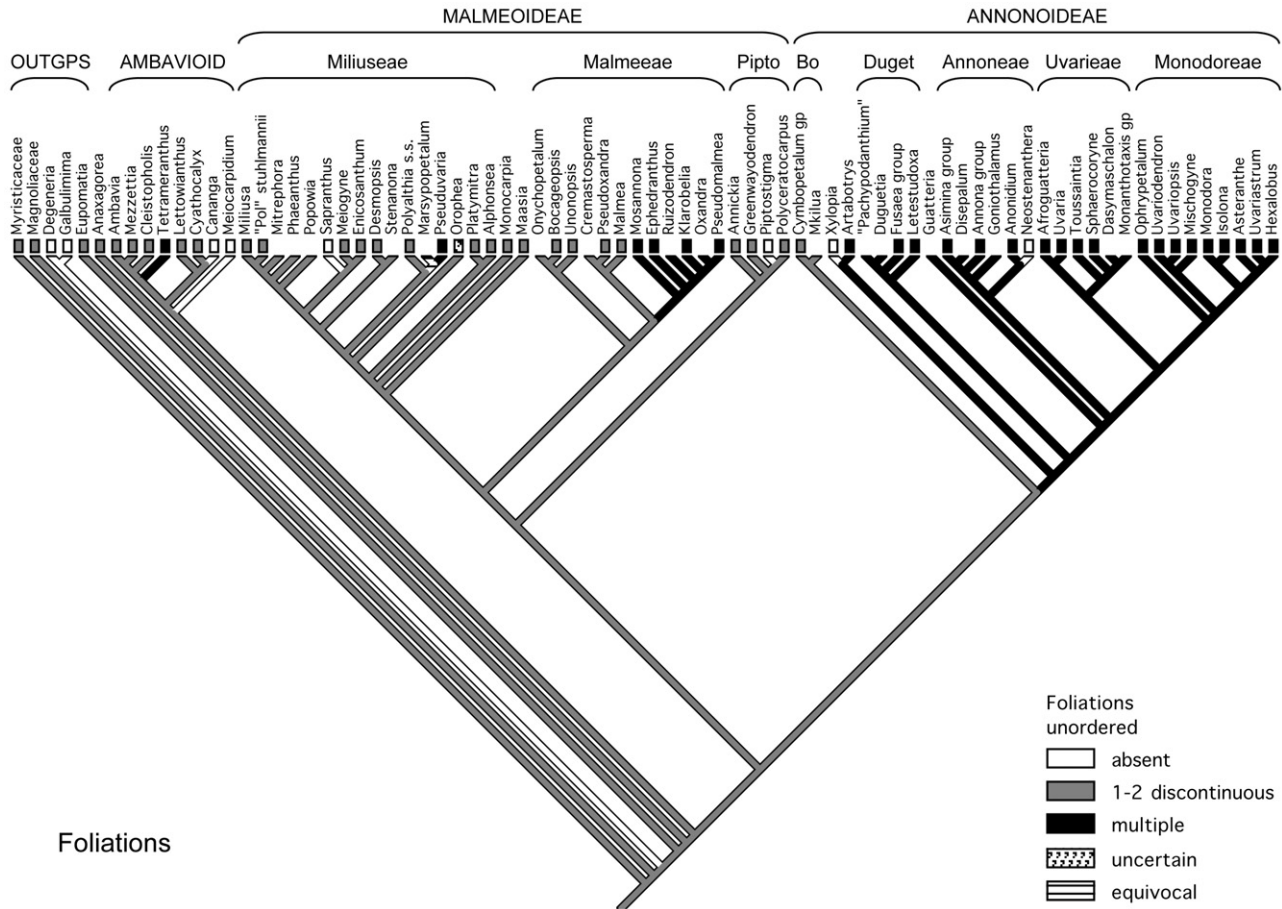


Figure 8. Phylogeny of Annonaceae and other Magnoliales, showing the most parsimonious course of evolution of nexine foliations.

Uvariastrum–Hexalobus clades. By contrast, the Bayesian analysis of Couvreur *et al.* (2008a) favoured intermediate structure as ancestral in Monodoreae (PP = 0.93), with three origins of columellae within the clade. If *Annickia* is linked with the Malmeeae–*Maasia*–*Monocarpia*–Miliuseae clade (Fig. 6), the most parsimonious ancestral state for Monodoreae is entirely unresolved: either granular, intermediate or columellar.

In Miliuseae, Le Thomas (1980/1981) noted *Miliusa* Lesch. ex A.DC. as another example of intermediate structure (Fig. 2F) and, because molecular analyses link it with the granular species ‘*Polyalthia*’ *stuhlmannii* (Fig. 5), it could represent an intermediate evolutionary stage between columellar and granular. Molecular data indicate that ‘*Polyalthia*’ *stuhlmannii* forms a clade with two species not included in our data set, ‘*P.*’ *cerasoides* (Roxb.) Benth. & Hook.f. ex Bedd. and ‘*P.*’ *pendula* Capuron ex G.E.Schatz & Le Thomas (Schatz & Le Thomas, 1990), which have a similar finely granular infratectum (Chaowasku *et al.*, 2008). Intermediate exines appear to have origi-

nated independently in *Encicosanthum* (including ‘*Polyalthia*’ *longifolia*), *Malmea*, *Pseudomalmea*, *Artabotrys* and *Sphaerocoryne*.

Together, these results confirm that granular infratectal structure was ancestral in Annonaceae, but the number of origins of columellar structure and subsequent changes are far from resolved. In any case, infratectal structure appears to be highly labile in Annonaceae, with several origins and/or losses of columellae. More reliable inferences may require new TEM data on unstudied taxa, developmental studies on problematic exines and/or better understanding of biases in different methods for reconstruction of character evolution.

Figure 7 shows the inferred evolution of the tectum, originally imperforate (or with microperforations visible only with SEM or TEM), then becoming finely perforate to reticulate, but with many reversals. As with infratectal structure (Figs 5, 6), the point at which the tectum became reticulate is sensitive to the position of *Annickia*. In Figure 7, several reversals to a continuous tectum are correlated with reversals to

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Figure 9. Phylogeny of Annonaceae and other Magnoliales, showing the most parsimonious course of evolution of a thickened outer nexine foliation.

granular infratectal structure: in Piptostigmataeae, *Sapranthus*, the '*Polyalthia*' *stuhlmannii*-*cerasoides*-*pendula* clade (Chaowasku *et al.*, 2008), *Xylopi*a, *Neostenanthera* and Uvarieae. *Ophrypetalum*, at the base of Monodoreae, is unusual in having free-standing columellae and therefore being intectate; a similar structure occurs in *Trigyna*e Schltld. and some species of *Bocagea* A.St.-Hil. and *Hornschuchia* Nees in Bocageae (Walker, 1971a, 1974a; Johnson & Murray, 1995). The fact that these intectate taxa are nested among groups with a reticulate tectum implies that they were derived from reticulate ancestors by the loss of connections between the heads of the columellae, as postulated by Walker (1974a). We scored *Ophrypetalum* as having the reticulate-perforate state, but if we had treated intectate as an additional state the same conclusion would follow.

Origin of a verrucate tectum (termed 'cerebroid' by Mols, 2004) is a synapomorphy of two separate clades, Miliuseae (e.g. Fig. 2G) and Duguetieae (*Duguetia* and *Letestudoxa* Pellegr.; modified to imperforate in *Fusaea* (Baill.) Saff.). This corresponds to what Le

Thomas (1980/1981) called a 'dislocated' tectum, which she interpreted as an intermediate stage in a trend from continuous to reticulate, correlated with a trend from granular to columellar infratectal structure. She cited *Miliusa* (Fig. 2F, G) and *Isolona* (in Monodoreae; Fig. 3H, I) as evidence, as they combine intermediate infratectal structure and a verrucate tectum. However, comparison of Figures 5 and 7 shows that most verrucate Miliuseae are still columellar, whereas most Monodoreae with an intermediate infratectum, except *Isolona* and some *Hexalobus* spp., are still reticulate. This implies that changes in the two characters were not consistently correlated. TEM studies might modify these conclusions; we have scored several Miliuseae as columellar based on LM, but distinguishing columellae and intermediate elements with LM can be problematic.

Two other tectal modifications have more restricted distributions. Perisaccate to subsaccate exine, with the tectum more or less detached from the nexine, is a character noted by Walker (1971a) that was not represented in our previous data sets but occurs in four

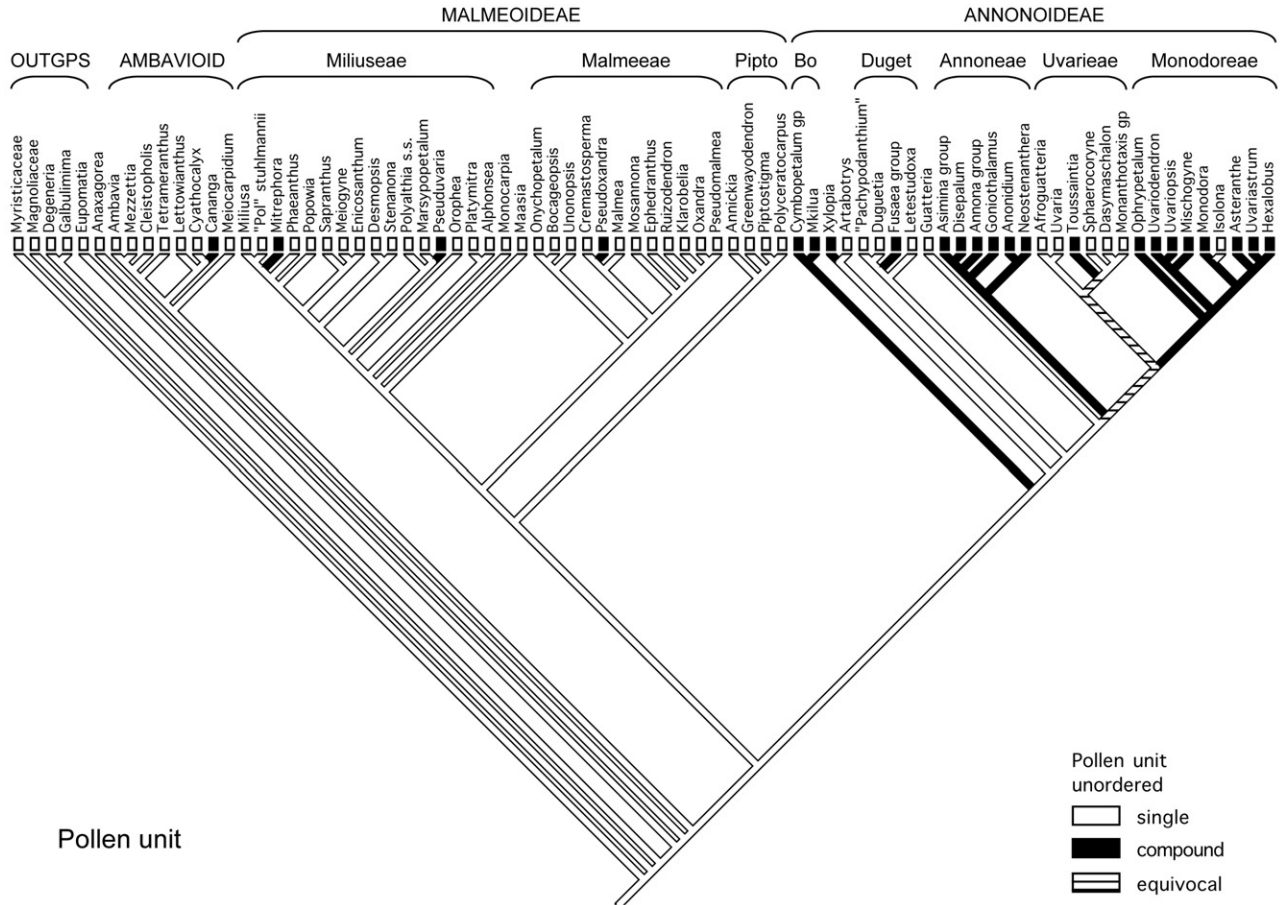


Figure 10. Phylogeny of Annonaceae and other Magnoliales, showing the most parsimonious course of evolution of tetrad (and polyad) pollen.

genera added in the present survey. This feature is restricted to Malmeeae; molecular relationships indicate that it is a synapomorphy of *Bocageopsis* and *Onychopetalum*, but a parallel advance in *Oxandra* and *Ruizodendron*. Spines embedded in a reduced granular exine, resulting in echinate sculpture, are a synapomorphy of the *Monanthotaxis* group and related Asian lianas (Uvarieae; Fig. 3D, E), including *Dasymaschalon* (Hook.f. & Thomson) Dalla Torre & Harms (Walker, 1971a, 1976b; Wang, 2009; Wang, Chalermglin & Saunders, 2009), but they originated independently in ‘*Pachypodanthium*’ (with an even more reduced exine: Le Thomas *et al.*, 1986), which is now included in *Duguetia* (Duguetieae; Chatrou, 1998; Chatrou, Koek-Noorman & Maas, 2000). As suggested by Le Thomas, Lugardon & Doyle (1994), the spines in ‘*Pachypodanthium*’ may be derived from verrucae of the sort seen in *Duguetia* and *Letestudoxa*. A similar scenario may be involved in *Dasymaschalon* and the *Monanthotaxis* group (plus the related echinate genera *Desmos* Lour. and *Friesodielsia* Steenis, not included

in this survey: Wang, 2009), which are linked with the verrucate genus *Sphaerocoryne*.

Nexine

Le Thomas (1980/1981) proposed that pollen of Annonaceae originally had no nexine, i.e. neither a foot layer nor foliations, as in *Piptostigma* Oliv. (Fig. 2C), but Figure 8 indicates that the ancestral pollen had one or two discontinuous nexine foliations, as in *Anaxagorea* (Fig. 2B). Such foliations appear to be a synapomorphy of Magnoliales and Laurales (Doyle, 2005), assuming that Magnoliales are linked with Laurales rather than Canellales, as indicated by increasingly strong molecular evidence (Sauquet *et al.*, 2003; Soltis *et al.*, 2005). The question of the homology of foliations, which is related to whether endexine and ectexine should be defined in terms of staining or development, has been debated by Gabarayeva (1995) and Doyle (2005), but this issue is not critical for understanding evolution in Annonaceae. Foliations were lost in several isolated lines, including *Meiocarpidium* Engl. & Diels,

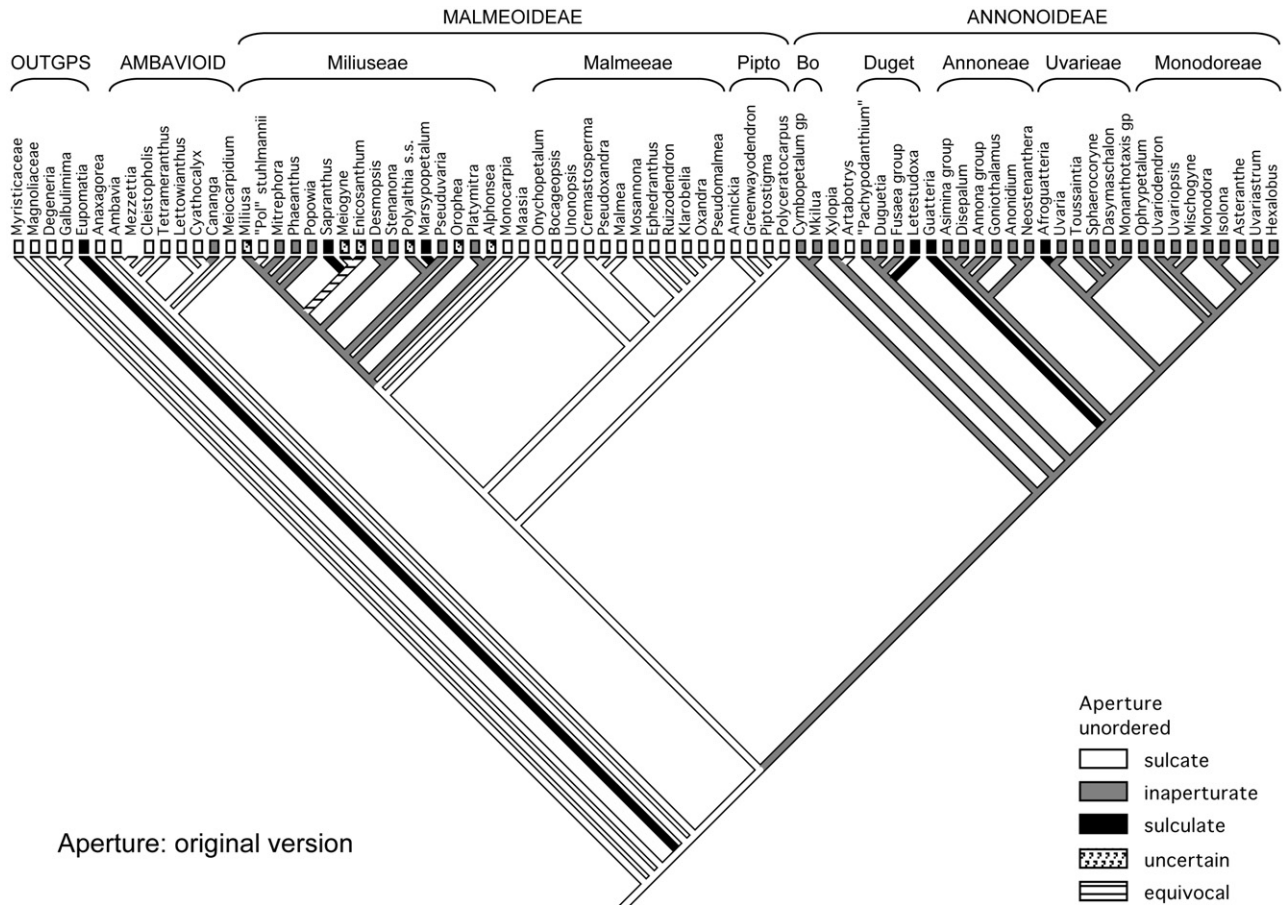


Figure 11. Phylogeny of Annonaceae and other Magnoliales, showing the most parsimonious course of evolution of aperture condition, as interpreted by Doyle & Le Thomas (1996).

Cananga (Fig. 2J), *Piptostigma* (Fig. 2C) and *Sapranthus*; elsewhere in Magnoliales, a parallel loss unites *Degeneria* and *Galbulimima*, correlated with reduction of exine thickness.

Multiplication of nexine foliations occurred in three isolated lines (*Tetrameranthus* in Ambavioideae, *Pseuduvaria* Miq. and *Orophea brandisii* Hook.f. & Thomson in Miliuseae) and two major clades (Fig. 8). One clade makes up approximately half of Malmeae (Waha, 1985; Le Thomas, 1998; A. Le Thomas & M. Suarez-Cervera, unpubl. data), including *Mosannona*, *Klarobelia* and *Pseudomalmea*, which were segregated from *Malmea* by Chatrou (1998), and *Ephedranthus*. Because *Malmea* and other early-diverging malmeoids have only one or two foliations, this character supports Chatrou's segregation of the new genera. Generally, the number of foliations in this clade is only slightly increased, varying between two and three around the grain (Fig. 2H). The second clade consists of most of Annonoideae, with the notable exception of the first-diverging Bocageae. In many of these taxa the foliations are numerous and highly convoluted (Fig. 3C, F, H, J).

We previously scored the *Cymbopetalum* group (Bocageae) as having multiple foliations (Doyle & Le Thomas, 1996), despite the absence of clear TEM data. However, Morawetz & Waha (1991) illustrated *Porcelia* Ruiz & Pav. as having only one or two wavy foliations. Low-magnification photos of *Cymbopetalum baillonii* R.E.Fr. by Tsou & Fu (2007) showed one prominent wavy foliation and a space below this where there might be additional thinner foliations, but higher-magnification photos of *C. brasiliensis* (Vell.) Benth. ex Baill., kindly provided by C.-H. Tsou (pers. comm.), show a thick outer foliation and only one thinner, discontinuous inner one. We have therefore rescored the *Cymbopetalum* group as having one or two foliations. This represents palynological support for the molecular position of Bocageae at the base of Annonoideae; the only other morphological support in the Doyle & Le Thomas (1996) data set is absence of the large micropylar plug that unites the remaining Annonoideae (with some reversals).

Figure 8 implies that foliations were lost twice within Annonoideae, in *Xylophia* (Fig. 3G) and *Neostenanthera*. However, whether this was attributable

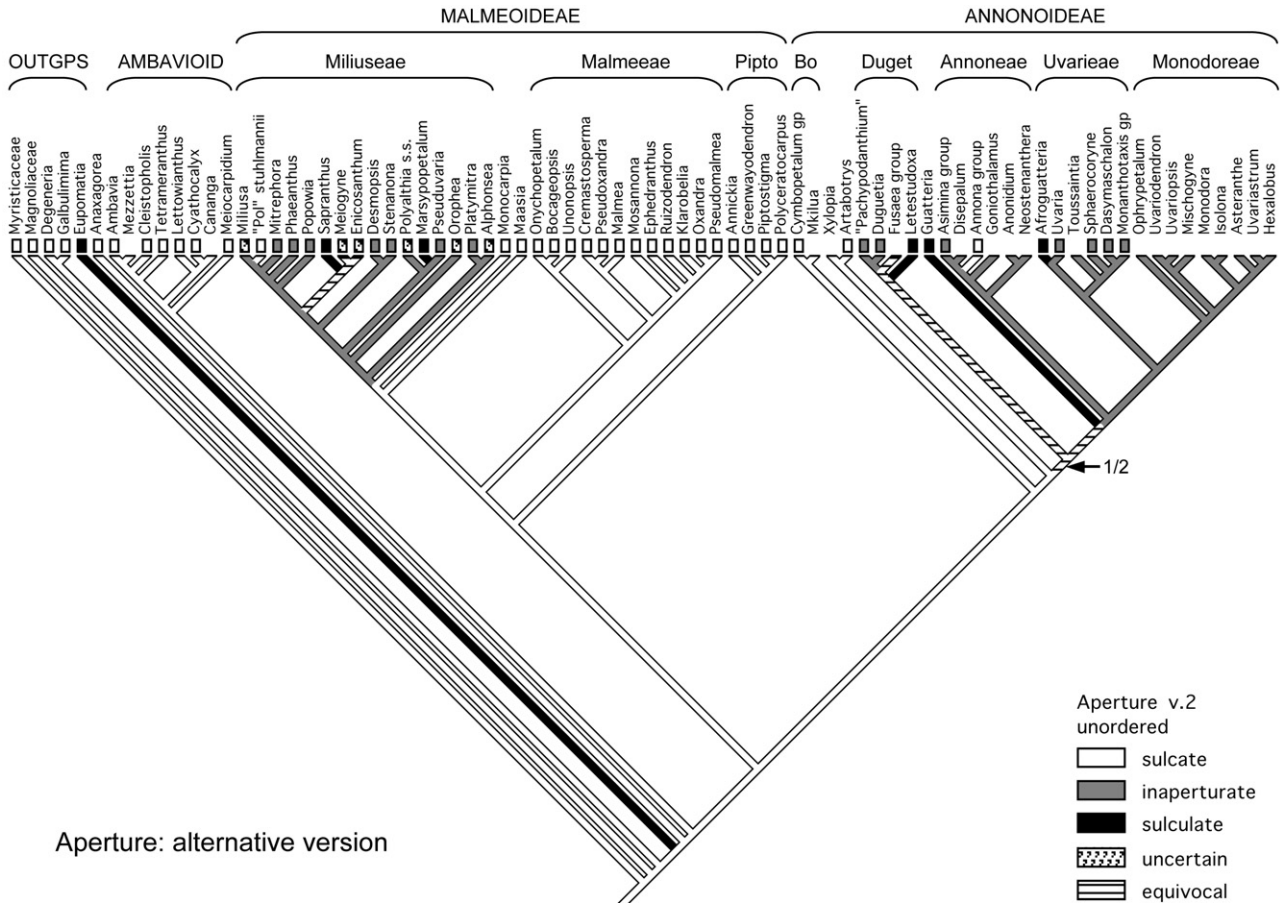


Figure 12. Phylogeny of Annonaceae and other Magnoliales, showing the most parsimonious course of evolution of aperture condition under an alternative interpretation, with taxa in which microspores are known to undergo rotation (*Annona*, *Cymbopetalum* group) scored as having a distal sulcus and other taxa with tetrads scored as inaperturate, if known not to undergo rotation, or as unknown.

to true loss or rather transformation of foliations depends on interpretation of exine homologies in these taxa, as discussed above. If the basal layer was derived not from fused granules, but rather from basally widened columellae, it may be that its inner part was derived from multiple foliations, like those in most other Annonoideae, which became obscured by deposition of exine material during development (Doyle *et al.*, 2000). To eliminate bias against this possibility, we have redefined the foliation character as unordered. These hypotheses should be testable by developmental studies.

Another nexine character noted by Le Thomas (1980/1981) is thickening of the outermost foliation (Fig. 9). Doyle & Le Thomas (1996) inferred that this change occurred independently in Malmeeae (e.g. Fig. 2H), Annonoideae with columellar tetrads (Fig. 3J) and a clade of Miliuseae consisting of the former *Ancana* and disulculate *Polyalthia* groups (now in *Enicosanthum*, *Meiogyne* and *Polyalthia s.s.*).

Closer examination of TEM figures of the groups in Miliuseae shows that the outer foliation is less thickened than in other taxa so scored, or not at all, and we have therefore rescored the relevant taxa as unthickened (see Appendix).

Our present analysis (Fig. 9) indicates that the outer foliation became thickened once in Annonaceae, in the common ancestor of Annonoideae and Malmeoideae. In Malmeoideae, all columellar monosulcate taxa studied with TEM have a thick outer foliation, including *Maasia* and *Monocarpia* Miq., which are successively closer outgroups to Miliuseae. With *Annickia* linked with other Piptostigmateae, this character was reversed in Piptostigmateae and Miliuseae, correlated with reversals to a continuous tectum and granular infratectal structure in the former and origin of a verrucate tectum in the latter. (If *Annickia* is sister to the Malmeeae–*Maasia*–*Monocarpia*–Miliuseae clade, as in Figure 6, it is equivocal whether the outer foliation was thickened

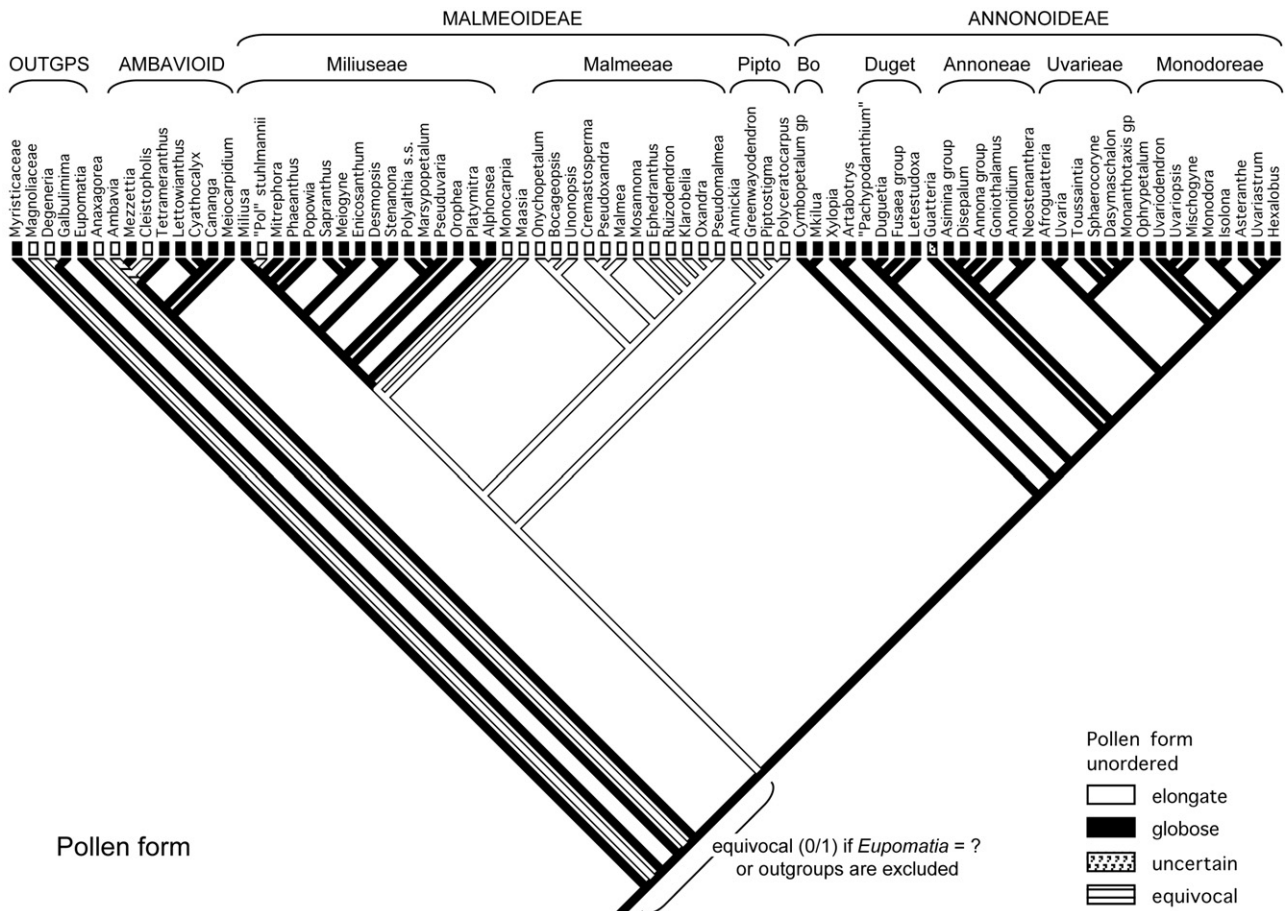


Figure 13. Phylogeny of Annonaceae and other Magnoliales, showing the most parsimonious course of evolution of pollen form.

once in the common ancestor of Malmeoideae and Annonoideae or twice, within Malmeoideae and on the line to Annonoideae.) Thickening of the outer foliation was reversed four times in Annonoideae: in Duguetieae, Uvarieae, *Isolona* and some *Uvariopsis* spp. Thus, thickening of the outer foliation occurred before the multiplication of foliations in both Malmeae and Annonoideae.

Tetrads and apertures

Perhaps the most vexing unresolved problems concern the evolution of tetrads and apertures and the relation of these two characters. As shown in Figure 10, tetrads arose independently in several isolated lines, namely *Cananga* in Ambavioideae, *Pseuduvaria* and *Mitrephora* (Blume) Hook.f. & Thomson in Miliuseae and *Pseudoxandra* in Malmeae, but most taxa with tetrads belong to Annonoideae. Tetrads occur in Bocageae (further aggregated into polyads in the *Cymbopetalum* group: Walker, 1971a; Johnson & Murray, 1995), *Xylophia*, the 'Fusaea group', Annoneae, *Toussaintia* and most Mon-

odoreae. Because these lines are interspersed with taxa with single grains (*Artabotrys*, Duguetieae, *Guatteria* and most Uvarieae), it is most parsimonious to assume that tetrads originated four to six times in Annonoideae. In their Bayesian analysis of this character, Couvreur *et al.* (2008b) also inferred that monads were ancestral in Annonoideae (PP = 0.86). On parsimony grounds, it is equivocal whether tetrads arose once in the common ancestor of the Annoneae–Uvarieae–Monodoreae clade and reverted to monads in Uvarieae, or arose independently in Annoneae and Monodoreae, but the Bayesian analysis of Couvreur *et al.* strongly favoured the former scenario. However, both methods agree that the single grains of *Isolona* in Monodoreae, which is linked with *Monodora* by its parasyncarpous gynoeceum, were derived from tetrads, as argued by Walker (1971a) and Le Thomas *et al.* (1986). Similar reversion to monads apparently occurred within the *Annona* group (e.g. the derived subgroup formerly treated as *Rollinia* A.St.-Hil.: Walker, 1971a; Richardson *et al.*, 2004). Tetrads in *Annona* are often very

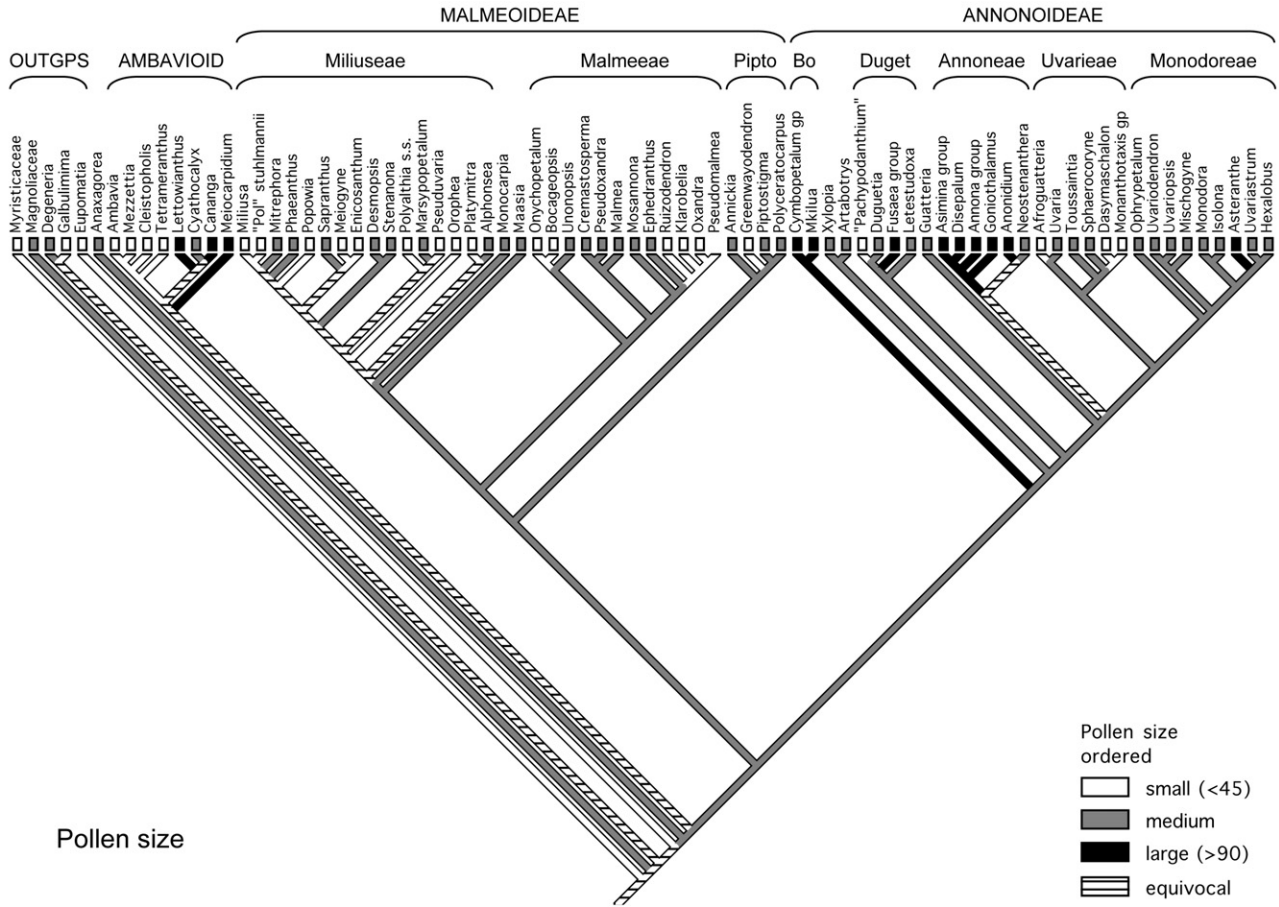


Figure 14. Phylogeny of Annonaceae and other Magnoliales, showing the most parsimonious course of evolution of pollen size. Small (0) = < 45 μm average size of monads; medium-sized (1) = 45–90 μm; large (2) = > 90 μm.

loose and the pollen may be dispersed as monads. Clearly this is a highly labile character, as underlined by the differences in tetrad development discussed further below.

In Figure 11 we reconstruct the evolution of the aperture character as defined by Doyle & Le Thomas (1996), with tetrads with a thin proximal exine scored as inaperturate. Loss of the ancestral sulcus occurred in *Cananga* (where it may be represented by a vestigial distal thinning: Doyle *et al.*, 2000; Fig. 2I, arrow) and unites two major clades, Miliuseae and Annonoideae. This tree implies that a sulcus reappeared in '*Polyalthia stuhlmannii*' in Miliuseae and *Artabotrys* in Annonoideae. Chaowasku *et al.* (2008) questioned whether '*P. stuhlmannii*' is monosulcate, as it has exine on the aperture membrane, but this is common in sulcate pollen and Le Thomas (1988) showed that the intine breaks through the exine in this area. *Artabotrys* is also problematic; it has single grains with a short thin area that Walker (1971a) and Le Thomas (1980/1981) interpreted as a vestigial sulcus, but its position has not been confirmed by develop-

mental studies. In Annonaceae, sulcate pollen (usually disulcate, but zonosulcate in *Letestudoxa* and *Guatteria*) always appears to be derived from inaperturate pollen, as postulated by Walker (1971a).

As noted in the Introduction, aperture conditions in Miliuseae have been a topic of much discussion. Mols (2004) concluded that most Miliuseae were inaperturate and disulcate pollen occurred only in *Sapranthus* and some but not all species of *Alphonsea* Hook.f. & Thomson, *Encicosanthum*, *Meiogyne* and *Polyalthia s.s.* However, this reflects an overly strict definition of disulcate that excludes forms with thin areas in the exine that are not visible with SEM (cf. Waha & Hesse, 1988), which satisfy the usual definition of an aperture as an area where the exine is modified for germination. With our scoring of taxa (see Appendix), Figure 11 indicates that shifts from inaperturate to disulcate occurred independently in or within *Alphonsea*, *Marsypopetalum* Scheff., *Miliusa*, *Orophea* Blume and *Polyalthia s.s.* and could be a synapomorphy of *Sapranthus*, *Meiogyne* and *Encicosanthum* (of which the latter two include both inap-

erturate and disulcate species). Such rampant parallelism suggests that some developmental predisposition for disulcate pollen was established in the common ancestor of Miliuseae. This is supported by the observations of Waha & Hesse (1988) and Chao-wasku *et al.* (2008) that many species have two 'germination zones' where the intine is modified but the exine is not.

Figure 11 assumes that the thin proximal exine in most tetrads is a consequence of the tetrad habit and is not homologous with the apertures of other taxa, as argued by Le Thomas (1980/1981). Proximal thinning is well documented outside Annonoideae, in the monosulcate monads of *Ambavia* (Le Thomas & Lugardon, 1975; Le Thomas, 1980/1981) and the tetrads of *Cananga* (with a possible vestigial sulcus: Doyle *et al.*, 2000; Fig. 2I) and *Pseuduvaria* (inaperturate: Su & Saunders, 2003). However, the remarkable discovery that the thin area in *Annona* and *Cymbopetalum* is formed at the distal pole and becomes proximal by rotation of the microspores (Tsou & Fu, 2002, 2007; Lora *et al.*, 2009) raises the possibility that it is homologous with a distal sulcus after all, as suggested by Tsou & Fu (2007). The broader significance of these observations is unclear, because in *Asimina* Waha (1987b) and Gabarayeva (1992, 1993) have shown that the exine is thicker from the beginning at the distal pole and the microspores do not rotate. These considerations are independent of whether the proximal thin area is functionally an aperture, as it appears to be in *Asimina* (Hesse *et al.*, 2009).

Given that only a handful of taxa have been studied developmentally, it would be premature to draw strong conclusions from these data, but as an heuristic exercise we have plotted an alternative aperture character on the tree in Figure 12, with the *Cymbopetalum* group and *Annona* scored as sulcate, the *Asimina* group as inaperturate and the vast majority of tetrads not yet studied developmentally as unknown. Now it is most parsimonious to interpret the thin areas in both the *Cymbopetalum* group and *Artabotrys* as retentions of the ancestral sulcus. This would mean that the name 'inaperturate clade', previously applied to Annonoideae, is a misnomer, but it would not mean that there was no change in the aperture at the base of the clade: the thin area in *Cymbopetalum* differs from a normal sulcus in being round and covering nearly half the grain, and the sulcus in *Artabotrys* is nearly round, so the clade would still be united by a change in shape of the sulcus. However, the fact that the tetrads of *Asimina* and the monads of *Duguetia* and most Uvarieae are inaperturate implies that the common ancestor of Annoneae, Uvarieae and Monodoreae was inaperturate. This in turn implies that the initially distal thinning in *Annona* is a reversal rather

than a retention of the original sulcus, as inferred in *Cymbopetalum*.

These observations underline the need for TEM and developmental studies in Miliuseae and Annonoideae. In the tetrad groups, broader developmental surveys are needed on the systematic distribution of microspore rotation. In monad taxa, developmental studies are needed to test whether the supposed vestigial sulcus of *Artabotrys* is distal. In the secondary monads of *Isolona*, the exine is also reduced on one side, which Le Thomas (1980/1981) assumed was proximal, but without developmental data. Many of Walker's (1971a, b, 1972) LM figures of monads that he described as inaperturate show variations in exine thickness around the grain that might represent vestiges of apertures or a reduced proximal exine.

Pollen form

Pollen form, divided by Doyle & Le Thomas (1996) into boat-shaped and globose, might be questioned as an independent character, as it is often correlated with aperture condition. Pollen is boat-shaped and monosulcate in *Anaxagorea*, Piptostigmateae, Malmeeae, *Maasia*, *Monocarpia* and '*Polyalthia stuhlmannii*', but globose throughout the largely inaperturate Annonoideae and in the inaperturate and disulcate taxa that make up most of Miliuseae. However, Ambavioideae are an exception, as they are basically monosulcate but vary between boat-shaped in *Cleistopholis* and *Ambavia* and globose in the other genera. The putatively sulcate pollen of *Artabotrys* is also globose. Similarly, form is not always correlated with aperture condition in other Magnoliales: Magnoliaceae and *Degeneria* are sulcate and boat-shaped, but *Galbulimima* is sulcate and globose and Myristicaceae are sulcate and vary between boat-shaped and globose, with globose reconstructed as ancestral (Sauquet & Le Thomas, 2003).

In Figure 13, the inferred ancestral state for pollen form in Annonaceae is globose, which is retained into Ambavioideae and Annonoideae, while the boat-shaped pollen of *Anaxagorea* and monosulcate members of Malmeoideae (Piptostigmateae, Malmeeae, *Maasia* and *Monocarpia*) is derived. Form differs from other pollen characters in that the inferred ancestral state varies with inclusion or exclusion of outgroups: with no outgroups, the ancestral state in Annonaceae is equivocal, and the boat-shaped pollen of *Anaxagorea* and Malmeoideae may be either derived from globose or retained from lower in Magnoliales. It is possible that the result obtained with outgroups is misleading because it is influenced by the globose pollen of *Eupomatia*, which is derived in being zonosulcate, raising the possibility that it became globose as a consequence of the shift in aperture type. In any case, pollen in Malmeoideae was

originally boat-shaped and became globose in Miliuseae, correlated with loss of the sulcus. Shifts from globose to boat-shaped occurred in *Ambavia* and *Cleistopholis* in Ambavioideae (either once with a reversal in *Mezzettia* Becc., or twice), without any change in aperture, and in '*Polyalthia*' *stuhlmannii* in Miliuseae, correlated with reappearance of a sulcus.

It should be acknowledged that the distinction between boat-shaped and globose is somewhat arbitrary and potentially subject to error because of expansion and contraction of the pollen. Detailed measurements of pollen prepared with consistent methods might provide more reliable and informative data.

Pollen size

Pollen size, meaning the size of monads in compound grains, is questionable as a cladistic character because it varies continuously, so that discrete states are hard to define. Size is also influenced by preparation method; grains studied with SEM often appear to be shrunken compared with those studied with LM (Walker, 1971a; Doyle & Le Thomas, 1994). Pollen of Annonaceae is generally large relative to that of most other angiosperms. Walker recognized limits between size classes at 50 and 100 μm , but plots by Doyle & Le Thomas (1994, 1996) suggested that there are more natural breaks near 45 and 90 μm .

Figure 14 shows that pollen size is highly homoplastic in Annonaceae, but despite this and the other problems noted some clades are fairly uniform and united by changes in size. The inferred ancestral state is medium-sized (45–90 μm , large from a general angiosperm perspective), as in *Anaxagorea*, early-branching members of Malmeoideae and many Annonoideae. Increases to > 90 μm unite Bocageeae and possibly Annoneae (if medium size in *Neostenanthera* is a reversal), but there were independent increases in the *Fusaea* group and *Asteranthe* and one or more in Ambavioideae (*Meiocarpidium*, *Lettowianthus* Diels and *Cananga*). Decreases to < 45 μm unite core Ambavioideae (*Tetrameranthus* through *Cleistopholis*), *Onychopetalum* plus *Bocageopsis* and the clade including *Oxandra* and *Ruizodendron* in Malmeae and the *Dasymaschalon*–*Monanthotaxis* clade in Uvarieae, which is notable for its reduced exine and spines. The situation in Miliuseae is highly confused: medium size may have been retained into the base of the clade, followed by several reductions to small pollen, or reduction to small size may be a synapomorphy of the clade, with several reversals to medium size.

SYSTEMATIC VALUE OF POLLEN CHARACTERS

General conclusions on pollen evolution are summarized in Figure 15, with sketches of reconstructed

pollen types placed on a tree showing the evolution of infratectal structure as inferred with parsimony (plus an alternative sketch based on the Bayesian analysis of Couvreur *et al.*, 2008a). This figure may serve as a framework for reviewing the value of pollen characters for higher-level systematics in Annonaceae. Although molecular trees imply that many pollen characters were subject to high levels of homoplasy, they often provide valid evidence for relationships.

Pollen and relationships

Anaxagorea and most Ambavioideae have monosulcate pollen and tectate–granular exine structure – features that are shared with outgroups of the Annonaceae and thereby support a position near the base of the family. However, there was more homoplasy in exine structure within Malmeoideae and Annonoideae than previously thought, such that exine structure shows some broad systematic correlations, but many exceptions at a finer scale. Thus, granular structure is apparently a reversal from columellar rather than primitive in the monosulcate pollen of '*Polyalthia*' *stuhlmannii*, the disulcate pollen of *Sapranthus* and the monosulcate pollen of most Piptostigmateae, assuming *Annickia* is part of this clade. However, the fact that *Greenwayodendron* has tectate–granular monosulcate pollen supports its removal from *Polyalthia* (Verdcourt, 1969) and is consistent with its position in Piptostigmateae, whether its exine structure is primitive or secondarily derived.

In the studies of Doyle & Le Thomas (1994, 1996), reticulate–columellar exine structure supported inclusion of *Annickia* in Malmeae, but, if molecular analyses are correct in linking *Annickia* with Piptostigmateae, the parsimony optimization in Figure 15 indicates that this exine structure is synapomorphic for all taxa other than *Anaxagorea* and Ambavioideae, i.e. the whole Malmeoideae–Annonoideae clade. However, if *Annickia* is linked with the Malmeae–*Maasia*–*Monocarpia*–Miliuseae clade (Fig. 6), which is more parsimonious in terms of morphology, it is equally parsimonious to assume that tectate–granular structure was retained up to the base of the Malmeoideae–Annonoideae clade and into the remaining Piptostigmateae. If so, reticulate–columellar structure and a thickened outer nexine foliation may be synapomorphies of *Annickia*, Malmeae, *Maasia* and *Monocarpia*, which were subsequently modified in Miliuseae. In either case, these features were established in the common ancestor of Malmeae, *Maasia*, *Monocarpia* and Miliuseae.

Within Malmeoideae, Miliuseae are united by an especially large number of pollen advances: loss of the sulcus, globose shape, a verrucate tectum and thinning of the outer foliation (cf. Mols, 2004). These are in fact the only morphological synapomorphies of the

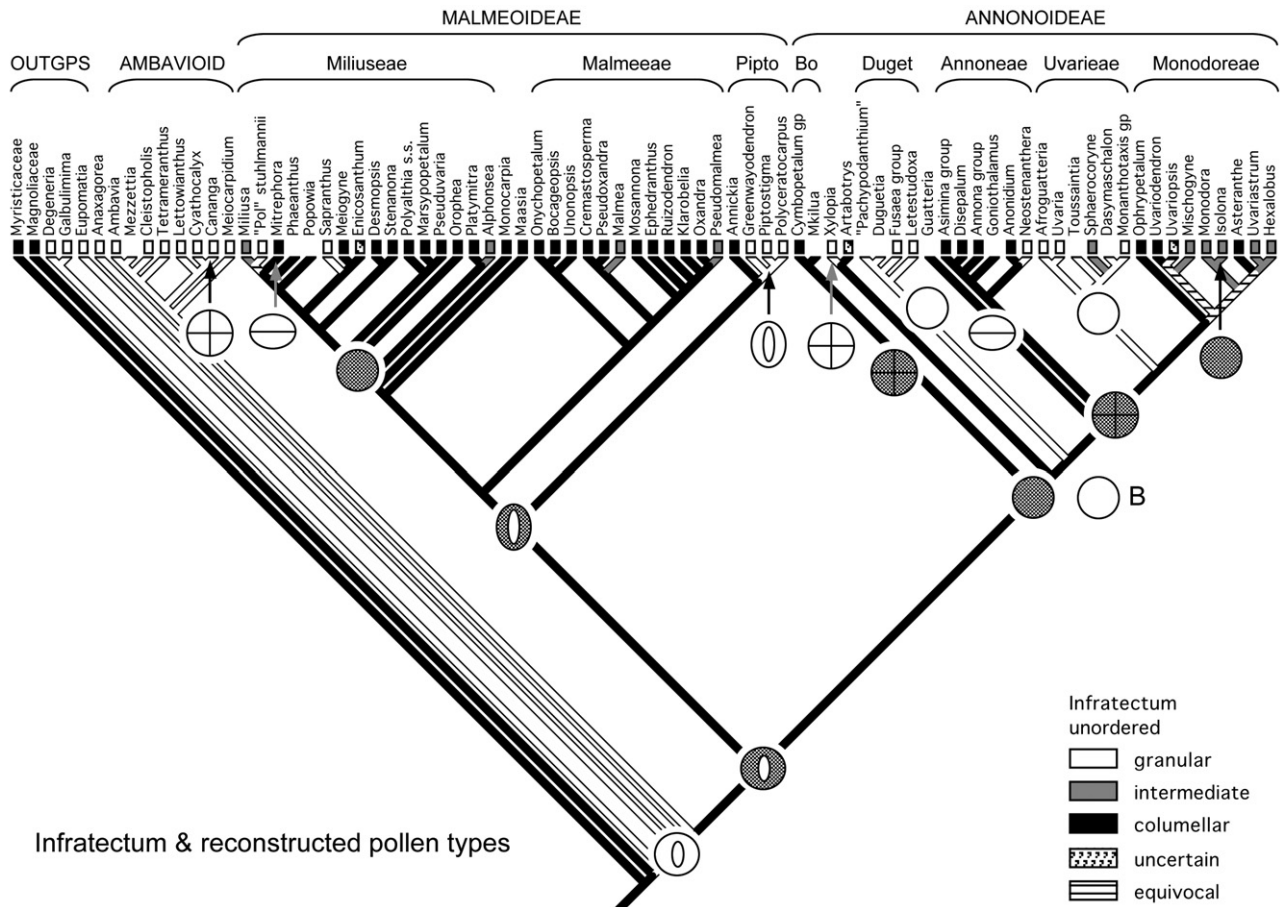


Figure 15. Phylogeny of Annonaceae and other Magnoliales, showing the most parsimonious course of evolution of infratectal structure, with sketches of major pollen types placed where they appear on the cladogram (B, morphology based on the Bayesian analysis of Couvreur *et al.*, 2008a). White shading of pollen sketches indicates granular infratectal structure; dark shading indicates columellar or intermediate infratectal structure.

clade in the Doyle & Le Thomas (1996) data set. However, evaluation of the systematic significance of inaperturate and disulcate pollen within Miliuseae requires more work at the TEM level. As recognized by Rogstad & Le Thomas (1989), the reticulate–columellar monosulcate pollen of *Maasia* (the former *Polyalthia hypoleuca* complex or columellar–sulcate *Polyalthia* group) supports its separation from other groups formerly assigned to *Polyalthia*, which are scattered within the miliusoids, consistent with their generally verrucate tectum and inaperturate or disulcate aperture condition. The sulcus and reticulate–columellar exine structure of both *Maasia* and *Monocarpia* are symplesiomorphies shared with Malmeae, which would be equally consistent with a position with or in Malmeae or on the line to Miliuseae. Multiplication of nexine foliations unites a clade in Malmeae that includes *Klarobelia*, *Mosannona* and *Pseudomalmea*, supporting the segregation of these genera from *Malmea* by Chatrou (1998).

Annonoideae are strongly supported by molecular data, but they have only two unequivocal morphological synapomorphies in the Doyle & Le Thomas (1996) data set, one of which is loss of the sulcus, or its modification into a round thin area, if the initially distal thinning in *Cymbopetalum* is homologous with a sulcus (the other is bimodal chromosome size, later reversed within the clade). Tetrads are homologous within many subgroups of this clade (Bocageae, Annoneae and Monodoreae), but molecular relationships imply that they originated at least four times. As in Malmeoideae, there is considerable homoplasy in exine structure. However, if the parsimony optimization in Figure 15 is correct, and columellar structure is ancestral, reversals to granular structure unite both Duguetieae and Uvarieae. The Bayesian analysis of Couvreur *et al.* (2008b) differed in concluding that granular structure was ancestral in Annonoideae but agreed that it was a reversal in Uvarieae. Multiplication of nexine foliations unites all Annon-

oideae except Bocageae, thereby supporting the position of the latter. Exine similarities in *Xylopia*, *Neostenanthera* and *Cananga* led Doyle & Le Thomas (1994, 1996) to the incorrect conclusion that these taxa were related, but closer examination reveals differences in detail that are consistent with the molecular position of *Cananga* in Ambavioideae, particularly the fact that its nexine is more clearly made up of fused granules (Doyle *et al.*, 2000). This feature is shared with its sister genus *Cyathocalyx* and arose independently in two other Ambavioideae, *Meiocarpidium* and *Cleistopholis*.

In some Annonaceae, the exine is so reduced and/or autapomorphic that it provides little or no evidence on relationships. The most striking case is *Guatteria*, which has zonosulcate pollen with no exine, only intine (Walker, 1971a; Morawetz & Waha, 1985). Because it lacks exine characters, which we therefore scored as unknown, the pollen of *Guatteria* could be almost equally well derived from any other type. Its zonosulcate aperture might support a relationship with some other sulcate group, but this has not been confirmed by phylogenetic analyses. In Figure 11, the zonosulculus of *Guatteria* is an autapomorphy; under the alternative interpretation of aperture homologies in Annonoideae (Fig. 12), it may be homologous with the zonosulculus of *Letestudoxa* in Duguetieae, but this is equivocal. However, in another group with a reduced exine, the *Dasymaschalon*–*Monanthes* clade, the globose inaperturate monads and granular exine structure are either consistent with or supportive of a position in Uvarieae, while modification of the reduced tectum into spines links the two groups with each other. Similarly, *Duguetia* (including '*Pachypodanthium*') apparently lost all its infratectal structure (Le Thomas *et al.*, 1986, 1994), but its residual verrucate tectum supports its relationship with *Letestudoxa*.

Measures of homoplasy

The systematic value of characters is often judged by the consistency index (CI), which provides an inverse measure of homoplasy in a set of characters on a tree. Doyle *et al.* (2000) found that pollen characters had an intermediate CI on a tree based on morphology (0.26, compared with 0.24 for vegetative characters, 0.24 for floral characters and 0.33 for fruit and seed characters), but the lowest CI on a tree based on both morphology and *rbcL* (0.18, vs. 0.23 for vegetative, 0.22 for floral and 0.33 for seed and fruit characters). Similarly, Mols (2004) found that pollen characters had the lowest CI (0.33) of any character set considered on a molecular tree of Miliuseae. However, CI is not a perfect measure of systematic value, as characters can have a low CI, because they undergo many changes across the tree, but still unite clades at a

local level, rather than simply being autapomorphies. The retention index (RI) corrects for this effect. In the Doyle *et al.* (2000) analysis, the RI was higher for pollen characters (0.58) than for vegetative (0.39) or floral (0.34) characters, exceeded only by fruit and seed (0.62) characters, suggesting that pollen has more systematic value than the CI might suggest.

With the present data set, some adjustment is needed to compare consistency indices for pollen and other characters because the many added taxa have not been scored for non-palynological characters. Increased numbers of taxa are generally correlated with lower CI (Donoghue & Sanderson, 1992), so in the present case CI for pollen is expected to fall with the addition of taxa, whereas CI for other characters should remain artificially high because the effective number of taxa is lower. Therefore, we calculated consistency indices for pollen characters (with the alternative aperture character removed to avoid redundancy) with all 73 taxa of Annonaceae and with taxa reduced to the original number of 42 in Doyle & Le Thomas (1996), with added taxa removed and *Meiogyne*, *Enicosanthum* and *Polyalthia* s.s. substituted for *Ancana*, *Polyalthia longifolia* and the disulcate *Polyalthia* group, respectively. The resulting numbers can be compared with those for other characters in the Doyle & Le Thomas (1996) data set on the tree of Chatrou *et al.* (2012), pruned to the relevant 42 taxa.

As in Doyle *et al.* (2000), pollen characters have the lowest CI of the four character sets considered (0.15 with all 73 taxa of Annonaceae and 0.19 with taxa reduced to 42, vs. 0.22 for vegetative, 0.23 for floral and 0.32 for fruit and seed characters). However, pollen characters have a much higher RI (0.61 with all 73 taxa, 0.54 with 42 taxa) than vegetative (0.31) and floral (0.34) characters, almost as high as fruit and seed characters for the same smaller number of taxa (0.59). This suggests that, although pollen has a higher level of homoplasy across Annonaceae than many other characters (of which fruit and seed characters are least homoplastic), it does have considerable systematic value within portions of the tree. A third measure, rescaled consistency index (RC), is intended to combine the advantages of CI and RI; as might be expected, relative RC values for the different character sets follow an intermediate trend (0.09 with all 73 taxa and 0.10 with 42 taxa for pollen, 0.07 for vegetative, 0.08 for floral and 0.19 for fruit and seed characters).

PROSPECTS

As molecular results continue to become more robust and molecular studies reveal more cases in which morphology gave misleading evidence on relation-

ships, it is easy to regard morphology as obsolete as a source of phylogenetic information (Scotland, Olmstead & Bennett, 2003). However, the successes of molecular phylogenetics do not mean there is no longer any reason to study morphology. Even if morphology is not used as the basis for phylogeny reconstruction (except in integrating fossils into the phylogeny of living taxa: Wiens, 2004; Doyle, 2006, 2008; Doyle & Endress, 2010), cases such as microspore rotation, uncertain aperture position and differing degrees of development of sulculi illustrate how molecular results can reveal morphological problems that we might not even realize existed, but must be solved if we wish to understand the evolution of plants and other organisms in its full complexity.

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APPENDIX

CHARACTER DEFINITIONS (DL INDICATES DOYLE & LE THOMAS, 1996)

1. (DL 42). Pollen unit (0) single (monads), (1) compound (tetrads, polyads).
2. (DL 43). Proximal exine (0) normal, (1) reduced. New taxa scored following assumptions of Doyle & Le Thomas (1996).
3. (DL 44). Aperture (0) sulcate, (1) inaperturate, (2) sulcate.
4. (New). Same as in previous character, but with the *Annona* and *Cymbopetalum* groups scored as sulcate, the *Asimina* group as inaperturate and tetrads not studied developmentally as unknown (see text for discussion).
5. (DL 46). Pollen form (0) elongate (boat-shaped), (1) globose.
6. (DL 47). Tectum (0) imperforate or microperforate, (1) verrucate, (2) reticulate-perforate. Taxa with reduced tectum scored as unknown (?).
7. (New). Tectum (0) not separating from the nexine, (1) perisaccate or subsaccate. Taxa with reduced tectum scored as unknown (?).
8. (DL 48). Spines (0) absent, (1) present (echinate).
9. (DL 49). Infratectal structure (0) granular, (1) intermediate, (2) columellar.
10. (DL 50). Base of infratectum (0) undifferentiated, (1) fused granules.
11. (DL 51). Nexine foliations (0) absent, (1) 1–2, often discontinuous, (2) multiple, continuous, often contorted. Changed from ordered to unordered because of uncertainty on nature of the character transformation in *Xylopi*a and *Neostenanthera* (see text for discussion).
12. (DL 52). Outer foliation (0) undifferentiated, (1) thickened.
13. (DL 45). Pollen size (average) (0) small (< 45 µm), (1) medium, (2) large (> 90 µm) (ordered). Based on acetolysed material studied with LM (usually by Walker, 1971a, b, 1972) rather than SEM material, which often appears to be shrunken.

TAXA AND REFERENCES FOR SCORING

Outgroups (see pollen references in Doyle, 2005):

Myristicaceae.

Magnoliaceae.

Degeneria I.W.Bailey & A.C.Sm. (= Degeneriaceae).

Galbulimima F.M.Bailey (= Himantandraceae).

Eupomatia R.Br. (= Eupomatiaceae).

Annonaceae:

Afroguatteria Boutique: Walker (1971b) LM; Le Thomas (1980/1981) TEM; Le Thomas & Thanikaimoni (1987) LM; A. Le Thomas SEM (unpubl. data). Described by Walker as inaperturate, but shown by Le Thomas & Thanikaimoni to be disulcate.

Alphonsea Hook.f. & Thomson: Walker (1971a) LM; Le Thomas & Thanikaimoni (1987) LM, SEM; Mols (2004) LM, SEM; Chaowasku *et al.* (2008) LM, SEM, TEM. Described by Walker as inaperturate, but Le Thomas & Thanikaimoni showed that three species are disulcate, Mols found both types and Chaowasku *et al.* reported one (?) or two germination zones in the intine, so we score this character as (1/2). Chaowasku *et al.* (2008) described the infratectum as ‘coarsely granular’ and the nexine as ‘sometimes indistinctly lamellate’; their figures show our intermediate infratectum state and one or two nexine foliations with no thickening of the outer foliation.

Ambavia Le Thomas: Le Thomas (1972) LM, SEM; Le Thomas & Lugardon (1975) TEM; Le Thomas (1980/1981) LM, SEM, TEM; Le Thomas *et al.* (1986) SEM, TEM; Le Thomas (1988) TEM.

Anaxagorea A.St.-Hil.: Walker (1971a) LM; Walker (1974a, 1976b) SEM; Hesse, Morawetz & Ehrendorfer (1985) SEM, TEM; Gabarayeva (1995) TEM.

Annickia Setten & Maas (= *Enantia* Oliv.): Walker (1971a) LM; Le Thomas (1980/1981) SEM, TEM; Le Thomas & Thanikaimoni (1987) LM; A. Le Thomas SEM (unpubl. data), showing sparse short columellae.

Annona group (= *Annona* L., *Raimondia* Saff., *Rollinia* A.St.-Hil. and *Rolliniopsis* Saff.): Walker (1971a) LM; Walker (1976a, b) SEM; Lugardon & Le Thomas (1974) TEM; Le Thomas (1980/1981), Le Thomas *et al.* (1986) SEM, TEM; Waha (1987a) TEM; Tsou & Fu (2002) LM, TEM.

Anonidium Engl. & Diels: Walker (1972) LM; Le Thomas (1980/1981) SEM, TEM.

Artabotrys R.Br.: Walker (1971a) LM; Lugardon & Le Thomas (1974), Le Thomas & Lugardon (1976), Le Thomas (1980/1981, 1988) TEM, SEM (unpubl. data).

Asimina group (= *Asimina* Adans. and *Deeringothamnus* Small): Walker (1971a) LM; Walker (1974a, 1976b) SEM; Walker & Skvarla (1975), Waha (1987b), Gabarayeva (1992, 1993) TEM; Hesse *et al.* (2009) LM, SEM.

Asteranthe Engl. & Diels: Walker (1972) LM; Le Thomas & Lugardon (1972), Le Thomas (1980/1981) SEM, TEM; Couvreur *et al.* (2008a) SEM, TEM.

Bocageopsis R.E.Fr.: Walker (1971a) LM; Waha (1985) SEM, TEM; Le Thomas & Thanikaimoni (1987) LM; A. Le Thomas LM, SEM (unpubl. data).

Cananga (DC.) Hook.f. & Thomson: Walker (1971a) LM, SEM; Walker (1976b) SEM; Le Thomas (1980/1981), Doyle *et al.* (2000) SEM, TEM.

Cleistopholis Pierre ex Engl.: Walker (1971a) LM; Le Thomas (1980/1981) SEM, TEM; Le Thomas & Thanikaimoni (1987) SEM; Le Thomas (1988) TEM. Described by Walker as inaperturate, but shown by Le Thomas (1980/1981, 1988) and Le Thomas & Thanikaimoni (1987) to be sulcate.

Crematosperma R.E.Fr.: Walker (1971a) LM.

Cyathocalyx Champ. ex Hook.f. & Thomson (including *Drepananthus* Maingay ex Hook.f.: Wang & Saunders, 2006): Walker (1971a) LM (*Cyathocalyx* and *Drepananthus*); Wang (2004) LM, SEM, TEM; A. Le Thomas SEM (unpubl. data). Described as inaperturate by Walker (1971a), but his figures of *Cyathocalyx* and *Drepananthus* show a possible sulcus, and this is clearer in our unpublished SEM photos and figures of Wang (2004). Walker described *Cyathocalyx* (but not *Drepananthus*) as tectate-perforate, and some species illustrated by Wang have larger tectal perforations than other Ambavioideae, so we have scored the genus as polymorphic for tectum. Wang's figures show fused basal granules and 1–2 foliations.

Cymbopetalum group (*Bocagea* A.St.-Hil., *Cardiopetalum* Schltdl., *Cymbopetalum* Benth., *Froesiodendron* R.E.Fr., *Hornschuchia* Nees, *Porcelia* Ruiz & Pav. and *Trigynaea* Schltdl.): Walker (1971a) LM, SEM (polyads); Walker (1974a, b, 1976a, b) SEM; Johnson & Murray (1995) SEM; Le Thomas *et al.* (1986) SEM; Morawetz & Waha (1991) SEM, TEM; Tsou & Fu (2007) LM, TEM. Nexine foliations scored as 1–2 rather than multiple in Doyle & Le Thomas (1994, 1996) and outer foliation as thickened rather than unknown, based on Morawetz & Waha (1991) for *Porcelia* and unpublished TEM photos of *Cymbopetalum* by C.-H. Tsou (pers. comm.; see text for discussion).

Dasymaschalon (Hook.f. & Thomson) Dalla Torre & Harms: Walker (1971a) LM, SEM; Walker (1976b),

Wang (2009), Wang *et al.* (2009) SEM. A few species differ from the majority in lacking spines (Walker, 1971a; Wang, 2009), but Wang (2009) inferred that the echinate state is ancestral.

Desmopsis Saff.: Walker (1971a) LM; Walker & Skvarla (1975) TEM; Mols (2004) LM, SEM. Described by Walker & Skvarla as 'granular pollen with presumed incipient columellae,' but the infratectal elements are as rod-like as in many groups that we have scored as columellar.

Disepalum Hook.f.: Walker (1971a) LM (octads).

Duguetia A.St.-Hil.: Walker (1971a) LM; Le Thomas *et al.* (1986), Le Thomas *et al.* (1994) SEM, TEM; Waha (1987a) TEM; A. Le Thomas SEM (unpubl. data).

Enicosanthum Becc. (including *Polyalthia longifolia* (Sonn.) Thwaites and other members of 'Polyalthia' section *Monoon* (Miq.) Hook.f. & Thomson: Mols *et al.*, 2004; Xue *et al.*, 2011): Walker (1971a) LM (*E. grandifolium* (Elmer) Airy Shaw, inaperturate); Walker (1976b) SEM (*E. grandifolium*); Le Thomas & Lugardon (1976), Le Thomas (1980/1981) TEM, A. Le Thomas SEM ('*P. longifolia*') (unpubl. data); Waha & Hesse (1988) TEM ('*P. sclerophylla*' Hook.f. & Thomson); Le Thomas (1988) LM ('*P. rumphii*' (Blume ex Hensch.) Merr. and '*P. sclerophylla*'); Mols (2004) LM, SEM. Mols (2004) scored all species as inaperturate except '*P. longifolia*' and '*P. sclerophylla*', and these as polymorphic, but grains of '*P. rumphii*' and '*P. sclerophylla*' (and other uniovulate species not yet included in molecular analyses, such as '*P. fragrans*' (Dalzell) Hook.f. & Thomson, '*P. jenkinsii*' (Hook.f. & Thomson) Hook.f. & Thomson, '*P. laui*' Merr. and '*P. simiarum*' (Buch.-Ham. ex Hook.f. & Thomson) Benth. & Hook.f. ex Hook.f.) figured by Le Thomas (1988) are convincingly disulcate. Le Thomas & Lugardon (1976) and Le Thomas (1980/1981) described '*Polyalthia longifolia*' as monosulcate, but because it is nested within *Enicosanthum* (Mols *et al.*, 2004) we assume that the basic state for the genus is inaperturate or disulcate. Mols (2004) scored several members as echinate, including '*P. longifolia*', in which Le Thomas (1980/1981) noted spine-like projections from the tectal verrucae, but, because the ancestral state is equivocal on Mols's tree and these projections differ from the typical more isolated spines in other Annonaceae, we have scored *Enicosanthum* as lacking spines. '*P. longifolia*' has an intermediate infratectum, but Walker (1971a) showed apparent well-developed columellae in *E. grandiflorum*, so we have scored the group as polymorphic. The outer foliation character is known only in '*P. longifolia*'.

Ephedranthus S.Moore: Walker (1971a) LM; Waha (1985) SEM, TEM; Le Thomas *et al.* (1986) SEM; Le Thomas (1998) and A. Le Thomas & M. Suarez-

Cervera SEM, TEM (unpubl. data). Scored by Doyle & Le Thomas (1996) as having 1–2 nexine foliations, but because Waha described *Ephedranthus* as having an outer thickened foliation and an inner foliation that is often split, and Le Thomas (1998) described it as having one or two inner foliations and compared it with *Mosannonna* and *Klarobelia*, we have rescored it as having multiple foliations.

Fusaea group (= *Fusaea* (Baill.) Saff. and *Duckeanthus* R.E.Fr.): Walker (1971a) LM; Walker (1976b) SEM; Le Thomas *et al.* (1994) SEM, TEM.

Goniothalamus (Blume) Hook.f. & Thomson: Walker (1971a) LM; A. Le Thomas SEM (unpubl. data).

Greenwayodendron Verdc. (= *Polyalthia oliveri* Engl. and *P. suaveolens* Engl. & Diels): Walker (1972) LM; Le Thomas & Lugardon (1976), Le Thomas (1980/1981, 1988), Le Thomas *et al.* (1986) TEM (as '*P. suaveolens*').

Gutteria Ruiz & Pav. (including *Guatterrella* R.E.Fr., *Guatterriopsis* R.E.Fr. and *Heteropetalum* Benth.: Erkens *et al.*, 2007): Walker (1971a) LM; Morawetz & Waha (1985) LM, SEM, TEM; Le Thomas *et al.* (1986) SEM, TEM; Waha (1987a) TEM.

Hexalobus A.DC.: Walker (1971a) LM; Le Thomas & Lugardon (1972) SEM, TEM; Lugardon & Le Thomas (1974), Le Thomas & Lugardon (1976) TEM; Le Thomas (1980/1981) SEM, TEM; Couvreur *et al.* (2008a) SEM, TEM. Data of Couvreur *et al.* (2008a) confirm our scoring of the tectum as verrucate or perforate (1/2) and the infratectum as intermediate; even in *H. monopetalus* (A.Rich.) Engl. & Diels, described as columellar by Le Thomas & Lugardon (1976) and Le Thomas (1980/1981), Couvreur *et al.* found granules mixed with the columellae. Couvreur *et al.* (2008a) described the infratectum of some *Hexalobus* and *Isolona* species as granular, but the granules are large and often irregular and therefore fall in our intermediate state. Doyle & Le Thomas (1994, 1996) scored the outer foliation as not thickened, but Couvreur *et al.* (2008a) showed that it is less thickened than in *Asteranthe*, *Monodora* and *Uvariastrum* but thicker than in *Isolona*, so we have rescored it as thickened.

Isolona Engl.: Walker (1971a) LM; Le Thomas & Lugardon (1976) TEM; Le Thomas (1980/1981) SEM, TEM; Le Thomas *et al.* (1986) SEM; Couvreur *et al.* (2008a) SEM, TEM. Le Thomas *et al.* (1986) showed that the area of reduced exine is proximal in tetrads, but because it is not known whether or not microspore rotation occurs we have scored the alternative aperture character as unknown. Reconstruction of tectal evolution in the genus by Couvreur *et al.* (2008a) indicates that the ancestral tectum was either their type A or B, both of which correspond to our verrucate state. See *Hexalobus* for remarks on infratectal structure.

Klarobelia Chatrou (*Crematosperma anomalum* R.E.Fr. and '*Malmea*' spp.: Chatrou, 1998; includes *Pseudephedranthus* Aristeg.: Pirie *et al.*, 2006): Walker (1971a) LM (as '*Pseudephedranthus*'); Le Thomas (1998) and A. Le Thomas & M. Suarez-Cervera SEM, TEM (*K. candida* Chatrou, *K. inundata* Chatrou and *K. pumila* Chatrou) (unpubl. data). Fine to larger tectal perforations, distinct columellae, thickened outer and 1–3 inner foliations.

Letestudoxa Pellegr.: Walker (1971b) LM; Le Thomas (1980/1981) TEM; Le Thomas & Thanikaimoni (1987) LM, SEM. Described by Walker as inaperturate, but shown by Le Thomas & Thanikaimoni to have two furrows fused into a ring (zonasulculus).

Lettowianthus Diels: Walker (1972) LM; Walker (1976b) SEM; Le Thomas & Lugardon (1976), Le Thomas (1980/1981) SEM, TEM.

Maasia Mols, Kessler & Rogstad (Mols *et al.*, 2008; = '*Polyalthia hypoleuca* complex' of Rogstad, 1989): Walker (1971a) LM, SEM ('*P. glauca*' (Hassk.) F.Muell.); Walker (1976b) SEM; Rogstad & Le Thomas (1989) SEM, TEM; Mols (2004) LM, SEM.

Malmea R.E.Fr. (Chatrou, 1998): Walker (1971a) LM (*M. obovata* R.E.Fr.); Waha (1985) SEM, TEM ('*M. obovata*' = *M. manausensis* Maas & Miralha); Le Thomas (1998) and A. Le Thomas & M. Suarez-Cervera SEM, TEM (*M. dielsiana* R.E.Fr.) (unpubl. data). Large tectal perforations, reduced infratectum in *M. manausensis*; small perforations, rare large granules that often take on the aspect of irregular, thick columellae (scored as intermediate) and thick nexine with occasional gaps suggesting that it consists of two foliations in *M. dielsiana*.

Marsypopetalum Scheff. (including *Polyalthia crassa* R.Parker, *P. littoralis* (Blume) Boerl. and two other species: Xue *et al.*, 2011): Walker (1971b) LM; Mols (2004) LM, SEM; Xue *et al.* (2011) SEM. *Marsypopetalum* and '*P. littoralis*' described as inaperturate by Walker (1971b) and Mols (2004), but Xue *et al.* (2011) concluded that all species probably have disulculate or derived conditions.

Meiocarpidium Engl. & Diels: Walker (1971a) LM; Le Thomas (1980/1981) SEM, TEM. Described by Walker as having very loose tetrads, but shown by Le Thomas to have single grains.

Meiogyne Miq. (including *Ancana* F.Muell. and *Fitzalania* F.Muell.: Mols *et al.*, 2004): Walker (1971a) LM (*Meiogyne*, described as inaperturate); Walker (1971b) LM ('*Fitzalania*', described as inaperturate); Waha & Morawetz (1988) SEM, TEM ('*Ancana*' and '*Fitzalania*', described as disulculate); Mols (2004) LM, SEM, TEM (both inaperturate and disulculate species). Based on Waha & Morawetz (1988), Doyle & Le Thomas (1994, 1996) scored the *Ancana* group as having a thickened outer foliation, but re-examination of figures of '*Ancana*' and '*Fitzalania*'

in Waha & Morawetz shows that the outer foliation is only slightly or not at all thicker than the inner one, so we have scored *Meiogyne* as unthickened.

Mezzettia Becc.: Walker (1971a) LM; Chaowasku *et al.* (2008) LM, SEM, TEM. Considered inaperturate by Walker and Chaowasku *et al.*, but Walker's figure shows a possible sulcus and Chaowasku *et al.* show lower sculpture in part of the grain. Walker described the exine as columellar, but no columellae are visible below the verrucate tectum in TEM sections of Chaowasku *et al.*; they described the exine as lacking differentiation into a tectum and infratectum and the nexine as lamellated, but the exine is too thin and the magnification too low to rule out the presence of a thin layer of fine granules. Hence, we score the aperture and infratectum characters as unknown.

Miliusa Lesch. ex A.DC.: Walker (1971a) LM; Lugardon & Le Thomas (1974) TEM; Le Thomas (1980/1981) SEM, TEM; Mols (2004) LM, SEM; Chaowasku *et al.* (2008) LM, SEM, TEM. *Miliusa* has been interpreted as inaperturate (Walker, 1971a; Mols, 2004) and disulculate (Doyle & Le Thomas, 1996). However, although Chaowasku *et al.* (2008) described *Miliusa* as inaperturate with one (?) or two germination zones in the intine, and their SEM figures show no definite external aperture, their TEM section of *M. horsfieldii* (Benn.) Pierre (fig. 4E) shows that the exine at the germination zones is much thinner, so we score the genus as (1/2).

Mischogyne Exell: Walker (1972) LM; Le Thomas (1980/1981) TEM.

Mitrephora (Blume) Hook.f. & Thomson: Walker (1971a) LM; Mols (2004) LM, SEM; Weerasooriya & Saunders (2010) SEM. Described by Walker (1971a) as having distinct columellae, and columellae are visible in Walker (1971a: pl. 16, fig. 1).

Mkilua Verdc.: Johnson & Murray (1995) SEM; Doyle & Le Thomas (1995), based on TEM of M. Harley and B. Lugardon (unpubl. data).

Monanthotaxis group (*Monanthotaxis* Baill., including African species formerly assigned to *Popowia* Endl.; *Enneastemon* Exell, *Exellia* Boutique, *Friesodielsia* Steenis p.p and *Gilbertiella* Boutique): Walker (1971a) LM; Le Thomas & Lugardon (1974), Le Thomas (1980/1981) SEM, TEM.

Monocarpia Miq.: Walker (1971a) LM; Mols (2004) LM, SEM; Chaowasku, Keßler & van der Ham (2012) SEM, TEM. Based on figures in Chaowasku *et al.* (2012), the pollen is boat-shaped with distinct columellae, and the nexine appears to have a thickened outer foliation, as in *Maasia* (Rogstad & Le Thomas, 1989) and other columellar monosulcates.

Monodora Dunal: Walker (1971a) LM; Le Thomas (1980/1981) TEM; Couvreur *et al.* (2008a) SEM, TEM.

Mosannonna Chatrou ('*Malmea*' spp.: Chatrou, 1998): Walker (1971a) LM, SEM (as '*Malmea*' *costa-*

ricensis R.E.Fr. and '*Malmea*' *raimondii* (Diels) R.E.Fr.); Walker (1974a, 1976b) SEM ('*M.*' *costaricensis*); Le Thomas (1998) and A. Le Thomas & M. Suarez-Cervera SEM, TEM (*Mosannonna depressa* (Baill.) Chatrou) (unpubl. data). Small to larger tectal perforations, distinct columellae and small granules, thickened outer and 1–3 inner foliations.

Neostenanthera Exell: Walker (1971a) LM; Le Thomas (1980/1981) TEM, SEM (sparse microperforations) (A. Le Thomas, unpubl. data). Rescored as unknown for fused basal granules and thickened outer foliation because of homology problems discussed in text.

Onychopetalum R.E.Fr.: Walker (1971a) LM; Walker (1976a, b) SEM. Tectum perisaccate; unclear if perforate, so scored as unknown.

Ophrypetalum Diels: Walker (1972) LM; Walker (1974a, 1976b) SEM; Le Thomas (1980/1981) TEM; A. Le Thomas SEM, TEM (unpubl. data). We assume that the intact state, with free or sometimes apically connected columellae, is an autapomorphy derived from reticulate–columellar and have therefore scored the tectum as reticulate (see text for discussion).

Orophea Blume: Walker (1971a) LM ('*O.*' *luzonensis* Merr., with tetrads, belongs in *Pseuduvaria*, as suspected by Walker: Su & Saunders, 2003); Mols (2004) LM, SEM; Chaowasku *et al.* (2008) LM, SEM, TEM. Described by Walker (1971a) as inaperturate; Mols *et al.* scored six species as inaperturate and only *O. kerrii* Kessler as disulculate, but *O. polycarpa* A.DC. figured in Chaowasku *et al.* is clearly disulculate. TEM figures of Chaowasku *et al.* show variation between 1–2 nexine foliations and 4–5 foliations in *O. brandisii* Hook.f. & Thomson, with no thickening of the outer foliation.

Oxandra A.Rich.: Walker (1971a) LM. No visible perforations, tectum scored as unknown.

'*Pachypodanthium*' Engl. & Diels (now included in *Duguetia*: Chatrou, 1998; Chatrou *et al.*, 2000): Le Thomas *et al.* (1986) TEM; A. Le Thomas SEM (unpubl. data).

Phaeanthus Hook.f. & Thomson: Walker (1971a) LM. Described as smooth to weakly verrucate, but most grains figured are clearly verrucate, with no visible columellae.

Piptostigma Oliv.: Walker (1971a) LM; Le Thomas & Lugardon (1974), Le Thomas (1980/1981), Le Thomas *et al.* (1986) SEM, TEM.

Platymitra Boerl.: Walker (1971a) LM; Mols (2004) LM, SEM; A. Le Thomas SEM (unpubl. data); Chaowasku *et al.* (2008) LM, SEM, TEM. Apparently inaperturate with two germination zones in the intine only (Chaowasku *et al.*, 2008).

Polyalthia Blume s.s. (including *Haplostichanthus* F.Muell.: Mols *et al.*, 2004): Walker (1971b) LM ('*Haplostichanthus*,' described as inaperturate); Le Thomas

(1988) LM (*P. cauliflora* Hook.f. & Thomson and *P. obliqua* Hook.f. & Thomson, disulcate); Waha & Hesse (1988) SEM, TEM (*P. cauliflora*, inaperturate under SEM, one unthickened foliation); Waha & Morawetz (1988) SEM, TEM (*Haplostichanthus*, disulcate, 1–2 foliations, outer not thickened); Mols (2004) LM, SEM (*P. cauliflora*); A. Le Thomas SEM (unpubl. data) (*P. suberosa* (Roxb.) Thwaites).

Polyalthia stuhlmannii (Engl.) Verdc.: Le Thomas & Lugardon (1976), Le Thomas (1980/1981) TEM; Le Thomas (1988) TEM; A. Le Thomas SEM (unpubl. data). See text for discussion of aperture condition and exine structure.

Polyceratocarpus Engl. & Diels: Walker (1971a) LM; Le Thomas & Lugardon (1974), Le Thomas (1980/1981, 1988) TEM.

Popowia Endl.: Walker (1971a) LM; Mols (2004) LM, SEM; A. Le Thomas LM, SEM (unpubl. data), SEM. Mols (2004) scored *P. odoardi* Diels as echinate, but because this species is nested within *Popowia* in his tree we have scored the tectum as verrucate and lacking spines.

Pseudomalmea Chatrou ('*Malmea*' spp.: Chatrou, 1998): Le Thomas (1998) and A. Le Thomas & M. Suarez-Cervera SEM, TEM (unpubl. data) (*P. declina* (R.E.Fr.) Chatrou). Tectum with fewer and smaller perforations than in other taxa formerly assigned to *Malmea*, large rounded granules or columellae (scored as intermediate), thickened outer and 1–4 inner foliations.

Pseudoxandra R.E.Fr.: Walker (1971a) LM, SEM; Walker (1976b) SEM; Le Thomas *et al.* (1986) SEM, TEM.

Pseuduvaria Miq. (including *Petalolophus* K.Schum.: Mols *et al.*, 2004): Walker (1971a) LM (*Pseuduvaria* and '*Orophea*' *luzonensis*); Walker (1974a, 1976b) SEM ('*O.*' *luzonensis*); Su & Saunders (2003) SEM, TEM; Su & Saunders (2006) SEM.

Ruizodendron R.E.Fr.: Walker (1971a) LM.

Sapranthus Seem.: Walker (1971a) LM; Waha & Hesse (1988), Le Thomas *et al.* (1986) SEM, TEM; Le Thomas & Thanikaimoni (1987) LM.

Sphaerocoryne Scheff. ex Ridl.: Le Thomas (1980/1981) SEM, TEM; A. Le Thomas LM, SEM (unpubl. data) (as *Melodorum gracile* (Engl. & Diels) Verdc.: see text for discussion).

Stenanona Standl.: Walker (1971a) LM.

Tetrameranthus R.E.Fr.: Walker (1971a) LM; Hesse & Waha (1984) SEM, TEM; A. Le Thomas SEM, TEM (unpubl. data). Described by Walker as inaperturate, but shown by Hesse & Waha to have a reduced round aperture. The number of nexine foliations is at the limit between states: Hesse & Waha described two or more continuous foliations, but Le Thomas TEM photos show two; because the foliations are continuous we have placed them in the multiple state.

Toussaintia Boutique: Walker (1971b) LM; Le Thomas (1980/1981) TEM; A. Le Thomas SEM (unpubl. data).

Unonopsis R.E.Fr.: Walker (1971a) LM; Waha (1985) SEM, TEM.

Uvaria L.: Walker (1971a) LM; Walker (1976b) SEM; Lugardon & Le Thomas (1974) TEM; Le Thomas (1980/1981) SEM, TEM; Waha (1987a) TEM.

Uvariastrum Engl.: Walker (1971a) LM; Walker (1974a, 1976b) SEM; Lugardon & Le Thomas (1974), Le Thomas & Lugardon (1976), Walker & Skvarla (1975) TEM; Le Thomas (1980/1981), Le Thomas *et al.* (1986) SEM, TEM; Couvreur *et al.* (2008a) SEM, TEM.

Uvariadendron (Engl. & Diels) R.E.Fr.: Walker (1972) LM; Le Thomas (1980/1981) SEM, TEM.

Uvariopsis Engl.: Walker (1971a) LM; Lugardon & Le Thomas (1974), Le Thomas & Lugardon (1976) TEM; Le Thomas (1980/1981) SEM, TEM; Verdcourt (1986) SEM, TEM.

Xylophia L.: Walker (1971a) LM; Walker (1974a, 1976b) SEM; Le Thomas (1980/1981) SEM, TEM; A. Le Thomas TEM (unpubl. data). Rescored as unknown for fused basal granules and thickened outer foliation because of homology problems discussed in text.