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EVOLUTION OF ANGIOSPERM POLLEN. 2. THE BASAL ANGIOSPERMS[1]

Lu Lu,[2,3] *Alexandra H. Wortley,*[4] *De-zhu Li,*[2,3] *Hong Wang,*[2,3*] *and Stephen Blackmore*[4]

ABSTRACT

Recently, evolutionary relationships among the five major lineages of the basal angiosperms, i.e., Amborellanae, Nymphaeanae, Austrobaileyanae, Magnolianae, and Ceratophyllanae, still remain incompletely or controversially resolved. This study reviews and reevaluates the origin, evolution, and systematic significance of pollen morphology in basal angiosperms. Pollen grains from 34 species of 31 genera in 23 families of the basal angiosperms are investigated, using light microscopy (LM) and scanning electron microscopy (SEM), to illustrate the pollen diversity across the grade. Pollen data for 57 genera, representing all nine orders and 28 families of the basal angiosperms, were obtained from previous work and new investigations and were used to optimize character states onto recent molecular phylogenetic estimates. Two 18-character datasets were generated: a comprehensively coded dataset optimized with Fitch parsimony onto 12 recent phylogenies with differing topologies to evaluate the degree of pollen systematic significance of each topology, and a democratically coded dataset optimized by using Fitch parsimony, maximum likelihood, and hierarchical Bayesian inference onto a single recent phylogeny based on molecular data from Soltis et al. to evaluate the disparity of pollen evolutionary patterns among the three methods. Pollen morphology of the basal angiosperms is highly diverse, particularly in terms of pollen size, aperture number, aperture position, ectoaperture shape, tectum sculpture, and infratectal structure. Based on both datasets, the plesiomorphic pollen type for angiosperms was inferred to comprise monads of heteropolar, spheroidal, mono-apertural grains, with distal, colpate apertures, sculptured aperture membranes, and an infratectum and foot layer. Of the basal angiosperm phylogenies considered, when taxon sampling is taken into account, pollen characters provide greatest support for the topology represented in the three-mitochondrial-gene study from Qiu et al., in terms of number of synapomorphies plus what we term "likely synapomorphies" in which the subtending node is ambiguous and, therefore, the exact position of the synapomorphy is ambiguous. Thirty-six various lineages above family level are supported by pollen morphological synapomorphies or likely synapomorphies. We discuss the systematic significance of pollen morphology in basal angiosperm clades including the ANITA group (Amborellaceae, Nymphaeaceae, Schisandraceae, Trimeniaceae, and Austrobaileyaceae), Ceratophyllales, Chloranthales, and magnoliids, as well as in related lineages, i.e., monocots and basal eudicots. We also discuss present limitations in inferring the evolutionary history of pollen morphology in basal angiosperms.

Key words: Amborellales, ancestral character reconstruction, Austrobaileyales, basal angiosperms, Ceratophyllales, character evolution, Chloranthales, magnoliids, Nymphaeales, pollen morphology, systematic significance.

The basal angiosperms are defined as those species at the tips of the earliest diverging flowering plant lineages (i.e., all angiosperms with the exception of monocots and eudicots) and comprise a grade of separate evolutionary lineages, together containing a few hundred species. The nature of the basal angiosperms is intrinsically linked with the pattern, process, and timing of the origin of the angiosperms and is perhaps the most controversial topic in plant evolution, one that has generated an immense literature. Over the last 20 years, since the angiosperms have unequivocally been determined as a monophyletic

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group, they have been subject to the most intensive phylogenetic analyses ever conducted on any group of taxa, using a variety of methods and different combinations of molecular markers for clarifying relationships, particularly among extant basal angiosperms (reviewed by Qiu et al., 2006; Hansen et al., 2007; Jansen et al., 2007; Zavada, 2007; Soltis et al., 2007, 2008; Endress & Doyle, 2009).

An initial proposal of *Kadsura* Juss. (Schisandraceae) as the most primitive angiosperm by Delpino (1890) was refuted by recent studies, starting with Chase et al. (1993), whose analysis showed *Ceratophyllum* L. to be the basalmost extant angiosperm based on a large *rbc*L tree of seed plants. However, combined-gene analyses over the past decade or more have now confirmed, instead, the ANITA grouping (Amborellaceae, Nymphaeaceae, Schisandraceae, Trimeniaceae, and Austrobaileyaceae) as the basalmost grade of angiosperms (Mathews & Donoghue, 1999; Parkinson et al., 1999; Qiu et al., 1999; Soltis et al., 1999; Barkman et al., 2000; Graham & Olmstead, 2000). The relationships of the other earliest branching lineages, including magnoliids, Ceratophyllaceae, Chloranthaceae, monocots, and basal eudicots, have gradually been resolved with increasing confidence through a series of phylogenetic investigations (e.g., Chase et al., 1993; Qiu et al., 1999, 2000, 2005, 2006; Graham & Olmstead, 2000; Savolainen et al., 2000; Soltis et al., 2000, 2007, 2008; Zanis et al., 2002; Aoki et al., 2004; Hansen et al., 2007; Jansen et al., 2007; Moore et al., 2007; Endress & Doyle, 2009). By the time of the most recent Angiosperm Phylogeny Group classification (Angiosperm Phylogeny Group, 2009), the basal angiosperms were mainly agreed to comprise the five successively evolved major clades identified as the Amborellanae (Amborellales), the Nymphaeanae (Nymphaeales), the Austrobaileyanae (Austrobaileyales), the Magnolianae (Piperales, Canellales, Magnoliales, Laurales, and Chloranthales), and the Ceratophyllanae (Ceratophyllales).

Although these monophyletic clades within basal angiosperms have each been firmly ascertained from both molecular and morphological data, their systematic relationships remain incompletely resolved and controversially supported, depending on the genomes and reconstruction approaches used (Hansen et al., 2007; Moore et al., 2007). It will not be easy to determine whether Amborellanae alone is the first-branching angiosperm, or whether it groups with Nymphaeanae as a first branch (Qiu et al., 2000, 2006; Leebens-Mack et al., 2005; Jansen et al., 2007). No recent analyses have found consistent support for their relationships (reviewed by Hansen et al., 2007; Moore et al., 2007). The placements of the Ceratophyllales and Chloranthales in particular are highly unstable at present (reviewed by Qiu et al., 2006; Endress & Doyle, 2009). Thus, understanding the origin and early relationships of the angiosperms remains a formidable challenge.

Collating intensive morphological evidence can facilitate the understanding of these evolutionary uncertainties, which may not be resolved by molecular analyses in the short term. Although morphological investigations may not themselves solve the current impasse, it is useful to consider possible morphological support for alternative molecular tree topologies (Renner et al., 2000). Valuable morphological characters with systematic significance, such as floral morphology, ontogeny, and breeding systems, have been elucidated in detail for basal angiosperms over past decades (Donoghue & Doyle, 1989; Doyle et al., 1994; Doyle, 1996; Doyle & Endress, 2000; Floyd & Friedman, 2001; Endress, 2010, 2011). Palynology is considered a unique source of morphological information in that no other dataset can provide so great an amount of information from so little material in so short a time (Walker & Doyle, 1975). In previous studies, pollen characters provided insights into aspects of taxonomy and phylogenetics for many taxa within basal angiosperms, including the Winteraceae (Sampson, 1993, 2007), Calycanthaceae, Siparunaceae and Atherospermataceae (Renner, 1999), *Maloutchia* Warb. (Sauquet et al., 2003), Nymphaeaceae (Podoplelova & Ryzhakov, 2005), Annonaceae (Doyle & Le Thomas, 1997), Monimiaceae (Renner, 1998), and Schisandraceae (Wang et al., 2010).

The evolution of pollen morphology in the basal angiosperms has been debated for a long time (Muller, 1970; Walker, 1974, 1976; Walker & Doyle, 1975; Walker & Walker, 1984; Doyle & Hotton, 1991; Gabarayeva, 1991; Brenner, 1996; Friis et al., 1999, 2000; Sampson, 2000a, 2007; Furness & Rudall, 2004; Doyle, 2005; Zavada, 2007). There have been considerable controversies as to the nature of the most primitive angiosperm pollen grain and evolutionary trends (Walker & Doyle, 1975; Walker & Walker, 1984; Brenner, 1996; Doyle, 2005). Due to the continuing uncertainty in phylogenetic estimates, and incomplete or unavailable palynological data for certain extant and fossil taxa, the identification of plesiomorphic palynological characters, and related transitions in pollen morphological evolution, remain difficult. Nevertheless, our increasingly robust understanding of angiosperm phylogeny allows a more secure reconstruction of the morphological characters of extant angiosperms and their early

evolution than ever before (Endress & Doyle, 2009; Wortley et al., 2015).

In this paper, we investigate the diversity and evolution of pollen in basal angiosperms and related taxa using a combination of reanalyzed, previously published information and new data. This is set in the context of recent molecular phylogenetic hypotheses and follows the protocol outlined in Wortley et al. (2015). First, we optimize the distribution of selected pollen characters onto alternative molecular-based phylogenetic estimates from recent studies to infer plesiomorphic and synapomorphic (or likely synapomorphic) states for palynological characters. This enables us to interpret evolutionary patterns in terms of the inferred plesiomorphic pollen characters of angiosperms, and of the systematic value of palynological characters to elucidate some of the uncertain relationships among the early diverging lineages in divergent tree topologies. Second, we optimize the states of selected pollen characters onto a maximum likelihood tree generated from the molecular data of Soltis et al. (2011) using three methods, i.e., Fitch parsimony, maximum likelihood, and hierarchical Bayesian inference, to test the differences in inferred patterns of state change and the significance of disagreements among these methods. Finally, we discuss the constraints facing current research into plesiomorphic palynological characters and their evolution in basal angiosperm lineages.

Materials and Methods

study taxa

For new palynological observations, pollen grains from 34 species of 31 genera in 23 families of basal angiosperms were observed with light microscopy (LM) and scanning electron microscopy (SEM). Pollen samples were taken from the living collections at Kunming Botanic Garden (KBG) and Xishuangbanna Tropical Botanical Garden (HITBG), and the herbaria of the Kunming Institute of Botany, China (KUN), Royal Botanic Garden Edinburgh, U.K. (E), and the Australian National Herbarium, Australia (CANB) (cf. Appendix 1). Preparation of pollen grains for observation followed the acetolysis method of Erdtman (1960). To observe the stratification of the pollen exine, pollen grains used for LM examination were embedded in glycerine jelly on glass slides. Pollen size (the maxium diameter of a single grain) was measured under LM. To prepare specimens for SEM examination, acetolyzed grains were suspended in 95% ethanol for dehydration, then mounted on specimen stubs, and sputter-coated with gold-palladium. Pollen morphology (Figs. 1–6) was examined using a Hitachi (Tokyo, Japan) S-4800 SEM at 10 KV (KUN) and a Zeiss (Oberkochen, Germany) DSM 962 SEM at 15 KV (E). Image editing was accomplished using Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, California, U.S.A.).

taxon sampling and choice of base phylogenies

We used 12 base tree topologies from recent molecular phylogenies focusing on the basal angiosperms (Qiu et al., 1999, 2000, 2006; Graham & Olmstead, 2000; Mathews & Donoghue, 2000; Soltis et al., 2000, 2005, 2007, 2011; Müller et al., 2006; Jansen et al., 2007; Moore et al., 2007), which together represent all conflicting topologies found in the recent literature (Figs. 7–9). One topology was a modified combination of Jansen et al. (2007) and Moore et al. (2007), giving a topology similar to that of Soltis et al. (2011) but with different taxon sampling. Two topologies were taken from Qiu et al. (2006), i.e., one consensus tree of likelihood and parsimony analyses of eight genes, and one consensus tree of likelihood analyses of three mitochondrial genes.

All of these trees were modified by reducing the number of terminals, in particular within basal eudicots and monocots, which were not the focus of this study. All trees used gymnosperms as outgroups, with the exception of that from Mathews and Donoghue (2000), which was rooted without outgroups. The total number of terminals on all trees varied from 16 to 78, featuring 57 exemplar genera representing all nine orders and 28 families of the basal angiosperms, representing the breadth of morphological and palynological diversity within the group, with eight genus-level exemplars spanning four orders and eight families of the gymnosperms as outgroups, 10 genus-level exemplars spanning three orders and eight families of basal eudicots, and three genus-level exemplars spanning two orders and three families of monocots. We worked with genus-level terminals (cf. Appendix 1), and our systematic treatment follows the classification of the Angiosperm Phylogeny Group (2009) and Soltis et al. (2011).

In addition, to enable further comparative studies, a phylogenetic tree with branch lengths was generated based on molecular data of all 17 genic regions for 68 out of 640 taxa in total as utilized in Soltis et al. (2011; the most recent authoritative topology of angiosperms) that were obtained from TreeBASE (S11267, see <http://www.treebase.org>; see tree diagrams, Fig. 10). The 68 taxa were all covered within the 78 taxa described above (cf. Appendix 1), including 50 genera of basal angiosperms, seven genera of basal eudicots, three genera of monocots, and eight genera of gymnosperm as outgroups.

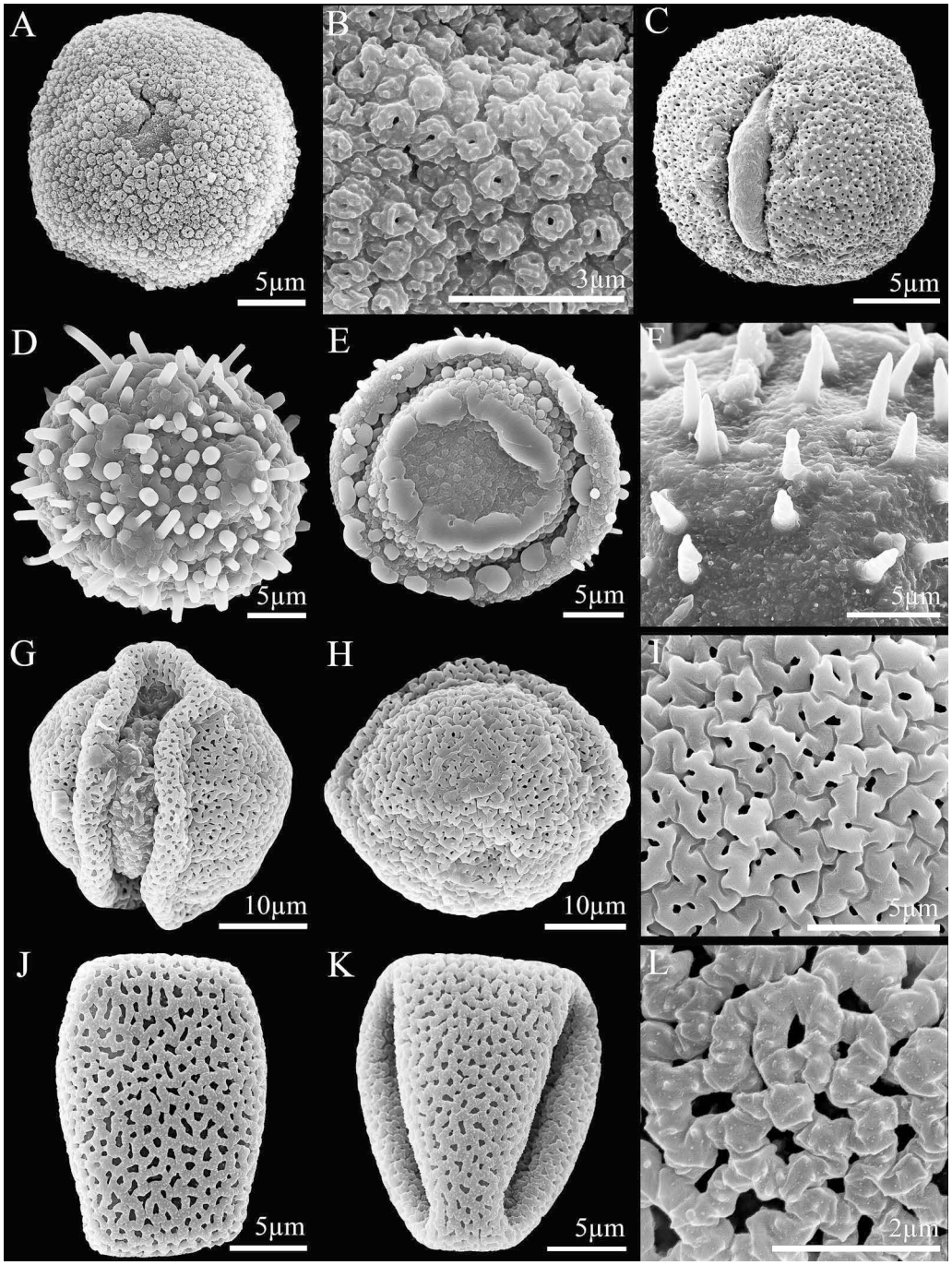


Figure 1. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). A, B. *Amborella trichopoda* Baill. —A. Distal polar view, showing monad, globose, monoporate pollen grain. —B. Detail showing *Amborella*-type tectum with spinulose supratectal elements. —C. *Trithuria australis* (Diels) D. D. Sokoloff, Remizowa, T. D. Macfarl. & Rudall, distal polar view, showing a distal colpus and perforate tectum with spinulose supratectal elements. D, E. *Nymphaea alba* L. —D. Distal polar view, showing zonate aperture located at the distal pole. —E. Proximal polar view, showing spheroidal shape, echinate and verrucate supratectal elements. —F. *Nuphar luteum* Walp., showing echinate and verrucate supratectal elements. G–I. *Austrobaileya scandens* C. T. White. —G. Distal polar view, showing heteropolarity, bilateral symmetry, and a distal colpus with sculptured aperture membrane. —H. Equatorial view, showing oblate shape. —I. Detail showing reticulate tectum with absence of supratectal elements. J–L. *Trimenia moorei* (Oliv.) Philipson. —J. Equatorial view, showing prolate shape and two colpi located at the equator. —K. Equatorial view. —L. Detail showing reticulate tectum with spinulose supratectal elements.

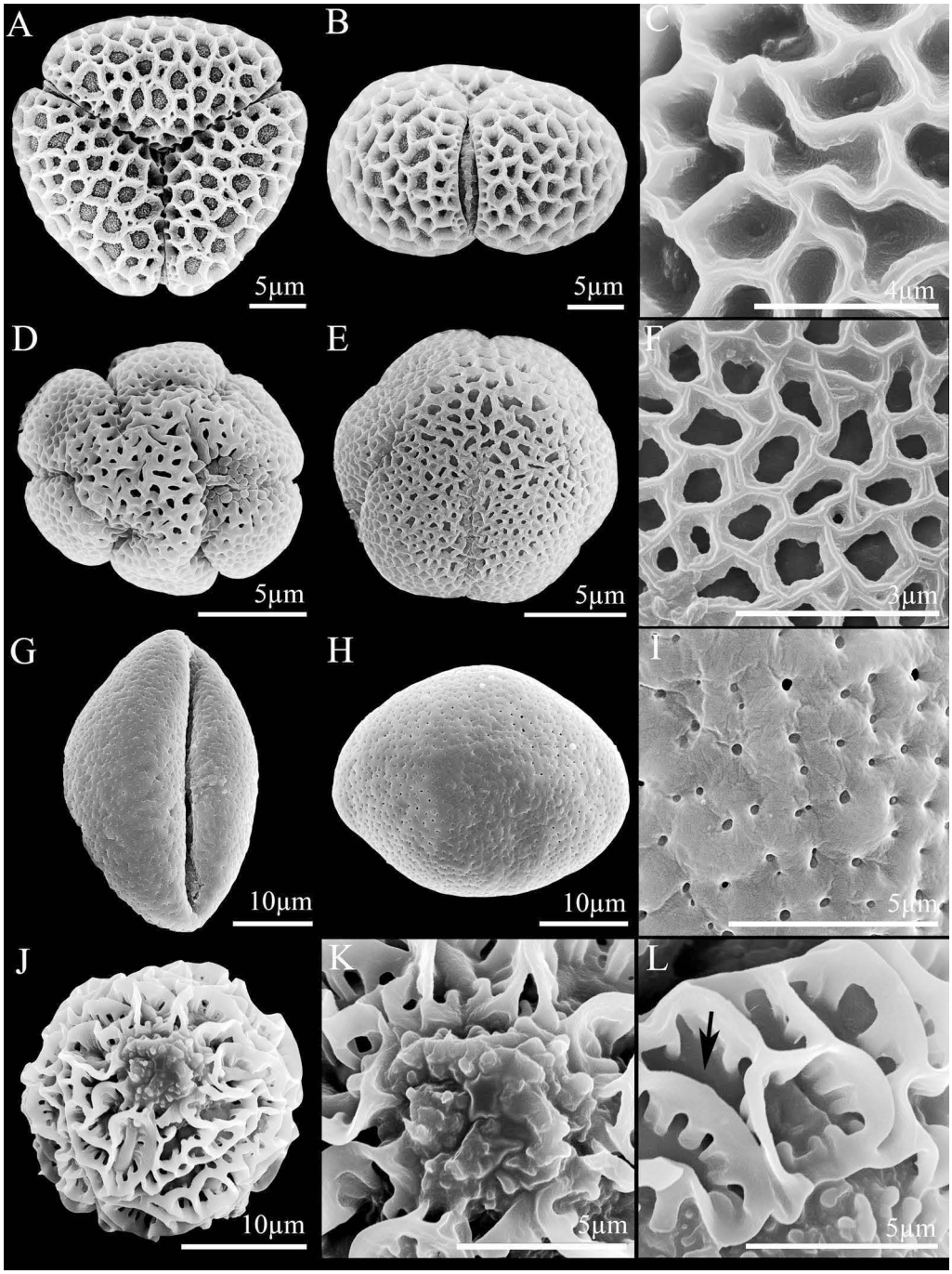


Figure 2. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). A, B. *Illicium micranthum* Dunn. —A. Polar view, showing isopolar, radially symmetrical, circular and syncolpate, triaperturate grains. —B. Equatorial view. —C. *Illicium macranthum* A. C. Sm., showing reticulate tectum. —D. *Chloranthus japonicus* Siebold, polar view, showing hexa-colpate apertures. E, F. *Chloranthus holostegius* (Hand.-Mazz.) S. J. Pei & Shan. —E. Polar view and partly equatorial view, showing hexa-colpate apertures. —F. Detail of reticulate tectum. G–I. *Canella* P. Browne, sp. indet. —G. Distal polar view, showing elliptic shape. —H. Proximal view. —I. Detail of perforate tectum. J–L. *Drimys piperita* Hook. f. —J. Tetrad. —K. Detail of porate aperture on a monad. —L. Detail of reticulate tectum, columellate infratectum; arrow indicates the foot layer.

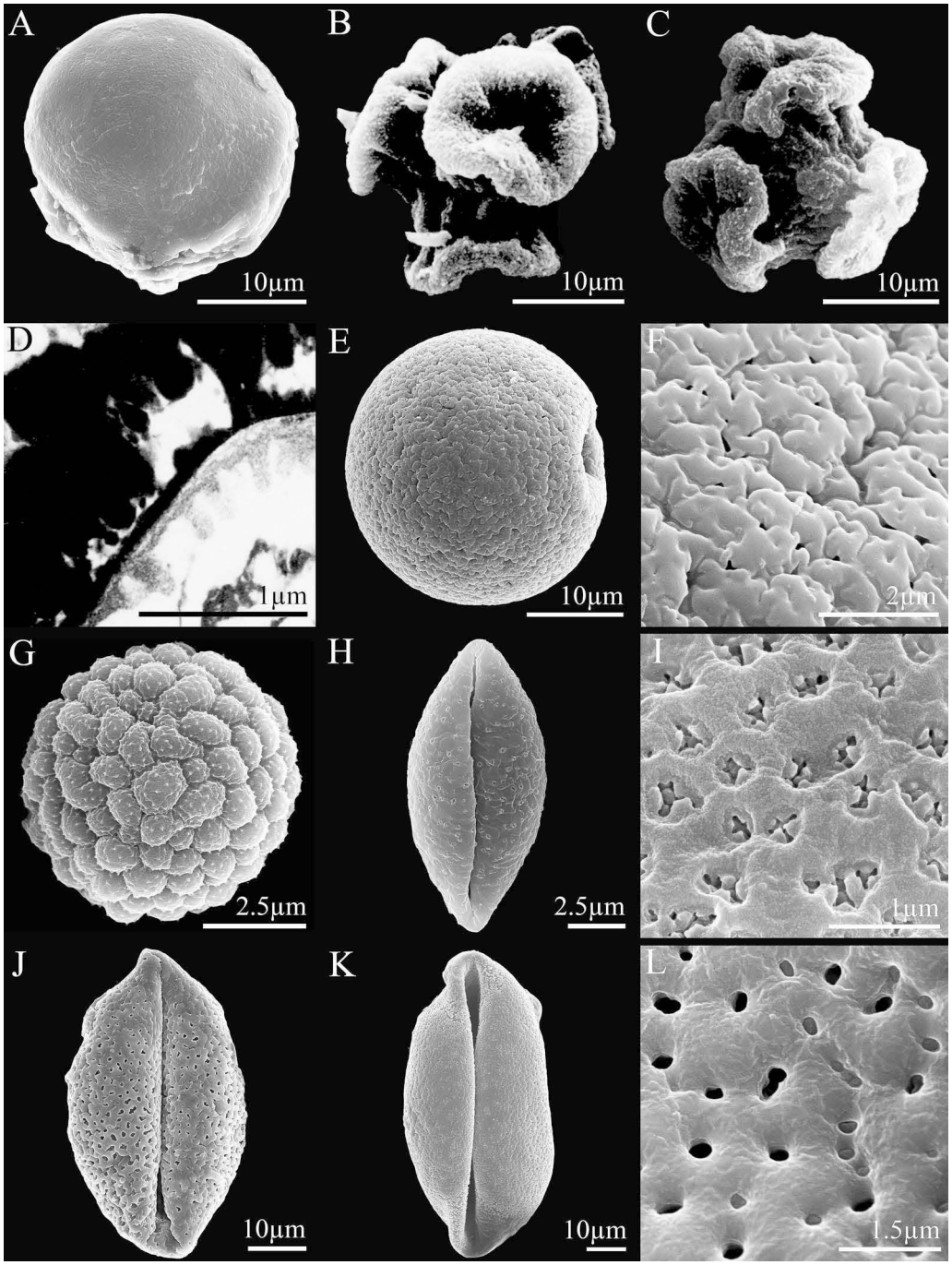


Figure 3. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). —A. *Hydnora abyssinica* A. Braun, equatorial view, showing a colpus located at the distal pole. B–D. *Lactoris fernandeziana* Phil.; images reprinted with permission from Zavada and Taylor (1986). —B, C. Tetrad. —D. TEM showing granulate infratectum. E, F. *Aristolochia contorta* Bunge. —E. Equatorial view, spheroidal pollen grain showing porate aperture. —F. Detail of rugulate tectum. —G. *Peperomia heyneana* Miq., showing apolar, radial, spheroidal, small-sized, inaperturate pollen grain and areolate tectum with spinulose supratectal elements. —H. *Saururus chinensis* (Lour.) Baill., distal polar view, showing boat-shaped pollen. —I. *Houttuynia cordata* Thunb., showing unique form of perforate tectum. —J. *Liriodendron tulipifera* L., distal polar view, showing boat-shaped pollen with large size. K, L. *Magnolia grandiflora* L. —K. Distal polar view, showing boat-shaped pollen. —L. Detail of perforate tectum.

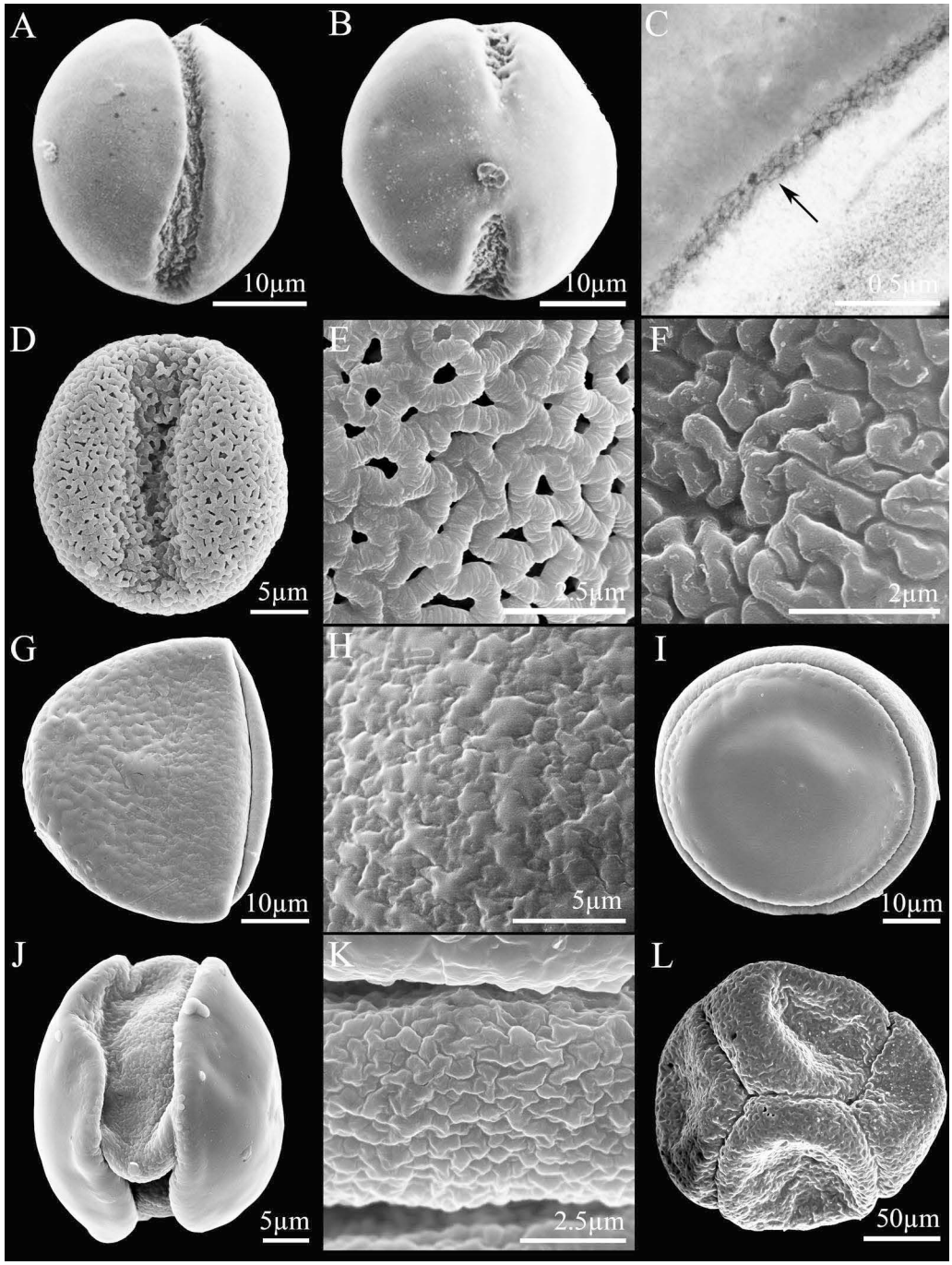


Figure 4. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). A–C. *Degeneria vitiensis* I. W. Bailey & A. C. Sm.; reprinted with permission from Sampson (2000a). —A. Distal polar view. —B. Proximal polar view. —C. TEM showing the near homogeneous nature of the exine with small granules; arrow indicates absence of a foot layer. D, E. *Knema globularia* (Lam.) Warb. —D. Distal polar view, showing subspheroidal shape, elliptic polar view, medium size, and a colpus. —E. Detail of reticulate tectum. —F. *Myristica* Gronov., sp. indet., showing rugulate tectum. G, H. *Galbulimima baccata* F. M. Bailey. —G. Equatorial view, showing a colpus located at the distal pole. —H. Detail of rugulate tectum. I–K. *Eupomatia laurina* R. Br. —I. Polar view, showing imperforate tectum. —J. Equatorial view, showing zonate aperture. —K. Detail of sculptured aperture membrane. —L. *Annona muricata* L., tetrad with very large-sized monads.

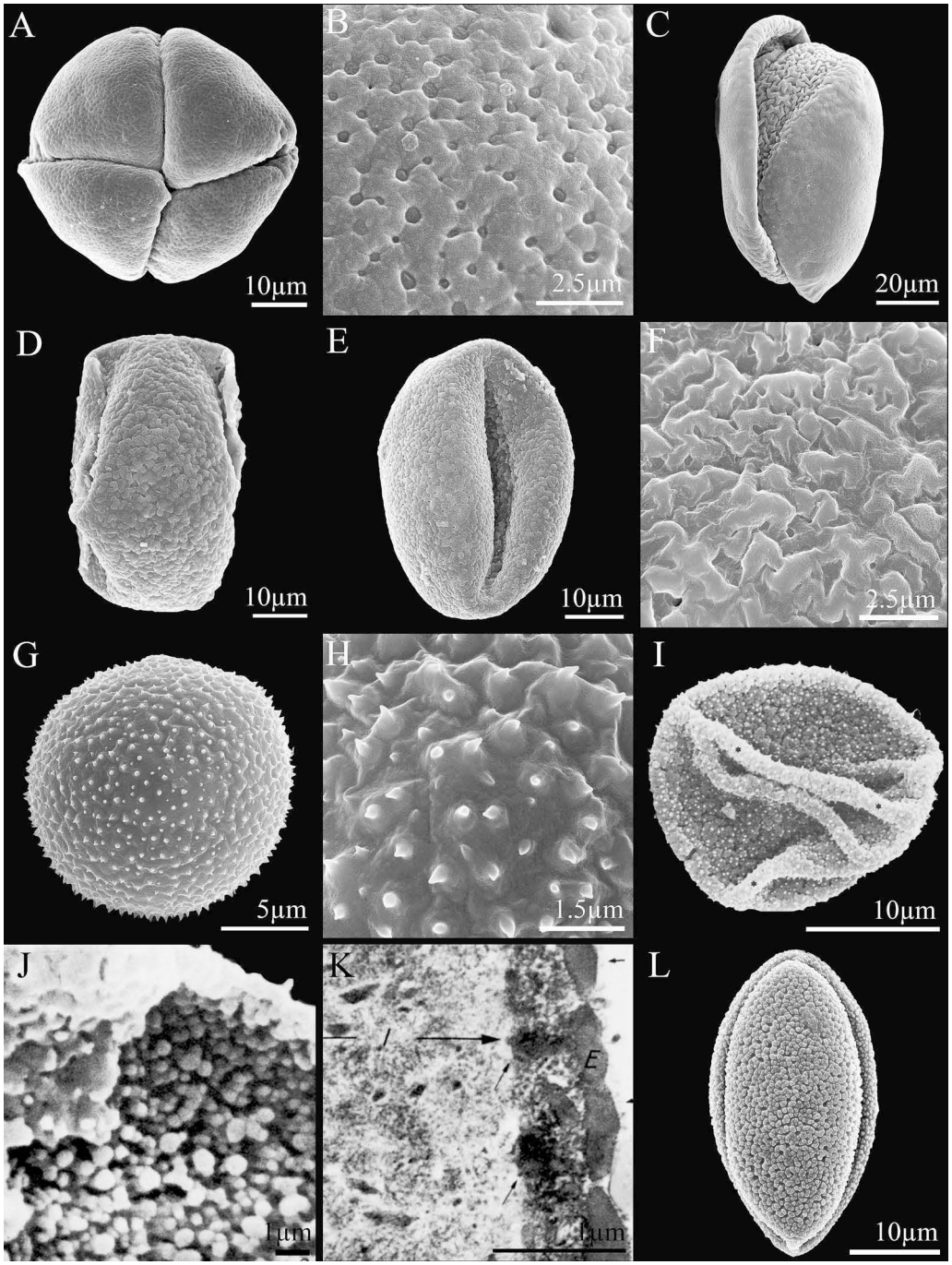


Figure 5. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). A, B. *Annona glabra* L. —A. Tetrad with medium-sized monads. —B. Detail of perforate tectum. —C. *Cananga odorata* (Lam.) Hook. f. & Thomson var. *fruticosa* (Craib) J. Sinclair, equatorial view, showing a monad with zonate aperture. D–F. *Calycanthus chinensis* (W. C. Cheng & S. Y. Chang) P. T. Li. —D. Equatorial view, showing bi-aperturate grain, apertures located at the equator. —E. Equatorial view, showing a colpus. —F. Detail of rugulate tectum. G, H. *Siparuna guianensis* Aubl. —G. Spheroidal, inaperturate pollen grain. —H. Detail of echinate supratectal elements. I–K. *Gomortega keule* (Molina) Baill., images reprinted with permission from Hesse and Kubitzki (1983). —I. Inaperturate grain. —J. TEM showing collumellate infratectum. —K. TEM showing exine structure: a channeled outer layer of intine (I) and exine (E) that is made up of tectal (short arrows) and columellar elements (longer arrows). —L. *Atherosperma moschatum* Labill., equatorial view, showing two colpi located at distal and proximal poles.

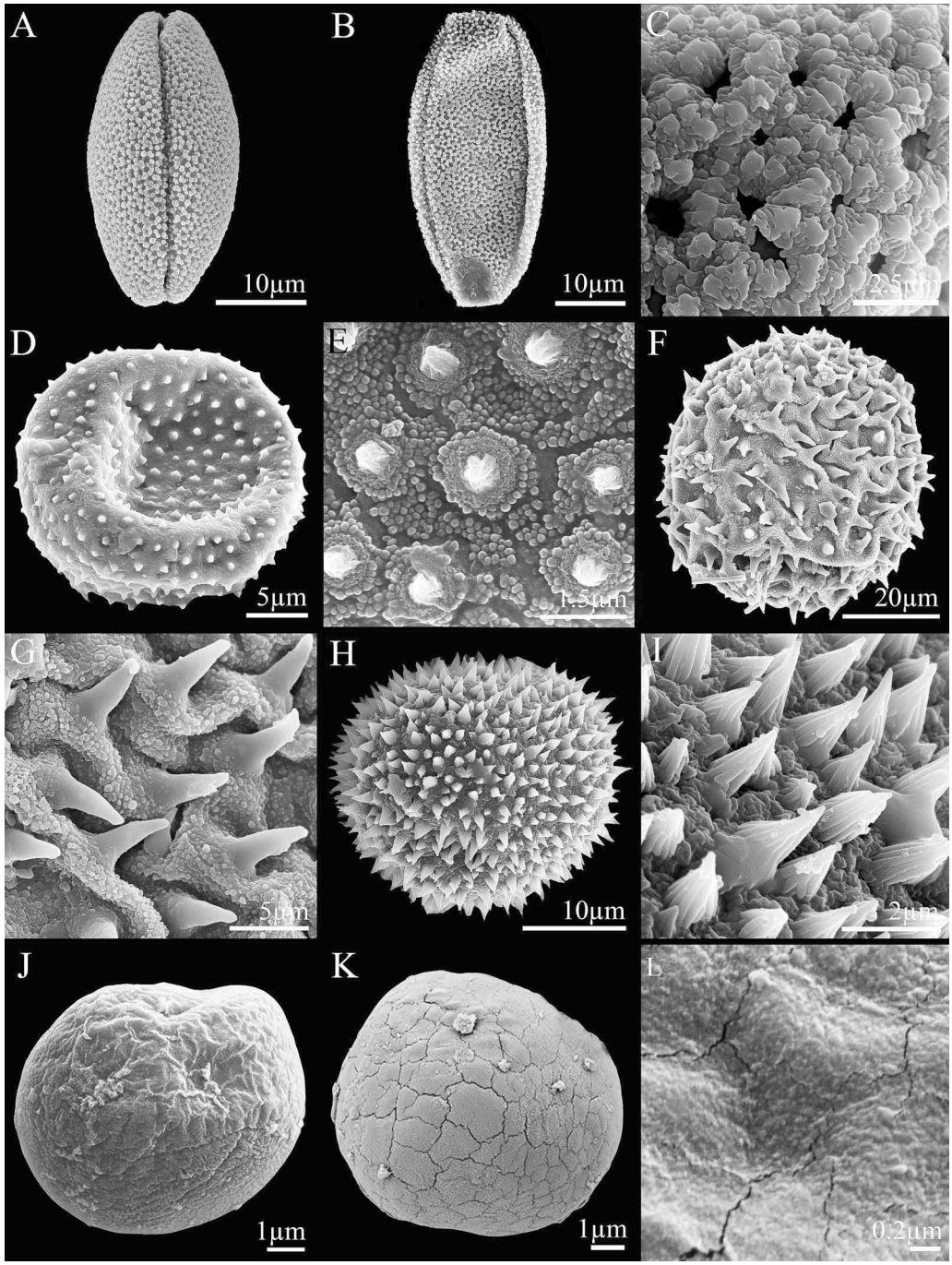


Figure 6. SEMs of pollen of the basal angiosperms, with arrangement following the topology of Soltis et al. (2011). —A. *Atherosperma moschatum* Labill., polar view, showing a colpus. —B. *Doryphora sassafras* Endl., equatorial view, showing two colpi located at distal and proximal poles. —C. *Daphnandra tenuipes* G. Perkins, detail of rudimentary tectum with verrucate supratectal elements. D, E. *Laurus nobilis* L. —D. Inaperturate pollen grain. —E. Detail of echinate and verrucate supratectal elements. F, G. *Hernandia didymantha* Donn. Sm. —F. Showing spheroidal and inaperturate pollen grain. —G. Detail of echinate and verrucate supratectal elements. H, I. *Peumus boldus* Molina. —H. Showing spheroidal and inaperturate pollen grain. —I. Detail of echinate supratectal elements. J–L. *Ceratophyllum* L., sp. indet. —J, K. Spheroidal and inaperture pollen grain. —L. Detail of exine surface (exine reduced to a very thin layer).

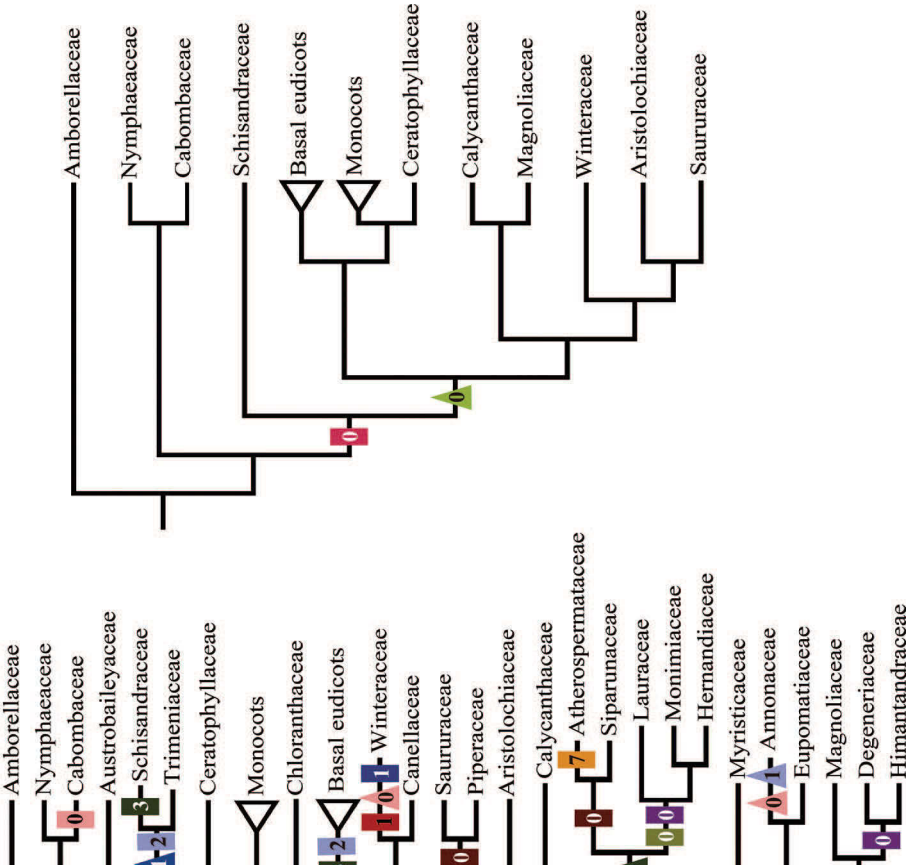
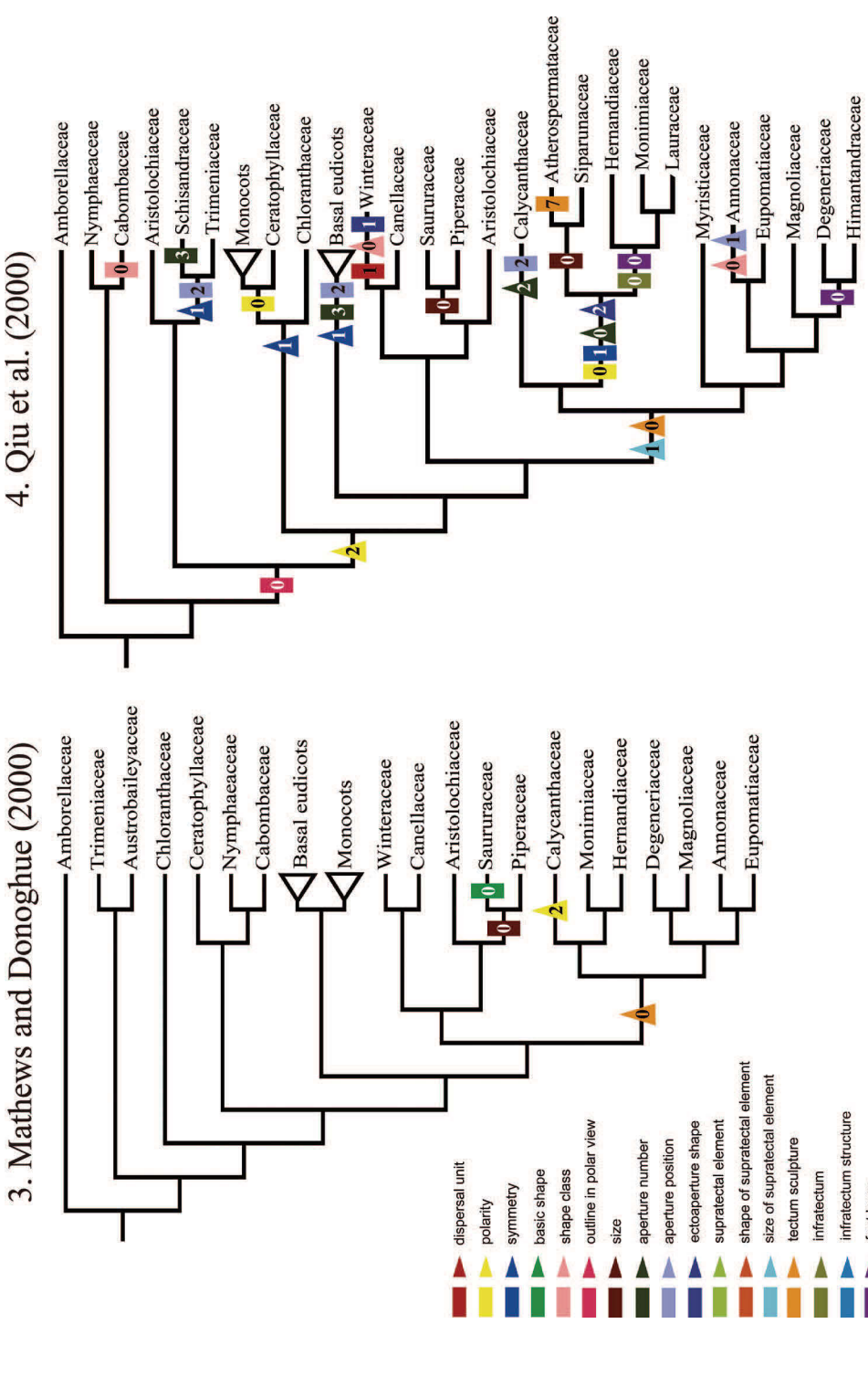


Figure 7. Synapomorphies and likely synapomorphies inferred from a comprehensive dataset of pollen characters analyzed with Fitch parsimony, labeled on the branches of a range of phylogenetic topologies for basal angiosperms. Topologies in this figure are from Qiu et al. (1999), Graham and Olmstead (2000), Mathews and Donoghue (2000), and Qiu et al. (2000). Synapomorphies are shown as rectangles and likely synapomorphies as triangles, with shape color indicating pollen characters and the number within indicating character state change, following Appendix 2. As shown in Appendix 2, pollen character 10, aperature membrane, is not shown because no synapomorphies were found for this character.



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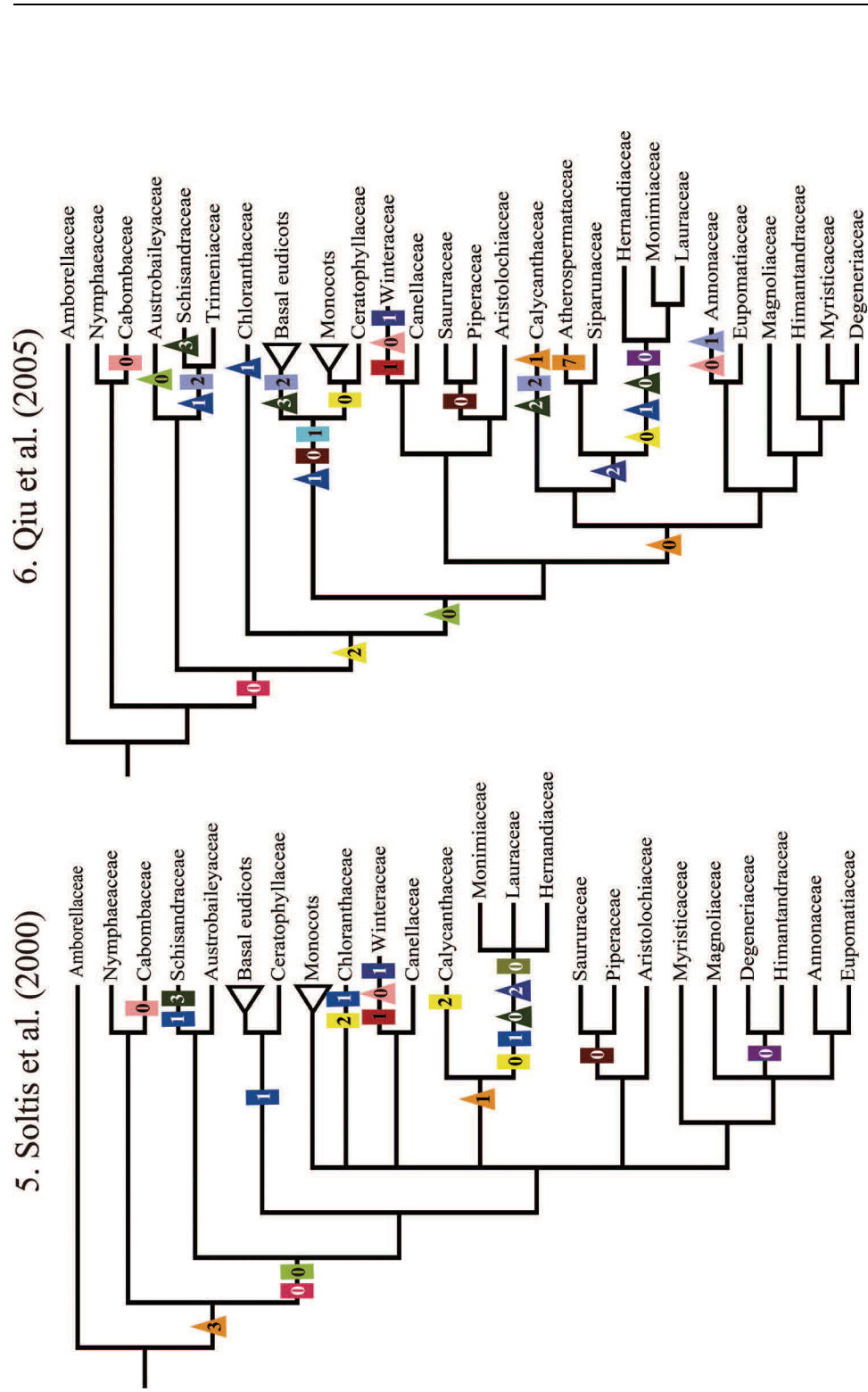

Figure 8. Synapomorphies and likely synapomorphies inferred from a comprehensive dataset of pollen characters analyzed with Fitch parsimony, labeled on the branches of a range of phylogenetic topologies for basal angiosperms. Topologies in this figure are from Soltis et al. (2000), Qiu et al. (2005), Müller et al. (2006), and Qiu et al. (2006, three genes). Synapomorphies are shown as rectangles and likely synapomorphies as triangles, with shape color indicating pollen characters and the number within indicating character state change, following Appendix 2. As shown in Appendix 2, pollen character 10, aperature membrane, is not shown because no synapomorphies were found for this character.

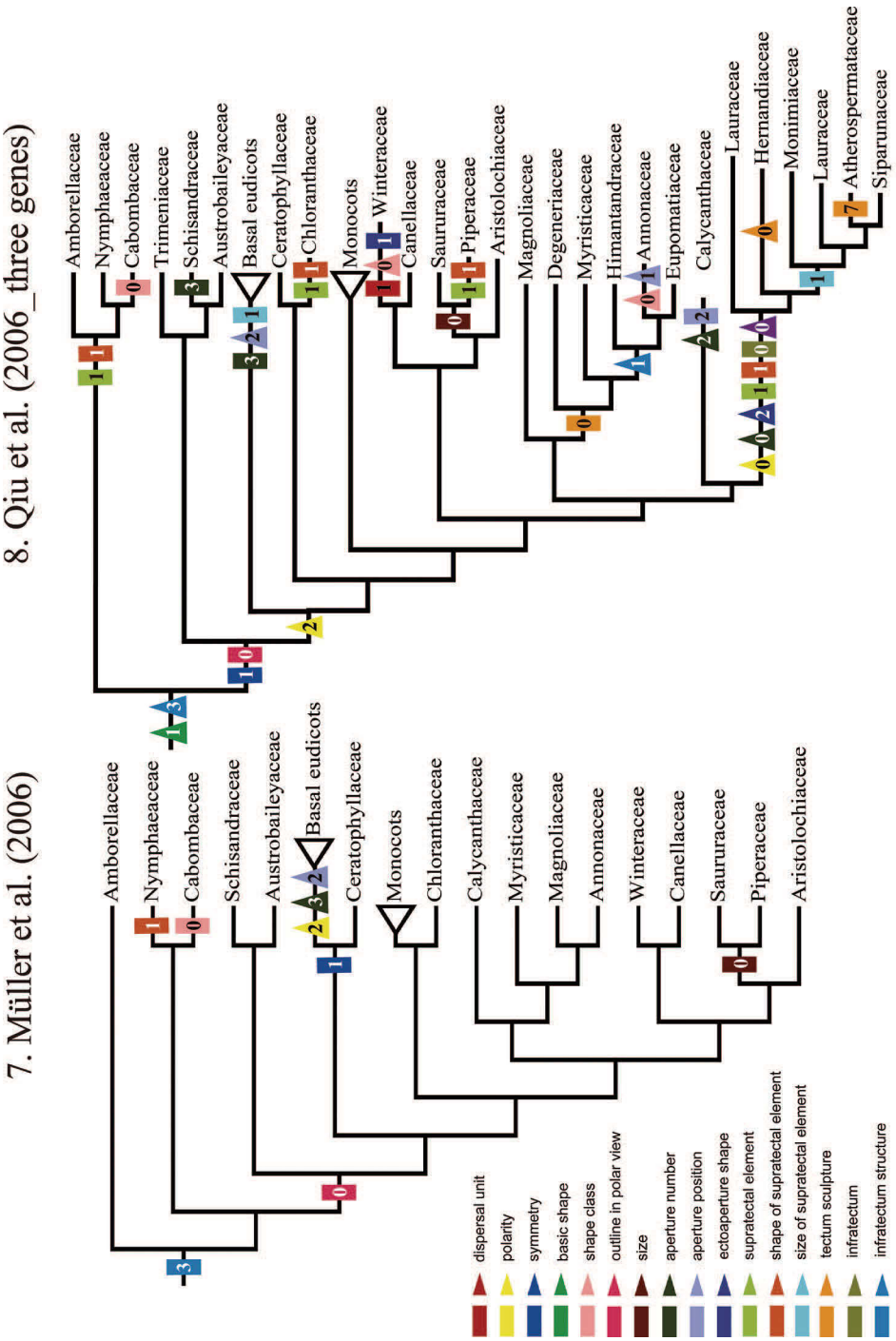

Figure 8. Continued.

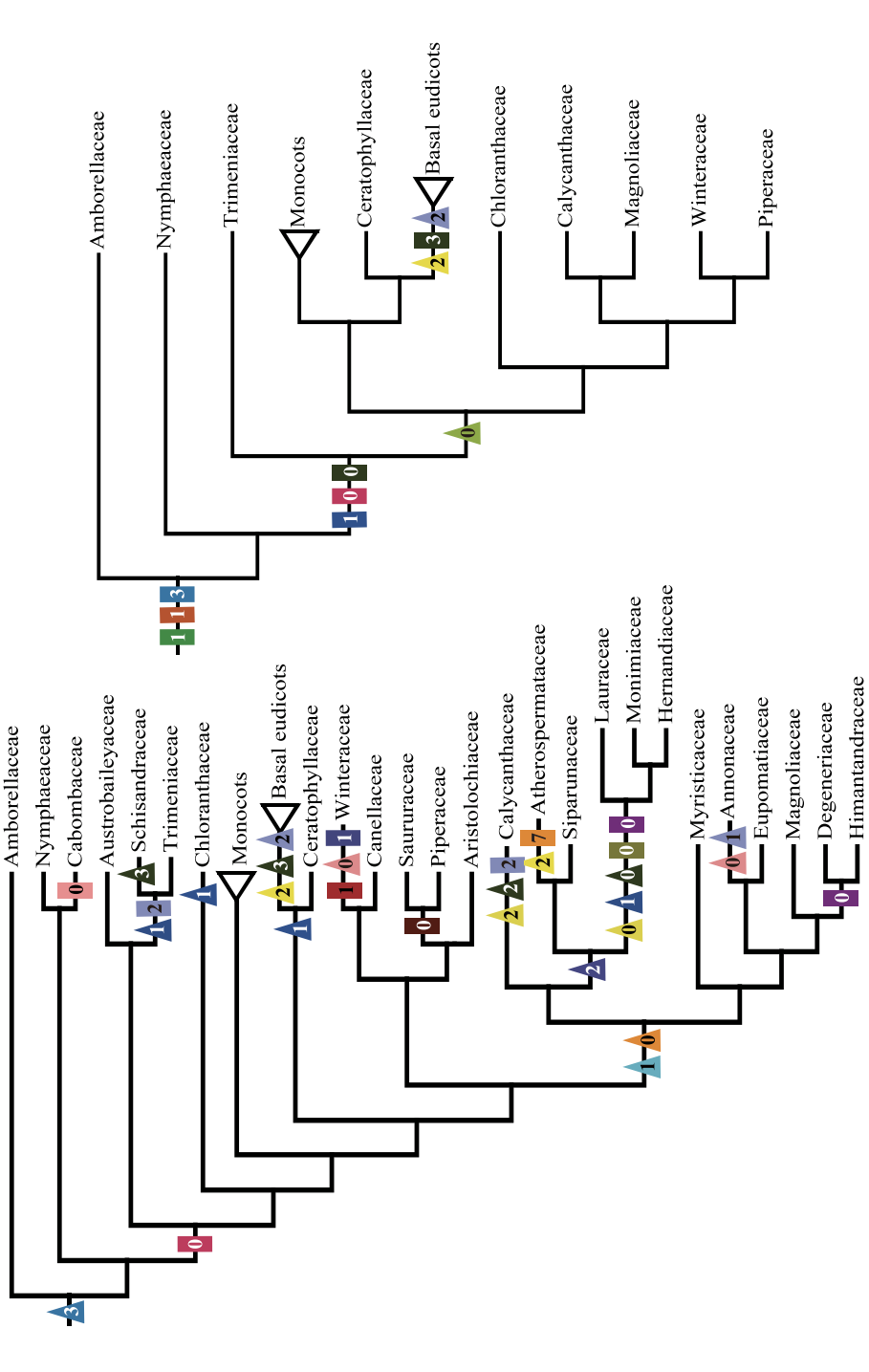

Figure 9. Synapomorphies and likely synapomorphies inferred from a comprehensive dataset of pollen characters analyzed with Fitch parsimony, labeled on the branches of a range of phylogenetic topologies for basal angiosperms. Topologies in this figure are from Qiu et al. (2006, eight genes), a modified combination of Jansen et al. (2007) and Moore et al. (2007), Soltis et al. (2007), and Soltis et al. (2011). Synapomorphies are shown as rectangles and likely synapomorphies as triangles, with shape color indicating pollen characters and the number within indicating character state change, following Appendix 2. As shown in Appendix 2, pollen character 10, aperture membrane, is not shown because no synapomorphies were found for this character.

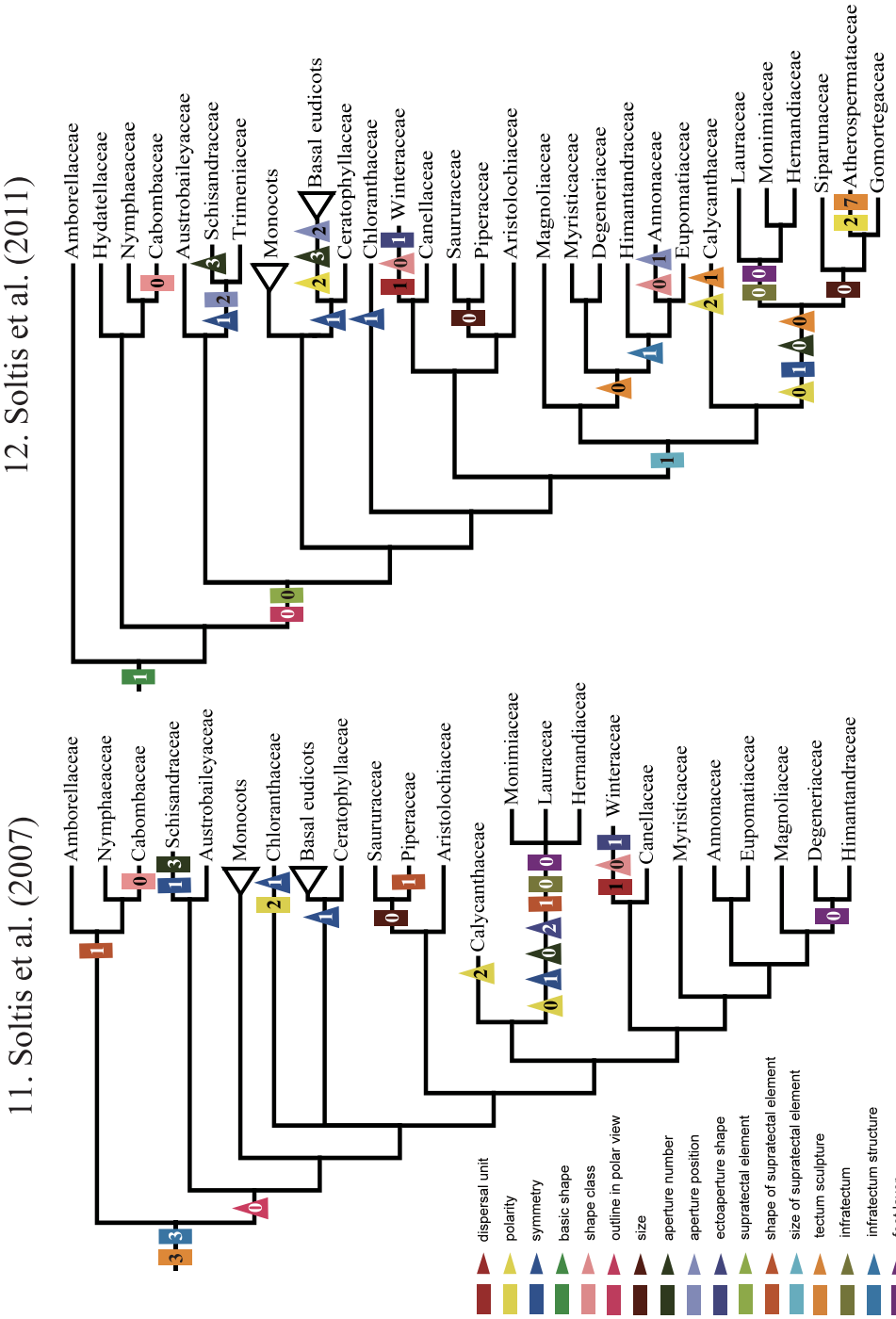

Figure 9. Continued.

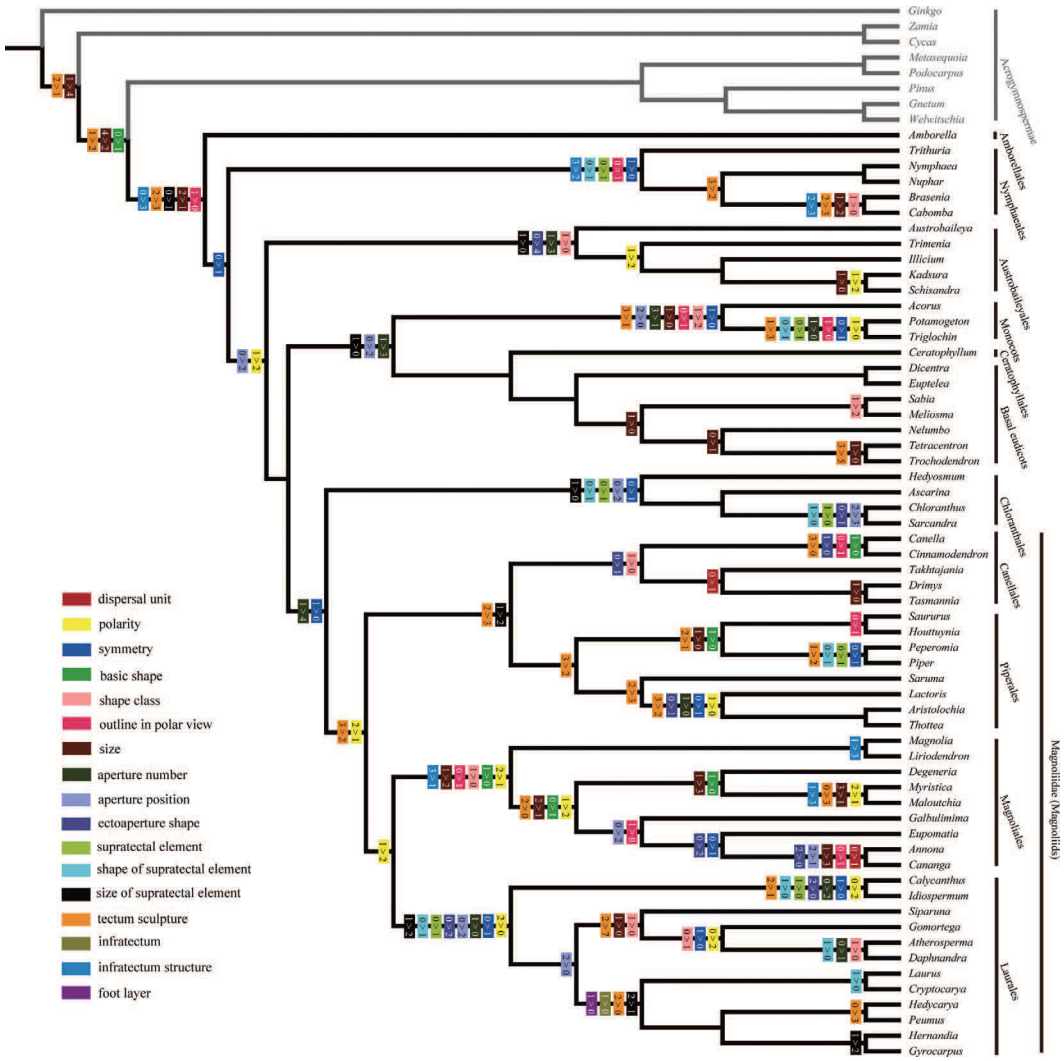

Figure 10. Inferences of unambiguous character state changes for the 18 pollen characters analyzed in this study, reconstructed using hierarchical Bayesian inference, with democratic coding method on a maximum likelihood (DML) phylogeny generated based on the molecular data of Soltis et al. (2011). Changes are mapped assuming a single transition between differing states at adjacent nodes. Pollen characters and the numbers within indicate character state changes, following Appendix 2. As shown in Appendix 2, pollen character 10, aperature membrane, is not shown because no changes were found for this character.

The new tree was reconstructed using maximum likelihood method (ML) with RAxML (Stamatakis, 2006). A bootstrap analysis (1000 replicates) was performed simultaneously with the ML analysis (option, -f a) under the GTRGAMMA model. Because the topology of the generated tree differed slightly from that of Soltis et al. (2011), we used constraints to ensure a match to Soltis et al.

POLLEN CHARACTERS AND CODING STRATEGY

Pollen morphological data were taken from the literature (mostly SEM and transmission electron microscopy [TEM] data), from websites, such as PalDat – Palynological Database, an Online Publication on fossil and recent Pollen and Spores (<http://www.paldat.org/>), and from the new observations presented here. Literature-based data were extracted directly from published images and measurements rather than descriptions to avoid discrepancies due to misinterpretation and conflicting terminology. The terminology used here follows that of Wortley et al. (2015) and Punt et al. (2007). Eighteen pollen characters were scored (cf. Appendix 2 for the coded character states): dispersal unit,

polarity, symmetry (in polar view), basic shape (in equatorial view), shape class, outline in polar view (amb), size (diameter of largest axis), aperture number, aperture position, aperture membrane, ectoaperture shape, supratectal element presence, shape of supratectal elements, size of supratectal elements, tectum sculpture, infratectum presence, infratectum structure, and foot layer presence. These cover all the variable and heritable characters commonly noted in LM, SEM, and TEM observations in previous and present studies of basal angiosperms. Coding strategy followed that of Wortley et al. (2015), with the exception of supratectal elements, for which a size division was defined at 0.5 μm in this study, because this minimized instances of polymorphic data points for our taxa more effectively than a 1-μm boundary. Characters were treated as unordered, binary or multistate characters, with inapplicable states coded as "-" and unknown data as "?". We generated both comprehensive (including polymorphic states; 78 genera of basal angiosperms, gymnosperms, monocots, and basal eudicots from all 12 studied topologies) and democratic (removing polymorphic states by coding for the most common state; including 68 genera of basal angiosperms, gymnosperms, monocots, and basal eudicots from the topology of Soltis et al. [2011]) matrices according to Wortley et al. (2015). In coding democratic states for size of supratectal element, those with both size $<$ 0.5 μm and $\geq$ 0.5 μm were evenly distributed on a grain as a state. Data matrices are shown in Tables 1 and 2.

METHODS FOR ANALYZING CHARACTER EVOLUTION

We used two strategies for optimization of pollen characters to infer ancestral states and synapomorphic traits, and possible evolutionary history: (1) analysis of the comprehensive dataset with Fitch parsimony on multiple phylogenetic estimates (hereafter abbreviated as CFP), and (2) analysis of the democratic dataset with Fitch parsimony, maximum likelihood, and hierarchical Bayesian inference (hereafter abbreviated as DFP, DML, and DHB, respectively).

Because phylogenetic estimates for the basal angiosperms remain controversial, the first strategy optimized the comprehensive dataset using Fitch parsimony (CFP) onto 12 trees with alternative positions of the major early-diverging lineages, using Mesquite 2.75 (Maddison & Maddison, 2011).

Because the above topologies were branch-length free, they could only be analyzed by non-model-based methods (i.e., parsimony). In order to perform model-based methods (ML and Bayesian methods), we also optimized pollen data for the 18 characters upon the new ML tree of the 64 taxa based on the data of Soltis et al. (2011). Because ML methods are presently not applicable to datasets including polymorphic data points, these analyses were conduced on a democratic matrix. Characters were then optimized, using Fitch parsimony (FP), maximum likelihood (ML), and Bayesian inference (HB) on this ML tree. FP and ML optimizations (using the Mk-1 model) were conducted in Mesquite 2.75; HB optimization was performed by using BayesMultistate in Bayestraits 1.0 (available from <http://www.evolution.rdg.ac.uk>) and implementing the reversible-jump hyperprior method. An exponential prior distribution was found to be most appropriate for all characters (seeded from a uniform 0–10 distribution, 0–30 distribution, or 0–40 distribution depending on the character). We used rate variations ranging from nine to 300 (set to obtain the acceptance value between 20% and 40%), a sampling frequency of 300 generations, and a burn-in period of 10,000 generations for a total of 5,000,000 generations. For ML and HB analyses, we took the state with the highest likelihood or probability at each node to represent the state of this node in phylogenetic reconstructions. If the likelihood or probability of the most likely state was found to be $\geq$ 95%, this state was considered to be strongly indicated as the likely reconstructed state for the node.

In this study we highlight both synapomorphies and likely synapomorphies. A likely synapomorphy is defined as a derived character state shared by all included taxa and their most recent common ancestor, in instances where the next node subtending the node of their most recent common ancestor underwent an ambiguous change. In such instances, this character state could be reconstructed as both a synapomorphy for the clade comprising these taxa, or for a larger clade that comprised these taxa and all their sister groups under the Most Parsimonious Reconstruction (MPR) mode implemented in Mesquite 2.75 (Lu et al., 2009, 2010).

RESULTS

PALYNOLOGICAL OBSERVATIONS

Pollen morphology of 34 species of 31 genera in 23 families observed under LM and SEM were described below and presented to show all 18 diverse pollen characters and their states in the basal angiosperms. Pollen SEM images from previous literature of three species, i.e., *Degeneria vitiensis* I. W. Bailey & A. C. Sm., *Gomortega keule* (Molina) Baill., and *Lactoris fernandeziana* Phil. (see below), for which pollen

Table 1. Comprehensive data matrix of pollen morphological characters for basal angiosperms (see text for details).

Acorus L.	0101210101000-1131
Akebia Decne.	0211200321000-{1,3}131
Amborella Baill.	0{0,1}{0,1}1{0,1}1{0,1}{0,1}01{0,1}1106131
Anemopsis Hook. & Arn.	0100-00101000-{1,2}131
Annona L.	{0,1}10{0,1}01{1,2,3}{0,1}1-{0,1}00-{1,2,3}131
Aristolochia L.	0{0,1}11{0,1}{0,1}{1,2}{0,1}0?{0,1}00-{0,1,2,3}131
Asarum L.	0211{0,1}0{1,2}{0,2,3,4}21{0,1,2}101{1,2}13{0,1}
Ascarina J. R. Forst. & G. Forst.	0111{0,1}0{0,1}10101103131
Atherosperma Labill.	0201{0,1}{0,1}{0,1}{1,2}{0,1}1{0,2}10{0,1}7131
Austrobaileya C. T. White	0101101101000-{2,3}131
Brasenia Schreb.	0101011101011031{2,3}1
Cabomba Aubl.	010101{1,2}101010-1131
Calycanthus L.	020{0,1}10{1,2}{1,2}{0,2}1000-{1,2,3}131
Cananga (DC.) Hook. f. & Thomson	1211013{0,1}{1,2}1{0,1,2}00–0111
Canella P. Browne	{0,1}100-{0,1}1101000-{0,1,3}131
Ceratophyllum L.	0011{1,2}0{0,1}0—00-00-0
Chimonanthus Lindl.	0201{0,1}11221000-{1,2,3}131
Chloranthus Sw.	021110{0,1}421{0,1}1103131
Cinnamodendron Endl.	0100-{0,1}1101000-0131
Cinnamomum Schaeff.	001110{0,1,2}0—11{0,1}10-0
Cissampelos L.	0211{0,1}00320000-3131
Cryptocarya R. Br.	0011{0,1}{0,1}10—{0,1}01{0,2}0-0
Cycas L.	0100-{0,1}{1,2}101000-{0,1,3}101
Daphnandra Benth.	0201{0,1}{0,1}{0,1}1{0,1}1{0,2}10{0,1}7131
Degeneria I. W. Bailey & A. C. Sm.	020{0,1}112101000-01{1,2}0
Dicentra Bernh.	0211{1,2}0{0,1}{3,4}21{0,4}00-{1,3}131
Doryphora Endl.	0201{0,1}{0,1}{0,1}{1,2}{0,1}1{0,2}10{0,1}7131
Drimys J. R. Forst. & G. Forst.	110100{0,1}101100-3131
Eupomatia R. Br.	0211{0,1}01121200-01{1,2}0
Euptelea Siebold & Zucc.	0211{0,1,2}01{3,4}{2,3}1000–3131
Galbulimima F. M. Bailey	0001101101000-{0,2}1{1,2}0
Ginkgo L.	010{0,1}1{0,1}1101000-2101
Gnetum L.	00111{0,1}00—11{0,1}2110
Gomortega Ruiz & Pav.	00111000—1102131
Gyrocarpus Jacq.	00111010—{0,1}1{0,1}00-0
Hedycarya J. R. Forst & G. Forst.	{0,1}{0,1}{0,1}1{0,1}{0,1}{0,1}{0,1}012110{1,2}0-0
Hedyosmum Sw.	0211101{1,4}{0,2}101103131
Hernandia L.	00111020—11{0,1}00-0
Hortonia Wight & Arn.	021110{0,1}0—11050-0
Houttuynia Thunb.	0100-00101000-{1,2}131
Idiospermum S. T. Blake	0201{0,1}1122-000-1??1
Illicium L.	0211{0,1}01321{0,4}00–3131
Kadsura Juss.	0111{0,1}0{0,1}3{0,2}1{0,4}00–3131
Knema Lour.	010{0,1}11{1,2}101{0,1}00-{2,3}131
Lactoris Phil.	1101{0,1}{0,1}0101{0,1}00–21{1,3}1
Laurus L.	001110{0,1}0—11{0,1}00-0
Liriodendron L.	0100-1{1,2}101000-{1,2,3}131
Magnolia L.	0100-1{1,2}1010{0,1}01{1,2}1{1,2,3}1
Mahonia Nutt.	00–110{1,2}{3,4}31{0,3,4}00-{1,2,3}131
Maloutchia Warb.	010{0,1}11{0,1,2}101{0,1}111{1,2}1{1,2,3}1
Meliosma Blume	0211{1,2}00321000-{1,3}131
Metasequoia Hu & W. C. Cheng	010110{0,1}0—00–110
Myristica Gronov.	010{0,1}11{1,2}101000-{2,3}131
Nelumbo Adans.	0{1,2}1110{1,2}{1,2,3,4}{0,2}1{0,2}00-{2,3}131
Nuphar Sm.	010{0,1}1111010111212{0,1}
Nymphaea L.	0{1,2}{0,1}{0,1}1{0,1}{1,2}1{0,2}{0,1}{0,2}11{0,1}{0,3}1{1,2,3}{0,1}
Peperomia Ruiz & Pav.	0011100{0,1}01{0,1}1104131
Peumus Molina	00111010—11130-0
Pinus L.	0100–121000100{2,3}101

Table 1. Continued.

Piper L.	01{0,1}1{0,1}{0,1}0{0,1}01011{0,1}{0,1,2}131
Podocarpus L'Hér. ex Pers.	0100-{0,1}{1,2}10101102101
Potamogeton L.	0011{0,1}0{0,1}0—1103131
Sabia Colebr.	0211{1,2}00321000-{1,3}131
Sarcandra Gardner	0211{1,2}01{0,4}3?100-3131
Saruma Oliv.	0101{0,1}11{0,1}01000-{0,1,2,3}131
Saururus L.	0100-{0,1}0101000-{1,2}131
Schisandra Michx.	0111{0,1}0{0,1}3{0,2}1{0,4}00–3131
Siparuna Aubl.	0011{0,1}{0,1}00—11101{1,2}1
Takhtajania Baranova & J.-F. Leroy	1101001101100-{1,2,3}131
Tasmannia R. Br. ex DC.	110100{0,1}101100-3131
Tetracentron Oliv.	0211100321000-5131
Thottea Rottb.	0011{0,1}{0,1}{1,2}0—00–113{0,1}
Triglochin L.	001110{0,1}0—110313{0,1}
Trimenia Seem.	0211{1,2}00{0,2,4}{2,3}1{0,1}{0,1}10{1,2,3}131
Trithuria Hook. f.	010{0,1}1{0,1}{0,1}10101101131
Trochodendron Siebold & Zucc.	0211{0,1}00321000-3131
Welwitschia Hook. f	0100-1{1,2}100000-5110
Zamia L.	010{0,1}114101000-1101

samples were unavailable, were permitted to be reprinted in our study (cf. Figs. 1–6).

Amborella trichopoda Baill. (Amborellales, Amborellaceae, Fig. 1A, B) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (oblate to subspheroidal); amb usually elliptic; size small to medium (ca. 15–30 μm); monoporate, aperture located at the distal pole, with sculptured aperture membrane; supratectal elements spinulose; tectum unique, *Amborella*-type.

Trithuria australis (Diels) D. D. Sokoloff., Remizowa, T. D. Macfarl. & Rudall (Nymphaeales, Hydatellaceae, Fig. 1C) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped or globose (spheroidal); amb elliptic to circular; size small to medium (ca. 12–27 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; supratectal elements spinulate; tectum perforate.

Nymphaea alba L. (Nymphaeales, Nymphaeaceae, Fig. 1D, E) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (oblate to spheroidal); amb elliptic to circular; size small to medium (ca. 23–32 μm); zonate, aperture located at the distal pole, with sculptured aperture membrane; supratectal elements spinulate and verrucate; tectum imperforate.

Nuphar luteum Walp. (Nymphaeales, Nymphaeaceae, Fig. 1F) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (oblate to subspheroidal); amb usually elliptic; size medium (ca. 35–42 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; supratectal elements spinulate and verrucate; tectum rugulate.

Austrobaileya scandens C. T. White (Austrobaileyales, Austrobaileyaceae, Fig. 1G–I) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (spheroidal); amb usually circular; size medium (ca. 38–45 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum reticulate.

Trimenia moorei (Oliv.) Philipson (Austrobaileyales, Trimeniaceae, Fig. 1J–L) has pollen dispersed as monads, isopolar or subisopolar, radially symmetrical; globose (prolate); amb circular; size small (ca. 10–15 μm); inaperturate or bi-colpate, apertures located at the equator, with sculptured aperture membrane; supratectal elements spinulose; tectum reticulate.

Illicium micranthum Dunn (Austrobaileyales, Schisandraceae, Fig. 2A, B) has pollen dispersed as monads, isopolar, radially symmetrical; globose (oblate); amb circular; size medium (ca. 45–50 μm); trisyncolpate, apertures centered at the equator, with sculptured aperture membrane; tectum reticulate.

Illicium macranthum A. C. Sm. (Austrobaileyales, Schisandraceae, Fig. 2C) has pollen dispersed as monads, isopolar, radially symmetrical; globose (oblate); amb circular; size medium (ca. 25–30 μm); trisyncolpate, apertures located at the equator, with sculptured aperture membrane; tectum reticulate.

Chloranthus japonicus Siebold (Chloranthales, Chloranthaceae, Fig. 2D) has pollen dispersed as monads, isopolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 15–20 μm); hexa-colpate, apertures located at the equator,

Table 2. Democratic data matrix of pollen morphological characters for basal angiosperms (see text and Table 2 for details).

Acorus L.	0101210101000-1131
Amborella Baill.	010101010111106131
Annona L.	110101211-000-2131
Aristolochia L.	001110100?-00-2131
Ascarina J. R. Forst. & G. Forst.	011110010101103131
Atherosperma Labill.	02010001–121027131
Austrobaileya C. T. White	0101101101000-2131
Brasenia Schreb.	010101110101103131
Cabomba Aubl.	0101012101010-1131
Calycanthus L.	0201101221000-1131
Cananga (DC.) Hook. f. & Thomson	121101311-000-0111
Canella P. Browne	0100-11101000-3131
Ceratophyllum L.	00111010—00-00-0
Chloranthus Sw.	021110142101103131
Cinnamodendron Endl.	0100-11101000-0131
Cryptocarya R. Br.	00111010—10120-0
Cycas L.	0100-11101000-1101
Daphnandra Benth.	02010001–121027131
Degeneria I. W. Bailey & A. C. Sm.	0200-12101000-0110
Dicentra Bernh.	0211100321000-3131
Drimys J. R. Forst. & G. Forst.	1101000101100-3131
Eupomatia R. Br.	0211001121200-0110
Euptelea Siebold & Zucc.	0211101321000-3131
Galbulimima F. M. Bailey	00011101101000-2110
Ginkgo L.	0100-11101000-2101
Gnetum L.	00111100—1122110
Gomortega Ruiz & Paz.	00111000—1102131
Gyrocarpus Jacq.	00111010—11200-0
Hedycarya J. R. Forst & G. Forst.	00111000011211010-0
Hedyosmum Sw.	021110142101103131
Hernandia L.	00111020—11200-0
Houttuynia Thunb.	0100-00101000-1131
Idiospermum S. T. Blake	020111122-000-1??1
Illicium L.	0211001321400-3131
Kadsura Juss.	0111000321400-3131
Lactoris Phil.	1101100101100-2111
Laurus L.	00111010—11200-0
Liriodendron L.	0100-12101000-2131
Magnolia L.	0100–1210101011131
Maloutchia Warb.	0100–1110111112121
Meliosma Blume	0211200321000-3131
Metasequoia Hu & W. C. Cheng	01011010—00–110
Myristica Gronov.	0100-11101000-3131
Nelumbo Adans.	0211101321000-3131
Nuphar Sm.	010111110101112121
Nymphaea L.	020110110021123121
Peperomia Ruiz & Pav.	0011100001-1104131
Peumus Molina	00111010—11130-0
Pinus L.	0100–1210001002101
Piper L.	011110010101122131
Podocarpus L'Hér. ex Pers.	0100–1210101102101
Potamogeton L.	00111000—1103131
Sabia Colebr.	0211200321000-3131
Sarcandra Gardner	021110143?100-3131
Saruma Oliv.	0101111101000-3131
Saururus L.	0100-10101000-1131
Schisandra Michx.	0111000321400-3131
Siparuna Aubl.	00110000—1110121
Takhtajania Baranova & J.-F. Leroy	1101001101100-3131
Tasmannia R. Br. ex DC.	1101000101100-3131
Tetracentron Oliv.	0211100321000-5131
Thottea Rottb.	00111010—00–1131
Triglochin L.	00111000—1103131
Trimenia Seem.	021120022111102131
Trithuria Hook. f.	010110010101101131
Trochodendron Siebold & Zucc.	0211100321000-3131
Welwitschia Hook. f	0100-12100000-5110
Zamia L.	0100114101000-1101

with sculptured aperture membrane; tectum reticulate.

Chloranthus holostegius (Hand.-Mazz.) S. J. Pei & Shan (Chloranthales, Chloranthaceae, Fig. 2E, F) has pollen dispersed as monads, isopolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 13–16 μm); hexa-colpate, apertures located at the equator, with sculptured aperture membrane; tectum reticulate.

Canella winterana (L.) Gaertn. (Canellales, Canellaceae, Fig. 2G–I) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped or globose (oblate); amb elliptic; size medium (ca. 28–33 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum perforate.

Drimys piperita Hook. f. (Canellales, Winteraceae, Fig. 2J–L) has pollen dispersed as tetrads, radially symmetrical, globose (spheroidal), size of each grain small to medium (ca. 22–30 μm); the individual grains each heteropolar, bilaterally symmetrical; globose (oblate); amb circular; monoporate, the aperture located at the distal pole, with sculptured aperture membrane; tectum reticulate.

Hydnora abyssinica A. Braun (Piperales, Hydnoraceae, Fig. 3A) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (oblate to subspheroidal); amb circular; size small (ca. 20–25 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; supratectal elements verrucate; tectum rugulate.

Lactoris fernandeziana (Piperales, Aristolochiaceae, Fig. 3B–D), reprinted SEM images from Zavada and Taylor (1986), has pollen dispersed as tetrads, radially symmetrical and globose (spheroidal), the size of each grain small (ca. 15 μm); the individual grains heteropolar and bilaterally symmetrical; globose (oblate); amb elliptic or circular; monoporate or monocolpate, aperture located at the distal pole, with

sculptured aperture membrane; tectum microrugulate.

Aristolochia contorta Bunge (Piperales, Aristolochiaceae, Fig. 3E, F) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (spheroidal); amb circular; size medium (ca. 27–30 μm); monoporate, aperture located at the distal pole, with sculptured aperture membrane; tectum rugulate.

Peperomia heyneana Miq. (Piperales, Piperaceae, Fig. 3G) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 5–8 μm); inaperturate; supratectal elements spinulose; tectum areolate.

Saururus chinensis (Lour.) Baill. (Piperales, Saururaceae, Fig. 3H) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped; amb elliptic; size small (ca. 10–15 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum perforate.

Houttuynia cordata Thunb. (Piperales, Saururaceae, Fig. 3I) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped; amb elliptic; size small (ca. 18–20 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum perforate.

Liriodendron tulipifera L. (Magnoliales, Magnoliaceae, Fig. 3J) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped; amb elliptic; size large (ca. 50–57 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum perforate to finely reticulate.

Magnolia grandiflora L. (Magnoliales, Magnoliaceae, Fig. 3K, L) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; boat-shaped; amb elliptic; size large (ca. 78–85 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum perforate.

Degeneria vitiensis (Magnoliales, Degeneriaceae, Fig. 4A–C), reprinted SEM images from Sampson (2000a), has pollen dispersed as monads, isopolar, bilaterally symmetrical; boat-shaped or globose (subspheroidal); amb elliptic; size large (ca. 50–60μm); monocolpate, aperture located at the distal pole, sometimes nearly encircling the grain, with sculptured aperture membrane; tectum imperforate.

Knema globularia (Lam.) Warb. (Magnoliales, Myristicaceae, Fig. 4D, E) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (subspheroidal); amb elliptic; size medium (ca. 25–28 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum reticulate.

Myristica Gronov., sp. indet. (Magnoliales, Myristicaceae, Fig. 4F) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (subspheroidal); amb elliptic; size medium (ca. 25–30 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum rugulate.

Galbulimima baccata F. M. Bailey (Magnoliales, Himantandraceae, Fig. 4G, H) has pollen dispersed as monads, heteropolar, bilaterally symmetrical; globose (subspheroidal); amb circular; size medium (ca. 38–43 μm); monocolpate, aperture located at the distal pole, with sculptured aperture membrane; tectum rugulate.

Eupomatia laurina R. Br. (Magnoliales, Eupomatiaceae, Fig. 4I–K) has pollen dispersed as monads, isopolar, radially symmetrical; globose (spheroidal); amb circular; size medium (ca. 43–47 μm); zonate, the aperture located at the equator, with sculptured aperture membrane; tectum imperforate (psilate).

Annona muricata L. (Magnoliales, Annonaceae, Fig. 4L) has pollen dispersed as tetrads, radially symmetrical and globose (spheroidal), size of each grain very large to gigantic (ca. 160–220 μm); the individual grains heteropolar and bilaterally symmetrical; globose (oblate); amb elliptic; monoporate, the aperture located at the proximal pole; tectum reticulate.

Annona glabra L. (Magnoliales, Annonaceae, Fig. 5A, B) has pollen dispersed as tetrads, radially symmetrical and globose (spheroidal), size of each grain medium to large (ca. 45–65 μm); the individual grains heteropolar and bilaterally symmetrical; globose (oblate); amb elliptic; monoporate, the aperture located at the proximal pole; tectum perforate.

Cananga odorata (Lam.) Hook. f. & Thomson var. *fruticosa* (Craib) J. Sinclair (Magnoliales, Annonaceae, Fig. 5C) has pollen dispersed as monads, isopolar, radially symmetrical; globose (oblate); amb elliptic; size very large (ca. 105–110 μm); zonate, the aperture located at the equator, with sculptured aperture membrane; tectum perforate.

Calycanthus chinensis (W. C. Cheng & S. Y. Chang) P. T. Li (Laurales, Calycanthaceae, Fig. 5D–F) has pollen dispersed as monads, isopolar, bilaterally symmetrical; globose (oblate); amb elliptic; size medium to large (ca. 45–52 μm); bi-colpate, apertures located at the equator, with sculptured aperture membrane; tectum rugulate.

Siparuna guianensis Aubl. (Laurales, Siparunaceae, Fig. 5G, H) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 14–16 μm); inaperturate; supratectal elements spinulate; tectum imperforate.

Gomortega keule (Laurales, Gomortegaceae, Fig. 5I–K), reprinted SEM images from Hesse and Kubitzki (1983), has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 23 μm); inaperture; supratectal elements spinulate; tectum rugulate.

Atherosperma moschatum Labill. (Laurales, Atherospermataceae, Figs. 5L, 6A) has pollen dispersed as monads, isopolar, bilaterally symmetrical; globose (oblate); amb elliptic; size medium (ca. 33–37 μm); bi-colpate, apertures located at distal and proximal poles, with sculptured aperture membrane; supratectal elements verrucate; tectum rudimentary.

Doryphora sassafras Endl. (Laurales, Atherospermataceae, Fig. 6B) has pollen dispersed as monads, isopolar, bilaterally symmetrical; globose (oblate); amb elliptic; size medium (ca. 40–44 μm); bi-colpate, apertures located at distal and proximal poles, with sculptured aperture membrane; supratectal elements verrucate; tectum rudimentary.

Daphnandra tenuipes G. Perkins (Laurales, Atherospermataceae, Fig. 6C) has pollen dispersed as monads, isopolar, bilaterally symmterical; globose (oblate); amb elliptic; size medium (ca. 37–40 μm); bi-colpate, apertures located at distal and proximal poles, with sculptured aperture membrane; supratectal elements verrucate; tectum rudimentary.

Laurus nobilis L. (Laurales, Lauraceae, Fig. 6D, E) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size small to medium (ca. 22–25 μm); inaperturate; supratectal elements spinulate and verrucate; tectum imperforate.

Hernandia didymantha Donn. Sm. (Laurales, Hernandiaceae, Fig. 6F, G) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size large (ca. 50–55 μm); inaperturate; supratectal elements spinulate and verrucate; tectum imperforate.

Peumus boldus Molina (Laurales, Monimiaceae, Fig. 6H, I) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size medium (ca. 26–30 μm); inaperturate; supratectal elements spinulate and verrucate; tectum reticulate.

Ceratophyllum,sp. indet. (Ceratophyllales, Ceratophyllaceae, Fig. 6J–L) has pollen dispersed as monads, apolar, radially symmetrical; globose (spheroidal); amb circular; size small (ca. 7–10 μm); inaperturate; exine nearly psilate.

POLLEN DIVERSITY

The pollen grains of basal angiosperms display a number of widely shared characteristics, including dispersal as monads, sculptured apertural membranes (if apertures are present), and an exine that comprises a tectum and infratectum plus a foot layer (except for Laurales). The taxa studied also display high diversity in pollen size, aperture number, aperture position, ectoaperture shape, tectum sculpture, and infratectal structure. Genera such as *Annona* L., *Aristolochia* L., *Asarum* L., *Calycanthus* L., *Hedycarya* J. R. Forst. & G. Forst., *Maloutchia*, *Nymphaea* L., and *Saruma* Oliv. showed greater infrageneric pollen variation than other studied genera, whereas *Drimys* J. R. Forst. & G. Forst., *Houttuynia* Thunb., *Laurus* L., and *Magnolia* L. were more consistent. The level of variation did not seem to depend, at generic level, upon taxonomic diversity, e.g., pollen grains of the medium-sized genus *Nymphaea* (ca. 50 species) were highly variable, whereas those of the large genus *Magnolia* (ca. 210 species) were relatively homogeneous. At family level, certain families, including the Aristolochiaceae, Myristicaceae, and Nymphaeaceae, showed more palynological variation than others such as the Hernandiaceae, Lauraceae, Saururaceae, Schisandraceae, and Winteraceae.

Supratectal elements, in particular, were highly diverse in the basal angiopserms. Based on our observations, five main types of character combinations were present: (1) sparsely large spinulose (with large, pointed, well-separated spines, commonly surrounded by tiny granules; cf. Fig. 6E, G, I); (2) densely large spinulose (large, pointed, closely packed spines, usually with expanded bases; cf. Fig. 5H); (3) small spinulose (small spinules present on a rugulate, reticulate, striate or *Amborella*-type tectum; cf. Fig. 1B, L); (4) verrucate (Fig. 6C); and (5) striate. Most taxa in the Laurales and Nymphaeaceae displayed the sparsely large spinulose type, and in the Piperaceae, the densely large spinulose type. The Amborellales, Chloranthales, and Hydatellaceae were characterized by the small spinulose type, the Atherospermataceae by the verrucate type, and *Cabomba* Aubl. by the striate type.

ANCESTRAL STATE RECONSTRUCTION

Using comprehensive coding with Fitch parsimony (CFP), a total of 216 character reconstructions (18 characters across 12 tree topologies) were conducted (Figs. 7–9). These results imply a number of plesiomorphic states for angiosperms, with several of them occurring on all 12 tree topologies, such as dispersal as monads, the pollen grains heteropolar, spheroidal in shape, monoaperturate, aperture distal, colpate, aperture membrane sculptured, supratectal element size < 0.5 μm, infratectum present, and foot

layer present. A further three character states were reconstructed as unambiguously plesiomorphic for angiosperms in most (10 or 11) topologies: pollen symmetry bilateral, outline in polar view elliptic, and pollen size medium (25–49 μm). Certain characters did not produce an unambiguously reconstructed state at the root of the angiosperms on most topologies: basic pollen shape, supratectal element presence and supratectal element shape, as well as tectum sculpture and infratectum structure.

Seventeen out of the 18 characters studied, the exception being aperture membrane ornamentation, were inferred to provide a synapomorphy or likely synapomorphy for at least one lineage of the basal angiosperms, across the various possible tree topologies, using CFP (Figs. 7–9). Among those characters, pollen polarity, symmetry, aperture number, supratectal element presence, supratectal element size, and tectum sculpture provided synapomorphies or likely synapomorphies for more than five families or higher lineages of basal angiosperms, whereas pollen dispersal unit, basic shape, outline in polar view, ectoaperture shape, supratectal element shape, infratectum, and infratectum structure provided synapomorphies or likely synapomorphies for only one or two evaluated families or higher lineages. Based on these analyses, 36 families or higher lineages could be defined by synapomorphies or likely synapomorphies in pollen characters. The following groups were defined by at least three pollen synapomorphies or likely synapomorphies in our sample: (1) angiosperms; (2) Atherospermataceae; (3) the clade comprising the Austrobaileyales and all later-diverging angiosperms; (4) basal eudicots; (5) Calycanthaceae; (6) Chloranthales; (7) the clade comprising Hernandiaceae, Lauraceae, and Monimiaceae; (8) the clade of Laurales with Calycanthaceae excluded; (9) the clade comprising Laurales and Magnoliales; and (10) Winteraceae. The clade comprising the Hernandiaceae, Lauraceae, and Monimiaceae was defined by at least five pollen synapmorphies or likely synapomorphies, and the clade of Laurales with Calycanthaceae excluded was defined by nine. Other lineages, such as the Austrobaileyales, Cabombaceae, Laurales, Nymphaeaceae, and Saururaceae, displayed only one palynological synapomorphy or likely synapomorphy. Across the 12 studied topologies (cf. Figs. 7–9 herein), that of Qiu et al. (2006, three genes) was reconstructed with 22 synapomorphies plus 14 likely synapomorphies (36 in total) for related taxa. The topologies from Qiu et al. (1999, 2000, 2005, 2006, eight genes) and Soltis et al. (2000, 2007, 2011) were reconstructed with a total of 20 to 32 synapomorphies plus likely synapomorphies. The trees from Graham and Olmstead (2000); Mathews and Donoghue (2000); Müller et al. (2006); and Jansen et al. (2007) and Moore et al. (2007) displayed only a few synapomorphies or likely synapomorphies (two, four, nine, and 10, respectively) due to the lower number of taxa, and therefore nodes, in these trees.

Fourteen of the 18 pollen characters also displayed unambiguous plesiomorphic states for the root of the angiosperms with democratic coding under Fitch parsimony (DFP), maximum likelihood (DML), and hierarchical Bayesian optimization (DHB). These reconstructed plesiomorphic states were simlar to the CFP analysis, with the exception of supratectal element size (< 0.5 μm) and pollen size medium (25–49 μm), as well as supratectal elements verrucate, the tectum sculputure rugulate, and the infratectum structure alveolate. Unambiguous or probable plesiomorphic states for the other four characters were not consistently reconstructed across the three methods. For instance, basic shape was reconstructed as boat-shaped using DFP and DML, but as globose using DHB. Ancestral pollen size was recovered as medium using DML, but large using DHB, and ambiguous under DFP. Supratectal elements were reconstructed as present using DFP, absent under DML, and ambiguous under DHB, and the size of supratectal elements was < 0.5 μm, using DFP and DHB, but ambiguous under DML.

We compared the results of democratic Fitch parsimony (DFP), democratic maximum likelihood (DML), and democratic Bayesian inference (DHB) to examine differences in character evolutionary patterns inferred by the three methods (using the most probable state found at each node in DML and DHB). Among the 18 characters studied, only dispersal unit evolved unequivocally at all nodes with all three methods. Pollen polarity, symmetry, basic shape, outline in polar view, aperture number, shape of supratectal element, and foot layer displayed a number of nodes with equivocal changes in DFP; pollen infratectum only in DML; pollen size in both DFP and DHB; aperture membrane, supratectal element presence, and size of supratectal element in both DML and DHB; and the rest of the 18 characters, in all three methods. Generally, DFP generated more ambiguous states for a character than DML or DHB, but for those characters with a number of inapplicable states (gaps), such as supratectal element size, DML and DHB tended to generate more states with equal possibility at a node. Synapomorphies for the clades at different family level or above from DFP, DML, and DHB analyses were summarized (Table 3).

Table 3. Synapomorphies inferred from a democratic dataset of pollen characters for basal angiosperms analyzed with Fitch parsimony (DFP), maximum likelihood (DML), and hierarchical Bayesian inference (DHB). Pollen characters (†) and their states (§) are numbered in this table following Appendix 2. No synapomorphies were found for pollen character 10, aperature membrane. Superscripts refer to the number of the method used: [1]DFP, [2]DML, [3]DHB.

Taxa/Pollen character no.	1†	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Angiosperms			$1^{\S 3}$	1^{12}		0^{23}									3^{2}		3^{123}	
Annonaceae	1^{123}						3^{3}		1^{123}		2^{3}							
Aristolochiaceae		1^{3}						0^{3}			1^{3}							
Atherospermataceae														0^{1}				
Atherospermataceae + Gomortegaceae + Siparunaceae					0^{3}		0^{123}								7^{3}			
Austrobaileyales					0^{3}			3^{3}			4^{3}							
Austrobaileyales and all later-diverging angiosperms		2^{3}							2^{3}									
Cabombaceae					0^{123}		2^{3}											
Cabombaceae + Nymphaeaceae												1^{2}	1^{123}					
Calycanthaceae								2^{123}	2^{12}						1^{123}			
Canellaceae															0^{3}			
Canellales					0^{3}						1^{3}							
Ceratophyllales + basal eudicots + monocots								3^{3}										
Chloranthales								4^{2}				1^{2}	1^{123}					
Chloranthales + magnoliids								4^{3}										
Degeneriaceae + Myristicaceae							2^{3}											
Hernandiaceae														1^{1}				
Hernandiaceae + Lauraceae + Monimiaceae																0^{123}		0^{123}
Laurales		0^{23}						0^{23}			2^{3}				0^{2}			
Laurales + Magnoliales														1^{1}				
Laurales with Calycanthaceae excluded												1^{2}	1^{123}					
Magnoliaceae							2^{123}											
Magnoliales					0^{3}		2^{3}										1^{3}	
Magnoliales with Magnoliaceae excluded															0^{3}			
Nymphaeaceae																	2^{12}	
Nymphaeales																	2^{3}	
Piperaceae												1^{2}	1^{123}					
Piperaceae + Saururaceae							0^{123}								1^{23}			
Schisandraceae + Trimeniaceae									2^{12}									
Schisandraceae (with *Illicium* excluded)							0^{123}											
Winteraceae	1^{123}																	

In contrast to democratic maximum likelihood (DML), democratic Bayesian inference (DHB) generated a greater number of unambiguous character state changes at internal nodes for each of the 18 pollen characters, except for dispersal unit, aperture membrane, infratectum, and foot layer, for which all three methods generated an equal number of character state changes. Ten internal nodes were characterized by at least five characters that underwent unambiguous state change throughout the phylogeny (ingroup) with DHB. These nodes are the roots of the clades that comprise all sampled angiosperms, Nymphaeales, monocots, the clade comprising *Potamogeton* L. and *Triglochin* L., Chloranthales, the clade comprising *Aristolochia*, *Lactoris* Phil., and *Thottea* Rottb., Magnoliales, Annonaceae, Calycanthaceae, and Laurales. This last group displayed the most number of state changes, i.e., nine (Fig. 10).

Discussion

Systematic significance of pollen morphology in the basal angiosperms

In this study, synapomorphies or likely synapomorphies were inferred for various lineages of the

basal angiosperms for 17 out of the 18 pollen characters studied (the only exception being ornamentation of the aperture membrane). A comprehensively coded matrix was used with Fitch parsimony (CFP), and for all 18 pollen characters studied a democratically coded matrix was used, with three methods of ancestral state reconstruction, including Fitch parsimony (DFP), maximum likelihood (DML), and Bayesian inference (DHB). These results indicate that pollen morphology can play an important role in supporting systematic relationships among basal angiosperms. Characters such as pollen grain polarity and symmetry, aperture number, supratectal element presence, and tectum sculpture provided the highest number of synapomorphies for families or higher taxa within basal angiosperms and indicate their value in taxonomy and systematics. When tree topologies from the recent literature were compared (cf. Figs. 7–9), it was clear that relationships are not yet clearly determined among basal angiosperms based on molecular data. Nevertheless, the topology of Qiu et al. (2006, three genes) is the best supported by pollen morphologies. This topology yielded the greatest number of taxa supported by pollen synapomorphies (22) and likely synapomorphies (14) under CFP and was the most congruent with the palynological evidence. The number of synapomorphies obtained did not seem to depend upon the number of terminals in the topology in our study. The phylogenetic conclusions of Qiu et al. (2006, three genes) placed the Amborellaceae as sister to Nymphaeales and the Chloranthaceae as sister to *Ceratophyllum*. Both relationships are also correspondingly demonstrated by the most recent angiosperm reconstructions of Qiu et al. (2010), with four mitochondrial genes *atp1*, *matR*, *nad5*, and *rps3*, and Zhang et al. (2012), with five highly conserved, low-copy nuclear genes *SMC1*, *SMC2*, *MSH1*, *MLH1*, and *MCM5*.

In the following discussion, we present the palynological data and its systematic significance for each of the major lineages within basal angiosperms, and within the ANITA group (Amborellales + Nymphaeales + Austrobaileyales). It has become clear through many studies (including the 12 phylogenetic trees used here) that the Amborellales, Nymphaeales, and Austrobaileyales (sensu Angiosperm Phylogeny Group, 2009) represent the earliest diverging lineages of extant angiosperms. Several pollen synapomorphies exist to support a relationship for the Austrobaileyales with later-diverging angiosperms, such as isopolarity (by democratic Bayesian inference: DHB), radial symmetry (by comprehensive data with Fitch parsimony [CFP] trees 8, 10 and democratic maximum likelihood [DML]), pollen circular outline in polar view (by CFP trees 1, 2, 4 to 10, 12, and democratic Fitch parsimony [DFP]), inaperturate pollen (by CFP tree 10), and supratectal elements absence (by CFP trees 5, 12). Hereafter, we refer to tree numbers specified in Figures 7 to 9 after the analytical method. This hypothesis is also supported by three likely synapomorphies in the Fitch parsimony analysis: isopolarity by CFP trees 4, 6, radial symmetry by CFP tree 1, and supratectal elements as absent in CFP tree 10. This provides evidence for the more basal positions of the lineages that lead to the Amborellales and Nymphaeales. Recent phylogenetic studies have suggested two main arrangements for the Amborellales with respect to the Nymphaeales. Either the Amborellales lies sister to all other angiosperms, as indicated by parsimony phylogenetic analyses (Soltis et al., 1999; Mathews & Donoghue, 2000; Moore et al., 2007), or the Amborellales lies sister to the Nymphaeales and this pair then lies sister to the other angiosperms, as indicated by likelihood and Bayesian methods (Qiu et al., 2006; Soltis et al., 2007). Sampson (2000a) has suggested that the isolated position of Amborellales as sister group to all other living angiosperms is reinforced by its unique pollen exine sculpturing. We find one additional synapomorphy from pollen data (radial symmetry, supported by DHB) and one likely synapomorphy (reticulate tectum sculpture, supported by CFP tree 5) to support this basally branching position of the Amborellales, with the Nymphaeales grouping with all later-diverging angiosperms). However, a sister relationship between the Nymphaeales and Amborellales is exhibited in the most palynologically favored reconstruction of Qiu et al. (2006, three genes) as well as that of Qiu et al. (2010). This is supported by the two (albeit related) synapomorphies of the supratectal elements as present (CFP tree 8) and the supratectal element shape as spinulate (CFP trees 8, 11).

Within the Nymphaeales, supratectal elements spinulate (comprehensive data with Fitch parsimony [CFP] tree 7) support the monophyly of the Nymphaeaceae, separated from Cabombaceae as in the Angiosperm Phylogeny Group (2009). The monophyly of the Cabombaceae is supported by two pollen synapomorphies: (1) oblate shape (CFP trees 1, 4 to 9, 11, 12, democratic Fitch parsimony [DFP], maximum likelihood [DML], and democratic Bayesian inference [DHB]) and (2) large size (50–99 μm, DHB). The Austrobaileyales is supported by a single likely synapomorphy, supratectal elements absent (CFP tree 6), and the three synapomorphies of oblate shape, pollen with three or multiples of three apertures, and syncolpate apertures, as indicated by DHB. The

interfamilial relationships within this order are also supported by pollen synapomorphies. The Schisandraceae is supported by the two synapomorphies of radial symmetry, as indicated in CFP trees 5, 11, and three (or multiples of three) apertures, as shown in CFP trees 1, 4, 5, 8, 11. The clade of Schisandraceae and Trimeniaceae, leaving the Austrobaileyaceae at the base of the order, is supported by a single likely synapomorphy, radial pollen symmetry (CFP trees 1, 4, 6, 9, 12) and one synapomorphy, equatorial apertures (CFP trees 1, 4, 6, 9, 12).

Ceratophyllales. The position of Ceratophyllales has been highly unstable (Qiu et al., 2006), with five main alternative hypotheses: (1) as sister to the basal eudicots (Soltis et al., 1999; Müller et al., 2006); (2) nesting within monocots (Soltis et al., 1997); (3) as sister to monocots (Qiu et al., 1999); (4) as sister to Chloranthaceae (Qiu et al., 2005, 2006 eight genes, 2010; Zhang et al., 2012); or (5) as the most basally branching of angiosperms (Chase et al., 1993; Savolainen et al., 2000). Hickey and Taylor (1996) suggested that Ceratophyllales was a poor candidate for the basalmost position suggested by Chase et al. (1993), a study that did not include *Amborella* Baill. The clade comprising basal eudicots, Ceratophyllales, and monocots is supported by the pollen synapomorphies of small size (comprehensive data with Fitch parsimony [CFP] tree 6) and three apertures (democratic Bayesian inference [DHB]), and one likely synapomorphy, radial symmetry (CFP tree 6). The clade comprising monocots and Ceratophyllales is supported by one synapomorphy, apolarity (CFP trees 4, 6), whereas the clade comprising basal eudicots and Ceratophyllales is supported by an alternative synapomorphy, radial symmetry (CFP trees 5, 7; also a likely synapomorpy in trees 9, 11, 12). Although there is support for a close affinity between basal eudicots, Ceratophyllales, and monocots, the topology of Qiu et al. (2006), exhibiting a sister relationship between Ceratophyllales and Chloranthales, is best supported by pollen evidence among all six studied trees with similar taxa sampling (i.e., Qiu et al., 1999, 2000, 2005, 2006 three/eight genes; Soltis et al., 2011) and is consistent with the topologies from Qiu et al. (2010) and Zhang et al. (2012). Furthermore, Endress and Doyle (2009) revealed that the relationship of Ceratophyllales to Chloranthales is supported by five unequivocal floral synapomorphies, whereas its relationship to eudicots is supported by only one unequivocal morphological synapomorphy.

Chloranthales. The Chloranthales, composed of the single family Chloranthaceae, is an isolated lineage dating back to the Early Cretaceous period (Qiu et al., 2006). In our study, the Chloranthales clade is well supported by the three synapomorphies of isopolarity (comprehensive data with Fitch parsimony [CFP] trees 5, 11; democratic maximum likelihood [DML]), supratectal elements present (CFP tree 8; DML), and supratectal elements spinulate (CFP tree 8; DML, democratic Bayesian inference [DHB]), and one likely synapomorphy, radial symmetry (CFP trees 5, 6, 9, 11, 12). The Chloranthales has been placed in a variety of positions in different molecular studies (Jansen et al., 2007), which include its grouping with monocots (Müller et al., 2006), nesting within the Austrobaileyales (Hansen et al., 2007), or as sister to the Ceratophyllales (Duvall et al., 2006; Mathews, 2006; Qiu et al., 2006). The placement of this order remains one of the most difficult issues for resolving relationships among the deep nodes of the angiosperm tree of life (Hansen et al., 2007). However, the options for its placement have been reduced by recent studies such as those from Qiu et al. (2005, 2006, 2010), Moore et al. (2007), Soltis et al. (2007), and Zhang et al. (2012). Despite this controversy, the Chloranthales have been most recently assigned as sister to magnoliids in the Angiosperm Phylogeny Group (2009) and Soltis et al. (2011). Some pollen evidence can be found for the varying positions of this order. There is one likely synapomorphy radial symmetry (CFP tree 4) supporting the clade comprising Ceratophyllales, Chloranthales, and monocots, and another likely synapomorphy, isopolarity (CFP tree 1) for the clade comprising basal eudicots, Chloranthales, and magnoliids. Only multiaperturate pollen (DHB) supports the monophyletic grouping of Chloranthaceae and magnoliids as indicated by Soltis et al. (2011). The best supported relationship in this study is that of Ceratophyllales as sister to Chloranthales.

Magnoliids. Magnoliids have been identified as a large monophyletic group (Angiosperm Phylogeny Group, 2009), but affinities among their member orders (Magnoliales, Laurales, Piperales, and Canellales) need further study, especially because there is still a lack of morphological characters that define relationships among the included lineages (Qiu et al., 2005, 2006). The morphological cladistic analysis of Doyle and Endress (2000) did not identify any synapomorphies for magnoliids as a whole, although there are several morphological features that suggest links among the four orders (Kubitzki et al., 1993). In our analysis, multiple apertures were the only synapomorphy found for the magnoliids (democratic Bayesian inference [DHB]).

Pollen character states were found to define well the relationships of some orders and their subclades within the magnoliids. Pollen synapomorphies define

Magnoliales (oblate shape, DHB) and Laurales (apolarity, democratic maximum likelihood [DML], DHB; zonate aperture and supratectal elements spinulate, DHB). In more recent phylogenetic analyses, Magnoliales appear as sister to Laurales, and Piperales as sister to Canellales, with the exceptions including the analyses by Müller et al. (2006) and Soltis et al. (2007). Magnoliales and Laurales share few morphological synapomorphies (Qiu et al., 2000), but a pollen synapomorphy of the supratectal element size $\geq$ 0.5 μm (comprehensive data with Fitch parsimony [CFP] tree 12; DFP), and a likely synapomorphy of the imperforate tectal ornamentation (CFP trees 1, 3, 4) were inferred for their sister relationship. Porate apertures and oblate pollen shape were inferred as synapomorphies for Canellales (DHB), but no pollen synapomorphy was found either for Piperales or for the clade comprising Canellales and Piperales. Within Canellales, Winteraceae were defined by the two synapomorphies of dispersal as tetrads (CFP trees 1, 4 to 6, 8, 9, 11, 12; democratic Fitch parsimony [DFP], DML, DHB) and porate apertures (CFP trees 1, 4 to 6, 8, 9, 11, 12), and one likely synapomorphy, oblate pollen shape (CFP 1, 4 to 6, 8, 9, 11, 12; DFP). Pollen morphology may play an important role in the systematics of Laurales since up to eight taxa were inferred to be supported by pollen synapomorphies or likely synapomorphies using CFP, DFP, DML, and DHB. The pollen morphology of Calycanthaceae is very distinct from other members of the Laurales in being isopolar (CFP 3, 5, 8, 9, 11, 12), and having two apertures (CFP trees 1, 4, 6, 8; DFP, DML, DHB) and perforate tectum ornamentation (CFP trees 6, 12; DFP, DML, DHB), all three characters being likely synapomorphies or synapomorphies for this family. The sister relationship between Calycanthaceae and other members of Laurales is well supported by up to nine synapomorphies or likely synapomorphies, such as pollen apolarity (CFP trees 1, 4, 5, 8, 11, 12), radial symmetry (CFP trees 1, 4, 5, 11, 12), inaperturate pollen (CFP trees 1, 4, 5, 8, 11, 12), a zonate aperture (CFP trees 4 to 6, 8, 9, 11; DFP, DML), supratectal elements present (CFP tree 8), supratectal elements spinulate (CFP tree 8), an imperforate tectum ornamentation (CFP tree 12), infratectum absent (CFP tree 8), and foot layer absent (CFP tree 8). The monophyletic group comprising Monimiaceae, Lauraceae, and Hernandiaceae is well supported by morphological and molecular data (Renner et al., 2000). It is also strongly supported by pollen synapomorphies or likely synapomorphies, which include pollen apolarity (CFP trees 6, 9), radial symmetry (CFP trees 1, 6, 9), inaperturate pollen (CFP trees 6, 9), imperforate tectum sculpture (DHB), infratectum absent (CFP trees 1, 4, 6, 9, 11, 12; DFP, DML, DHB), and foot layer absent (CFP trees 1, 4, 6, 9, 11, 12; DFP, DML, DHB). The Atherospermataceae can be defined by one likely synapomorphy, pollen isopolarity (CFP trees 9, 12), and three synapomorphies: oblate shape (DML), supratectal elements with both size ranges of $<$ 0.5 μm and $\geq$ 0.5 μm evenly distributed across the grain (DFP), and tectum comprising rudimentary or partly fused hemispherical processes (CFP trees 1, 4, 6, 8, 9, 12; DFP, DML). In addition, we infer a few synapomorphies or likely synapomorphies that support other clade groupings within Laurales such as the Hernandiaceae, and a clade comprising Gomortegaceae, Hernandiaceae, Lauraceae, and Monimiaceae; a clade comprising Atherospermataceae and Siparunaceae; a clade comprising Atherospermataceae, Lauraceae, and Siparunaceae; and a clade comprising Atherospermataceae, Gomortegaceae, and Siparunaceae (Figs. 7–9).

Relationships within the Magnoliales are problematic, particularly with respect to the two alternative basal groups that have been proposed, Myristicaceae (Qiu et al., 1999, 2000, 2006; Soltis et al., 2000, 2007) or Magnoliaceae (Qiu et al., 2006; Soltis et al., 2011). Pollen characters slightly support the latter hypothesis, suggesting that the Magnoliaceae is distinctive within the Magnoliales, since all other members of this order share the synapomorphy of imperforate tectum ornamentation (comprehensive data with Fitch parsimony [CFP] tree 8; democratic Bayesian inference [DHB]; also a likely synapomorphy in CFP tree 12). Two likely synapomorphies (oblate pollen shape [CFP trees 1, 4, 6, 8, 9, 12] and proximal aperture [CFP trees 1, 4, 6, 8, 9, 12]) as well as the three synapomorphies (pollen dispersal as tetrads [democratic Fitch parsimony (DFP), democratic maximum likelihood (DML), DHB], very large grain size [DHB], and proximal aperture position [DFP, DML, DHB]) were found for the Annonaceae. One likely synapomorphy, granulate infratectum (CFP trees 8, 12), was found for the clade comprising Annonaceae, Eupomatiaceae, and Himantandraceae, providing weak support for this clade. The sister relationship between the Himantandraceae and Degeneriaceae is supported by the synapomorphy of the absence of a foot layer (CFP trees 1, 4, 5, 9, 11). The Piperaceae and Saururaceae are subtended by the synapomorphies supratectal elements present (CFP tree 8), spinulate pollen (CFP trees 8, 11), and boat-shaped pollen (CFP tree 3). The sister relationship between these two families as found in most recent molecular studies (Soltis et al., 2011) is

further supported by small-sized pollen (CFP tree 1, 3 to 9, 11, 12; DFP, DML, DHB).

Related lineages: Monocots and basal eudicots. Several different placements have been suggested for monocots, ranging from sister to all remaining angiosperms other than the ANITA group, part of a clade with magnoliids and Chloranthales, sister to magnoliids, sister to eudicots, or part of a clade with Ceratophyllales plus eudicots (see Moore et al. [2007] for a review). A few pollen character states, i.e., small size, three apertures, radial symmetry, and apolarity, as discussed under Ceratophyllales, support the affinity of monocots with basal eudicots and Ceratophyllaceae.

The natural lineage of eudicots (Qiu et al., 2005) is well supported by two pollen synapomorphies, pollen with three or more apertures (comprehensive data with Fitch parsimony [CFP] trees 1, 4, but a likely synapomorphy in trees 6, 7; and democratic maximum likelihood [DML]) and equatorially positioned apertures (CFP trees 1, 4, 6, 7), and two likely synapomorphies, isopolarity (CFP trees 7 to 9; a synapomorphy in DML) and radial symmetry (CFP tree 4). The eudicots have been broadly characterized by the possession of tricolpate (or tricolpate-derived) pollen (Doyle & Hotton, 1991; Furness, 2007), but this aperture type has evolved independently several times in angiosperms. However, the tricolpate structures in members of the ANITA group mainly follow Garside's rule (Garside, 1946; Erdtman, 1952), whereas that of the eudicots follows Fischer's rule (Erdtman, 1952; Furness & Rudall, 2004).

In our analysis, the absence of pollen supratectal elements is a likely synapomorphy (comprehensive data with Fitch parsimony [CFP] trees 2, 6) that supports the clade comprising basal eudicots, monocots, and Ceratophyllaceae as sister to magnoliids. *Nelumbo* Adans. has historically been included in the Nymphaeaceae, but in recent molecular phylogenies, the genus nests within basal eudicots (Hayes et al., 2000). Early studies of its seed and embryo led researchers to describe *Nelumbo* as dicotyledonous (Mirbel, 1809). Pollen morphology supports an isolated position of *Nelumbo* in the basal eudicots with some unique characters such as large grain size (ca. 45–70 μm), one, two, or multiple apertures, and a zonate ectoaperture shape for the pollen (Perveen, 1999; Banks et al., 2007).

POSSIBLE EVOLUTION OF POLLEN MORPHOLOGY IN BASAL ANGIOSPERMS

The plesiomorphic palynological condition for the angiosperms has not been ascertained yet, although it has been debated across several decades. Several studies, such as those from Walker and Doyle (1975), Walker (1976), Walker and Walker (1984), as well as Zavada and Crepet (1986), suggested that the primitive conditions for angiosperm pollen were distally monosulcate, large- to medium-sized, boat-shaped, smooth-walled, with a granular infratectum. Using paleobotanical data from Israel, Brenner (1996) considered that the most ancestral pollen was inaperturate with strongly reduced columellae, and that this syndrome was at least as old as the reputed ancestral monosulcate condition. Sampson (2000a) concluded that the first angiosperm pollen grains were probably monosulcate, with a distal aperture and a homogeneous or partly granular extexine, but without a tectate-columellate structure. Later, Doyle and Endress (2000) and Doyle (2005) inferred that the ancestral angiosperms had globose, monosulcate pollen with columellae and a thin endexine, rather than boat-shaped monosulcate pollen, with a granular infratectum and no endexine.

In the present study, plesiomorphic states for pollen morphology within the basal angiosperms are inferred to be dispersal as monads, and grains that are heteropolar, spheroidal, and mono-aperturate, with distal, colpate apertures having sculptured membranes, as well as supratectal elements with spinulate shape and size < 0.5 μm, with an infratectum and foot layer (based on comprehensive Fitch parsimony [CFP]). From democratic Fitch parsimony, maximum likelihood, and Bayesian inference (DFP, DML, and DHB, respectively), pollen grains are also inferred as bilateral, elliptic, with a tectate-rugulate surface and alveolate infratectum alveolate. We consider that the earliest pollen type of the basal angiosperms is, therefore, likely to be characterized by most of these pollen features.

The likely aperture condition of early angiosperms has been widely debated. Arguments for an inaperturate condition have mostly been based on observations of basally branching groups, e.g., Laurales, Magnoliales, and monocots. Brenner (1996) considered that the tetrads of Winteraceae evolved from monoporate monads, which had been derived from inaperturate pollen. In addition, he suggested that the evolution of apertures from the inaperturate condition gave a reproductive advantage. Hickey and Taylor (1996) corroborated Brenner's (1996) inference that angiosperm pollen was primitively spherical and inaperturate. Furthermore, the analyses of Qiu et al. (2006) and Soltis et al. (2008) reinforced the hypothesis that some of the earliest angiosperms may have been aquatic, given that aquatic or moist habitats may have provided a refuge from competition

with terrestrial gymnosperms during the early evolution of angiosperms, and inaperturate pollen is frequently found correlated with aquatic or moist habitats (as in monocots), where it may be an adaptation for submarine pollination (Ducker et al., 1978), and with functional dioecy in some lineages of eudicots (Furness, 2007).

The trichotomosulcate pollen type is another significant trend in the early evolution of the angiosperm pollen aperture. Trichotomosulcate pollen occurs in some Nymphaeales, the Chloranthaceae, and magnoliids, and has been recovered in fossilized waterlily-type flowers from the Eocene flora of the Messel oil shales (Harley, 2004). Zonasulcate pollen has also been proposed as an ancestral state in the evolution of pollen apertures. Zonasulcate pollen has been inferred to have evolved independently in several basal angiosperm families, including the Nymphaeales, Magnoliales, Laurales, and some monocots. The zonasulcate condition may in fact be derived from the trichotomosulcate condition in pollen (Harley & Dransfield, 2003). A ringlike, equatorially located aperture (i.e., zonate pollen) has been found in gymnosperm *Circumpolles* (Pflug, 1953; Klaus, 1960) fossils from as early as the Triassic and Jurassic periods (Pozhidaev, 2000; Hesse & Zetter, 2005).

Based on recent molecular data, monoaperturate paleoherbs (Donoghue & Doyle, 1989) are no longer considered to be the earliest diverging of the angiosperms (Angiosperm Phylogeny Group, 2009), and in contrast, the pollen of the earliest-diverging angiosperm, *Amborella*, has been found to contain a mixture of inaperturate grains and grains with a reduced apertural area (cf. Sampson, 1993). Similarly, based on our analyses, the ancestral state for pollen aperture type was inferred to be a monoaperture rather than inaperturate, as indicated by all methodologies (comprehensive data with Fitch parsimony [CFP], democratic Fitch parsimony [DFP], democratic maximum likelihood [DML], and democratic Bayesian inference [DHB]). This is more consistent with previous studies that suggested the monosulcate condition as the basal type for the earliest angiosperms on the basis of cladistic (e.g., Doyle & Donoghue, 1986; Doyle & Endress, 2000) and character optimization methods (e.g., Doyle, 2005).

In terms of pollen size, the first angiosperm pollen has variously been proposed as small (Macphail et al., 1999; Heimhofer et al., 2007) or medium to large (Walker & Doyle, 1975; Walker, 1976; Walker & Walker, 1984; Zavada & Crepet, 1986). Our results on 11 out of 12 studied phylogenies (with the exception of Mathews & Donoghue [2000]), using comprehensive data with Fitch parsimony (CFP), democratic maximum likelihood (DML), and democratic Bayesian inference (DHB), suggest that medium (25–49 μm, most commonly) or large size (50–99 μm) is inferred as the ancestral state for the basal angiosperms.

Regarding the tectum sculpture of pollen grains, Walker and Doyle (1975) suggested that ancestral angiosperm pollen was more or less psilate. Hughes and McDougall (1987) discovered that one of the oldest angiosperm-like monosulcate grains had verrucate sculpture elements covered with minute spinules very much as seen in the Amborellales (Sampson, 1993). Friis et al. (2006) suggested that early angiosperms were insect pollinated and had pollen with a reticulate surface. The reticulate tectum might be an adaptation to insect pollination (Zavialova & Gomankov, 2009). In our analysis, perforate-fossulate, rugulate, reticulate, and an *Amborella*-type ornamentation were all found to be potential ancestral states for tectum sculpture in angiosperms under comprehensive data with Fitch parsimony (CFP). Of these, rugulate ornamentation was inferred as ancestral on the greatest number of trees (CFP trees 2, 5, 11), being unambiguously inferred as the ancestral state by democratic Fitch parsimony, maximum likelihood, and Bayesian inference (DFP, DML, and DHB, respectively). Therefore, we consider that a rugulate tectum was the most likely plesiomorphic angiosperm type.

An alveolate infratectum was revealed as a potential plesiomorphy for angiosperms in our study, particularly on trees rooted with gymnosperm outgroups. However, further evidence from either paleopalynology or character evolution analysis seems to support the columellate type (Brenner, 1996; Doyle & Endress, 2000; Doyle, 2005). In most members of the ANITA group, the ectexine is tectate-columellate (Sampson, 2000a). Furthermore, Heimhofer et al. (2007) found that the columellate-tectate pollen grains of the †*Clavatipollenites* Couper 1958 group represent a common constituent of early angiosperm pollen assemblages. Additional study of this character would be worthwhile.

LIMITATIONS IN INFERRING PALYNOLOGICAL EVOLUTION IN EARLY DIVERGING ANGIOSPERMS

Uncertainty in molecular phylogenies. Although great progress has been made in genetic and genomic approaches to phylogenetics over past decades, the precise phylogenetic relationships of the basal angiosperms remain unclear. Complex evolutionary processes such as rapid radiation in terms of both

species and morphology, polyploid speciation, and convergent evolution may have played a substantial role in the very early divergence of the angiosperms, making the discernment of basal angiosperm phylogeny even more difficult. The explosive radiation of angiosperms appears to have begun with the origin of the mesangiosperm clade, after the origin of crown group angiosperms, and with the divergence of the more basal ANITA lines (Moore et al., 2007; Endress & Doyle, 2009). Many of the early-diverging major clades, such as the magnoliids, monocots, Chloranthaceae, eudicots, and Ceratophyllaceae, branched in rapid succession (Soltis et al., 2008). Polyploidy has long been recognized as a major evolutionary force in many plant lineages, in particular the ferns and angiosperms; cytology and recent genomic studies have also provided evidence for a polyploid origin of angiosperms (reviewed by Soltis et al., 2008). In addition, evolutionary parallelism is implicated in the evolution of basal angiosperms and some gymnosperms, inferred by the similarity in the features of double fertilization and flowers or flower-like structures between basal angiosperms and Gnetales (reviewed by Qiu et al., 2000). Thus, both methodological inadequacies and potentially intricate evolutionary patterns render a stable phylogenetic framework with robust support unavailable at the present time. Nonetheless, by exploring a number of possible topologies, as herein, we were able to draw certain significant conclusions when tracing pollen evolutionary trends in basal angiosperms.

Character convergence and functional constraints in palynological evolution. The occurrence of evolutionary parallelism or convergence in pollen morphology in the basal angiosperms has been detected both in our analyses and in previous studies (Le Thomas, 1980; Harley, 2004; Furness, 2007; Lora et al., 2009). Changes of state in pollen characters such as aperture type and polarity are already known to have occurred a number of times (Harley, 2004). While monosulcate or inaperturate pollen is traditionally considered to be plesiomorphic for angiosperms (Walker & Doyle, 1975; Brenner, 1996; Doyle, 2005), such pollen may represent interesting examples of convergent evolution (Furness, 2007). Evolution of inaperturate pollen, in particular, is sometimes associated with an aquatic habit, parasitism, insectivory, heterostyly, anemophily, or the presence of pollinia (Furness, 2007). Such physical conditions are known to greatly affect pollen morphology in angiosperms (Tekleva & Krassilov, 2009; Zavialova & Gomankov, 2009) and would include the early branching lineages.

Pollination mechanisms, in particular, may impose constraints leading to convergent evolution of pollen morphological syndromes. Entomophilous pollen is generally characterized by compound apertures, a large grain size, and a reticulate tectum; anemophilous pollen may be distinguished by simple apertures, spherical grain shape, small size, a better-developed exine, with a dry surface and scabrate-areolate tectum; and hydrophilous pollen may be inaperturate, less rigid, with a thin, elastic, and reduced exine, sometimes functionally filiform, and produced in large amounts (reviewed by Ackerman, 2000). Tectate-columellate pollen has been of functional significance for the action of harmomegathy (Sampson, 2000a). Genome size may determine or correlate with characters such as pollen size (Knight et al., 2010).

Some characters of angiosperm pollen are known to have appeared independently in gymnosperms much earlier than the putative origin of the angiosperms (Zavialova & Gomankov, 2009). As a result of adaptive radiation during the earliest angiosperm evolution (Endress, 1986), character convergence may confound our understanding of pollen evolutionary patterns and the plesiomorphic pollen type for angiosperms.

Ontogenic mode. Gabarayeva and Grigorjeva (2012) highlighted the crucial importance of ontogenetic data for interpreting the nature of the exine, especially the infratectum and endexine, in basal angiosperm pollen. They pointed out that past misinterpretation of the infratectum as granular in the Magnoliaceae (e.g., Walker, 1976) may be due to the absence of ontogenetic data. In mature grains, sporopollenin accumulation on the tectum, columellae, and foot layer is so extensive that the rather short columellae often look like granules, and the endexine is often completely obscured (Gabarayeva & Grigorjeva, 2012). If the fundamental details of pollen ontogeny are not taken into account, then pollen diversity and character evolution in the basal angiosperms may in some cases be misinterpreted.

As with other reproductive characters such as the nature of the endosperm (Floyd & Friedman, 2001), pollen morphologies (e.g., structure of apertures, exine, and polarity) may exhibit high variability in developmental mechanisms, particularly during the early radiation of the angiosperms (Heslop-Harrison & Heslop-Harrison, 1992; Ressayre et al., 2002; Furness & Rudall, 2004; Furness, 2007; Lora et al., 2009). Blackmore et al. (2007) reviewed self-assembly processes in pollen wall ontogeny of the flowering plants, which are not under direct genetic control but play an important role in pollen wall patterning.

According to this study, almost all eudicots exhibit simultaneous microsporogenesis, with exceptions including a few basal eudicots such as the Nelumbonaceae and Proteaceae. By contrast, in basal angiosperms, microsporogenesis and aperture pattern are highly labile and exhibit both the common patterns of simultaneous and successive microsporogenesis as well as intermediate types. Both monosulcate and inaperturate pollen can occur in association with either simultaneous or successive microsporogenesis (Blackmore & Crane, 1998; Furness & Rudall, 2004). Therefore, similar pollen characters may result from different pollen developmental modes.

For example, the orientation of colpi relative to the tetrad in the tricolpate or tricolpate-derived pollen of Austrobaileyales and other basal eudicots follows Garside's rule and is, therefore, different in origin to that of eudicots that follow Fischer's rule (reviewed by Furness & Rudall, 2004). Monosulcate pollen in the Austrobaileyaceae and Chloranthaceae may result from simultaneous cytokinesis, whereas in the Magnoliaceae monosulcate pollen may result from simultaneous cytokinesis or intermediate simultaneous cytokinesis, and in *Galbulimima* F. M. Bailey (Himantandraceae) from successive cytokinesis (Harley, 2004).

Incomplete fossil information. Due to the pivotal systematic position of the basal angiosperms among the seed plants, fossil data play a vital role in phylogenetic reconstruction and outgroup determination. In addition to rapid taxonomic radiation, extinctions among early angiosperm lineages greatly confound relationships among modern basal angiosperms. The living sister group to all angiosperms is still debated (Qiu et al., 2000; Endress & Doyle, 2009), and fossil relatives remain mysterious, although new insights from paleobotany continue to reduce the phylogenetic gap between extant gymnosperms and angiosperms (Qiu et al., 2000; Soltis et al., 2005; Endress & Doyle, 2009: Friis et al., 2011). Incorporating fossils of inferred close relationship into analyses of extant angiosperms may facilitate prediction of the morphological characters present in the early angiosperms, determination of plesiomorphies, and resolution of the early evolution of angiosperms (Hermsen et al., 2006). However, as with previous studies (Doyle & Endress, 2000; Doyle, 2005), analyses such as ours cannot, at present, make use of extinct taxa while based on molecular phylogenetic estimates.

Infraspecific polymorphism in pollen characters. In certain basal angiosperms, pollen polymorphism occurs within species, e.g., *Trimenia papuana* Ridl. (Trimeniaceae), which has dimorphic pollen, with both inaperturate and polyporate types (Sampson, 2007). Similarly, pollen grains of the basalmost angiosperm, *Amborella trichopoda*, have been observed as ana-ulcerate or anasulcate; however, Bailey and Swamy (1948), Erdtman (1952), Walker (1976), and Sampson (1993) found that a high proportion of its pollen examined under SEM showed no signs of apertures. Infraspecific pollen polymorphism, particularly in this species, may render interpretations of ancestral character states as equivocal in deep nodes and have a significant effect upon determination of plesiomorphic characters for angiosperms, especially because a close outgroup cannot be defined.

Incorrect or ambiguous pollen data from previous studies. The pollen data used for character optimization onto molecular phylogenies, in both our study (see Table 1) and those of Doyle and Endress (2000) and Doyle (2005), were drawn mainly from palynological reports in the literature made over past decades. While these data are very useful, they should be used with caution, particularly if conflicting observations are noted. For instance, the pollen infratectum of *Welwitschia* Hook. f. and *Gnetum* L. (both gymnosperm outgroups in our study) is described as columellate in Hickey and Taylor (1996) but as granular in Tekleva and Krassilov (2009). This could be explained either as a polymorphic character or as an incorrect description in one or both cases. Furthermore, pollen data for some taxa in the older literature made use of only light microscope (LM) observations. Although it is often incredible how much information can be gleaned from careful LM observation, some characters such as the internal foramina may only be visible under TEM electron microscopy. Gullvåg (1966) suggested that, in the Coniferales, electron microscopy should be used in order to check the detailed characteristics of pollen types, because the light microscope is sometimes deceptive. Therefore, previous SEM and TEM observations on pollen grains are very substantial in the study.

Physical morphological transformation. Pollen grains possess a variety of physiological and structural adaptations to dry environments and possible dehydration, such as harmomegathy. The most striking of these adaptations is the ability of the pollen wall to fold onto itself to prevent further desiccation (Katifori et al., 2010). Folding processes (e.g., taking up a "boat-shaped" outline) can be critical to the survival of the pollen grain. Temporary changes in functional pollen morphology have been detected in gymnosperms (Cycadales and Gink-

goales), basal angiosperms (including the Nymphaeaceae, Ceratophyllaceae, Eupomatiaceae, Annonaceae, Monimiaceae, and Atherospermataceae), and eudicots, such as *Limnanthes* R. Br., *Pedicularis* L., *Acer* L., and *Hypericum* L. (Clarke, 1975; Pozhidaev, 1993; Tang et al., 1995; Ackerman, 2000; Zhang et al., 2000; Hesse & Zetter, 2005; Tekleva et al., 2007). In these taxa, pollen structure may be situationally modified in adaptation to different environments. The degree of hydration also influences the actual pollen grain shape (Halbritter & Hesse, 2004). For instance, dehydrated pollen of some members of the Cycadales (e.g., *Cycas* L.) and Ginkgoales (e.g., *Ginkgo biloba* L.) appears boat-shaped, with a folded aperture region resembling a sulcus, whereas in the hydrated state, pollen grains are more or less spheroidal, and apparently the aperture occupies almost half of the pollen grain surface (Tang et al., 1995; Zhang et al., 2000; Tekleva et al., 2007).

In basal angiosperms, there are a number of potential waterproofing mechanisms acting on the pollen wall of hydrophilous plants such as the Ceratophyllaceae to prevent them from bursting. For example, these plants have omniaperturate pollen with a thin, elastic exine that accommodates some increase in grain size (Thanikaimoni, 1986). Physical changes in the zona-aperturate pollen of some early flowering plants in wet environments may represent functional benefits for possible pollen tube formation or pollen germination (Hesse & Zetter, 2005). Boat-shaped pollen with a single aperture is commonly found in some gymnosperms and basal angiosperms (Ginkgoaceae, Cycadaceae, Zamiaceae, Saururaceae, Magnoliaceae, Degeneriaceae, Myristicaceae, and Annonaceae), although the zona-aperturate type has also been considered as a possible plesiomorphic angiosperm aperture condition (Pozhidaev, 2000; Harley & Dransfield, 2003). It is important to determine whether these pollen shapes are permanent and stable or merely appear under certain environmental conditions.

Future Prospects

It is crucial to continue testing hypotheses of angiosperm origins and to answer questions about the nature of the basalmost lineages (Lockhart & Penny, 2005) in several ways. First, although plastid phylogenomic approaches to resolving the relationships of the basal angiosperms (Jansen et al., 2007; Moore et al., 2007; Angiosperm Phylogeny Group, 2009) now appear to provide a more robust topology, this should be confirmed using more nuclear sequence data (Soltis et al., 2008). Further phylogenetic work is necessary, especially to resolve the relationships among the Ceratophyllales, Chloranthales, monocots, magnoliids, and eudicots. This will provide not only a better understanding of the speciation, extinction, and rapid diversification that occurred among the early angiosperms, but also a solid framework for studies of morphological evolution. Second, new discoveries of early angiosperm fossil taxa and their integration into the phylogeny of angiosperms would provide further insights to evaluate the implications of fossils for phylogeny and the origin of pollen morphology. Third, advancing knowledge of pollen ontogeny (microsporogenesis) and physiology is crucial to the correct interpretation of homology in pollen morphological characters.

Nevertheless, by careful analysis of existing literature and new data, on the basis of multiple possible phylogenetic estimates and using multiple coding strategies and analysis methods, we have been able to conduct a detailed and integrated analysis of pollen morphology of the basal angiosperms. This study provides evidence of plesiomorphic characters in pollen, synapomorphies for extant taxa and monophyletic groups, and insights into evolutionary patterns, as well as a basis for further study of both the pollen evolution and systematics of this group.

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Appendix 1. Phylogenetic position and genus-level terminals used in the phylogenetic analyses. The generic totals for families are sensu Angiosperm Phylogeny Group (2009) and Soltis et al. (2011). Fifty-seven genus-level exemplars represent nine orders and 28 families of basal angiosperms. Ten genus-level exemplars span three orders and eight families of basal eudicots; three genus exemplars are included for two orders and three families of monocots. Eight outgroup genera represent four orders and eight families of gymnosperms.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
BASAL ANGIOSPERMS			
AMBORELLALES			
Amborellaceae	1 (1)	[2,3]*Amborella* Baill.*	Sampson, 1993, 2000a; Hesse, 2001; Suo, 2004
		[1]*Amborella trichopoda* Baill.	New Caledonia, *Nakada s.n.* (CANBRHB of deposit)
AUSTROBAILEYALES			
Austrobaileyaceae	1 (1)	[2,3]*Austrobaileya* C. T. White	Endress & Honegger, 1980; Zavada, 1984; Sampson, 2000a
		[1]*Austrobaileya scandens* C. T. White (Fig. 1G–I)	Australia, Queensland, *L. S. Smith 4631* (CANB)
Schisandraceae	3 (3)	[2,3]*Illicium* L.	Lin, 1989; Liu & Yang, 1989; Doyle et al., 1990; Takahashi, 1994; Sampson, 2000a; Gabarayeva & Grigorjeva, 2004; Wang et al., 2010
		[1]*Illicium macranthum* A. C. Sm. (Fig. 2C)	China, *Y. M. Shui et al. 002758* (KUN)
		[1]*Illicium micranthum* Dunn (Fig. 2A, B)	China, *L. Zhang ZL05* (HITBG)

Appendix 1. Continued.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
		[2,3]*Kadsura* Juss.	Walker, 1974; Vijayaraghavan & Dhar, 1975; Praglowski, 1976; Lieux, 1980; Lan, 1984; Sampson, 2000a; Sun, 2000; Wang et al., 2010
		[2,3]*Schisandra* Michx.	Walker, 1974; Praglowski, 1976; Lieux, 1980; Sampson, 2000a; Sun, 2000; Wang et al., 2010
Trimeniaceae	1 (1)	[2,3]*Trimenia* Seem.	Sampson & Endress, 1984; Sampson, 1987, 2000a
		[1]*Trimenia moorei* (Oliv.) Philipson (Fig. 1J–L)	Australia, New South Wales, *P. H. Weston 2620* (E)
Canellales			
Canellaceae	6 (2)	[2,3]*Canella* P. Browne	Wilson, 1964; Sampson, 2000a
		[1]*Canella winterana* (L.) Gaertn. (Fig. 2G–I)	Dominican Republic, Barahona, *M. Fuertes 100* (E)
		[2,3]*Cinnamodendron* Endl.	Wilson, 1964; Sampson, 2000a
Winteraceae	5 (3)	[2,3]*Drimys* J. R. Forst. & G. Forst.*	Bailey & Nast, 1943; Lobreau-Callen, 1977; Praglowski, 1979; Sampson, 2000a; van der Ham & van Heuven, 2002
		[1]*Drimys piperita* Hook. f. (Fig. 2J–L)	Papua New Guinea, *J. R. Croft et al. LAE 65013* (E)
		[2,3]*Takhtajania* Baranova & J.-F. Leroy	Praglowski, 1979; Sampson, 2000a, 2000b; van der Ham & van Heuven, 2002
		[2,3]*Tasmannia* R. Br. ex DC.	Praglowski, 1979; van der Ham & van Heuven, 2002
Ceratophyllales			
Ceratophyllaceae	1 (1)	[2,3]*Ceratophyllum* L.	Díez et al., 1988; Takahashi, 1995
		[1]*Ceratophyllum* sp. indet. (Fig. 6J–L)	China, Gansu, *C. Schneider 1099* (E)
Chloranthales			
Chloranthaceae	4 (4)	[2,3]*Ascarina* J. R. Forst. & G. Forst.	Kuprianova, 1967; Todzia, 1988; Eklund et al., 2004
		[2,3]*Chloranthus* Sw.	Kuprianova, 1967; Todzia, 1988; Eklund et al., 2004
		[1]*Chloranthus holostegius* (Hand.-Mazz.) S. J. Pei & Shan (Fig. 2E, F)	China, Yunnan, *Germplasm Bank of Wild Species collection expedition 08CS162* (KUN)
		[1]*Chloranthus japonicus* Siebold (Fig. 2D)	China, Yunnan, *S. D. Zhang et al. APE015* (KUN)
		[2,3]*Hedyosmum* Sw.	Kuprianova, 1967; Todzia, 1988; Eklund et al., 2004
		[2,3]*Sarcandra* Gardner	Todzia, 1988; Eklund et al., 2004
Laurales			
Atherospermataceae	6 or 7 (3)	[2,3]*Atherosperma* Labill.	Sampson & Foreman, 1988
		[1]*Atherosperma moschatum* Labill. (Figs. 5L, 6A)	Australia, Victoria, *H. van Rees 048* (CANB)
		[2,3]*Daphnandra* Benth.	Sampson & Foreman, 1988; Sampson, 2000a
		[1]*Daphnandra tenuipes* G. Perkins (Fig. 6C)	Australia, New South Wales, *R. Schodde & H. C. Hayes 3572* (E)
		[2]*Doryphora* Endl.	Sampson & Foreman, 1988; Sampson, 2000a.
		[1]*Doryphora sassafras* Endl. (Fig. 6B)	Australia, New South Wales, *I. R. Telfora 1046* (CANB)
Calycanthaceae	4 (3)	[2,3]*Calycanthus* L.	Nicely, 1965; Li, 1990; Sampson, 2000a

Appendix 1. Continued.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
		[1]*Calycanthus chinensis* (W. C. Cheng & S. Y. Chang) P. T. Li (Fig. 5D–F)	China, Zhejiang, *S. D. Zhang et al. APE026* (KUN)
		[2]*Chimonanthus* Lindl.*	Nicely, 1965; Sampson, 2000a
		[2,3]*Idiospermum* S. T. Blake	Sampson, 2000a; Staedler et al., 2009
Gomortegaceae	1 (1)	[2,3]*Gomortega* Ruiz & Pav.	Hesse & Kubitzki, 1983
		[1]*Gomortega keule* (Molina) Baill. (Fig. 5I–K)	Hesse & Kubitzki, 1983
Hernandiaceae	4 (2) (APG notes 5, but Kew notes 4)	[2,3]*Gyrocarpus* Jacq.	Sampson, 2000a
		[2,3]*Hernandia* L.	Hesse & Kubitzki, 1983; Sampson, 2000a
		[1]*Hernandia didymantha* Donn. Sm. (Fig. 6F, G)	Costa Rica, *B. Hammel 11656* (KUN)
Lauraceae	50 (3)	[2]*Cinnamomum* Schaeff.	Kubitzki, 1981; van der Merwe & van Wyk, 1990; Tang & Shang, 1995; Sampson, 2000a; Premathilake & Nilsson, 2001
		[2,3]*Cryptocarya* R. Br.	Kubitzki, 1981; Raj & van der Werff, 1988; van der Merwe & van Wyk, 1990; Rowley & Vasanthy, 1993; Tang & Shang, 1995; Sampson, 2000a
		[2,3]*Laurus* L.*	Kubitzki, 1981; van der Merwe & van Wyk, 1990; Tang & Shang, 1995; Sampson, 2000a
		[1]*Laurus nobilis* L. (Fig. 6D, E)	China, Yunnan, *K. M. Feng 24109* (KUN)
Monimiaceae	22 (2)	[2,3]*Hedycarya* J. R. Forst. & G. Forst.	Sampson, 1997; Sampson, 2000a; Doyle, 2005
		[2]*Hortonia* Wight & Arn.	Sampson, 1993, 2000a; Doyle, 2005
		[2,3]*Peumus* Molina	Sampson & Foreman, 1990; Sampson, 1993, 1997; Doyle, 2005
		[1]*Peumus boldus* Molina (Fig. 6H, I)	Chile, Valparaíso, *P. Hechenleitner Vega et al. 12* (E)
Siparunaceae	2 (1)	[2,3]*Siparuna* Aubl.	Pignal et al., 1999.
		[1]*Siparuna guianensis* Aubl. (Fig. 5G, H)	Brazil, Amazonas, *P. H. Davis & D. F. Coelho D. 60375* (E)
MAGNOLIALES			
Annonaceae	129 (2)	[2,3]*Annona* L.	Walker, 1971a, 1971b; Le Thomas, 1980; Gottsberger & Silberbauer-Gottsberger, 1984; Doyle & Le Thomas, 1997; Sampson, 2000a
		[1]*Annona glabra* L. (Fig. 5A, B)	China, Yunnan, *L. Zhang ZL02* (HITBG)
		[1]*Annona muricata* L. (Fig. 4L)	China, Yunnan, *L. Zhang ZL01* (HITBG)
		[2,3]*Cananga* (DC.) Hook. f. & Thomson	Walker, 1971a; Le Thomas, 1980; Doyle & Le Thomas, 1997; Sampson, 2000a
		[1]*Cananga odorata* (Lam.) Hook. f. & Thomson var. *fruticosa* (Craib) J. Sinclair (Fig. 5C)	China, Yunnan, *L. Zhang ZL04* (HITBG)
Degeneriaceae	1 (1)	[2,3]*Degeneria* I. W. Bailey & A. C. Sm.	Dahl & Rowley, 1965; Sampson, 2000a
		[1]*Degeneria vitiensis* I. W. Bailey & A. C. Sm. (Fig. 4A–C)	Sampson, 2000a
Eupomatiaceae	1 (1)	[2,3]*Eupomatia* R. Br.	Hotchkiss, 1958; Woodland & Garlick, 1982; Sampson, 2000a
		[1]*Eupomatia laurina* R. Br. (Fig. 4I–K)	Australia, Queensland, Brisbane, 1875, *T. L. Bancroft s.n.* (E).
Himantandraceae	1 (1)	[2,3]*Galbulimima* F. M. Bailey	Sampson, 2000a

Appendix 1. Continued.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
		[1]*Galbulimima baccata* F. M. Bailey (Fig. 4G, H)	Australia, Queensland, *PLF 27536* (CANB)
Magnoliaceae	2 (2)	[2,3]*Liriodendron* L.*	Canright, 1953; Agababian, 1972; Praglowski, 1974; Wei & Wu, 1993; Xu & Kirchoff, 2008
		[1]*Liriodendron tulipifera* L. (Fig. 3J)	China, cult., *Y. Z. Chu KUN 63-34* (KUN)
		[2,3]*Magnolia* L.	Canright, 1953; Agababian, 1972; Praglowski, 1974; Sampson, 2000a; Xu & Kirchoff, 2008
		[1]*Magnolia grandiflora* L. (Fig. 3K, L)	China, cult., *Y. T. Liu 5058* (KUN)
Myristicaceae	20 (3)	[2]*Knema* Lour.	Nair, 1965; Sauquet & Le Thomas, 2003; Sauquet et al., 2003
		[1]*Knema globularia* (Lam.) Warb. (Fig. 4D, E)	China, Yunnan, *Y. H. Li 005098* (KUN)
		[2,3]*Maloutchia* Warb.	Walker & Walker, 1981; Sauquet & Le Thomas, 2003; Sauquet et al., 2003
		[2,3]*Myristica* Gronov.	Nair, 1965; Sauquet & Le Thomas, 2003; Sauquet et al., 2003
		[1]*Myristica*, sp. indet. (Fig. 4F)	Papua New Guinea, Milne Bay, *J. R. Croft et al. LAE 71132* (E)
Nymphaeales			
Hydatellaceae	1 (1)	[2,3]*Trithuria* Hook. f.	Remizowa et al., 2008
		[1]*Trithuria australis* (Diels) D. D. Sokoloff, Remizowa, T. D. Macfarl. & Rudall (Fig. 1C)	Australia, Western Australia, *T. D. Macfarlane & A. R. Annels 2283* (PERTH)
Cabombaceae	2 (2)	[2,3]*Brasenia* Schreb.	Osborn et al., 1991; Taylor & Osborn, 2006
		[2,3]*Cabomba* Aubl.*	Osborn et al., 1991; Taylor et al., 2008
Nymphaeaceae	6 (2)	[2,3]*Nuphar* Sm.*	Meyer, 1964, 1966; Sampson, 2000a; Taylor & Osborn, 2006
		[1]*Nuphar luteum* Walp. (Fig. 1F)	U.S.A., Utech, *W. W. Thomas 82–530* (KUN)
		[2,3]*Nymphaea* L.*	Meyer, 1964, 1966; Gabarayeva & El-Ghazaly, 1997; Perveen, 1999; Sampson, 2000a; Gabarayeva et al., 2001; Taylor & Osborn, 2006
		[1]*Nymphaea alba* L. (Fig. 1D, E)	China, cult. from Germany, *S. D. Zhang et al. APE021* (KUN)
Piperales			
Aristolochiaceae	5 to 8 (5)	[2,3]*Aristolochia* L.*	Nair, 1965; Sampson, 2000a; González et al., 2001; Mulder, 2003; Perveen & Qaiser, 2008
		[1]*Aristolochia contorta* Bunge (Fig. 3E, F)	China, cult., *S. D. Zhang APE016* (KUN)
		[2]*Asarum* L.*	Mi & Yang, 1991; Sampson, 2000a; González et al., 2001; Mulder, 2003
		[2,3]*Lactoris* Phil.	Zavada & Taylor, 1986; Sampson, 1995, 2000a
		[1]*Lactoris fernandeziana* Phil. (Fig. 3B–D)	Zavada & Taylor, 1986.
		[2,3]*Saruma* Oliv.	Dickison, 1992; Sampson, 2000a; González et al., 2001
		[2,3]*Thottea* Rottb.	González et al., 2001
Piperaceae	5 (2)	[2,3]*Peperomia* Ruiz & Pav.*	Nair, 1965; Lei & Liang, 1998; Sampson, 2000a

Appendix 1. Continued.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
		[1]*Peperomia heyneana* Miq. (Fig. 3G)	China, Yunnan, *Y. L. Li et al. YDDXS0406* (KUN)
		[2,3]*Piper* L.*	Nair, 1965; Huang & Huang, 1997; Lei & Liang, 1998; Sampson, 2000a; Suwanphakdee et al., 2008
Hydnoraceae	2 (1)	[1]*Hydnora* Thun.	original description
		[1]*Hydnora abyssinica* A. Braun (Fig. 3A)	Sudan, Blue Nile at Um Barona, near Wad Medani, *L. J. Musselman 198/6129* (E)
Saururaceae	5 (3)	[2]*Anemopsis* Hook. & Arn.	Xi, 1980; Liang, 1992; Smith & Stockey, 2007
		[2,3]*Houttuynia* Thunb.	Xi, 1980; Takahashi, 1986; Liang, 1992; Smith & Stockey, 2007
		[1]*Houttuynia cordata* Thunb. (Fig. 3I)	China, Yunnan, *Germplasm Bank of Wild Species collection expedition 09CS1221* (KUN)
		[2,3]*Saururus* L.	Xi, 1980; Liang, 1992; Sampson, 2000a; Smith & Stockey, 2007
		[1]*Saururus chinensis* (Lour.) Baill. (Fig. 3H)	China, Guangxi, cult., *S. D. Zhang APE011* (KUN)
Basal eudicots			
Proteales			
Nelumbonaceae	1 (1)	[2,3]*Nelumbo* Adans.*	Kuprianova & Tarasevich, 1983; Blackmore et al., 1995; Kreunen & Osborn, 1999; Perveen, 1999; Banks et al., 2007.
Sabiaceae	3 (2)	[2,3]*Sabia* Colebr.	Furness et al., 2007
		[2,3]*Meliosma* Blume	Furness et al., 2007
Trochodendrales			
Trochodendraceae	2 (2)	[2,3]*Tetracentron* Oliv.	Praglowski, 1975; Wang et al., 1984; Zavada & Dilcher, 1986
		[2,3]*Trochodendron* Siebold & Zucc.	Praglowski, 1975; Wang et al., 1984; Zavada & Dilcher, 1986
Ranunculales			
Berberidaceae	15 (1)	[2]*Mahonia* Nutt.*	Nowicke & Skvarla, 1981; Blackmore & Heath, 1984
Menispermaceae	70 (1)	[2]*Cissampelos* L.	Harley & Ferguson, 1982
Lardizabalaceae	8 or 9 (1)	[2]*Akebia* Decne.*	Blackmore et al., 1995; Wei & Li, 1995
Papaveraceae	20 (1)	[2,3]*Dicentra* Bernh.*	Stern, 1962; Blackmore et al., 1995
Eupteleaceae	1 (1)	[2,3]*Euptelea* Siebold & Zucc.	Praglowski, 1975; Wang et al., 1984; Zavada & Dilcher, 1986
Monocots			
Alismatales			
Potamogetonaceae	6 (1)	[2,3]*Potamogeton* L.*	Wang, 1990; Perveen, 1999
Juncaginaceae	4 (1)	[2,3]*Triglochin* L.*	Wang, 1990
Acorales			
Acoraceae	1 (1)	[2,3]*Acorus* L.	Rudall & Furness, 1997; Wang & Zhao, 2002
Gymnosperm outgroups			
Gnetales			
Gnetaceae	1 (1)	[2,3]*Gnetum* L.	Gullvåg, 1966; Yao et al., 2004
Welwitschiaceae	1 (1)	[2,3]*Welwitschia* Hook. f.*	Gullvåg, 1966; Rydin & Friis, 2005
Pinales			
Pinaceae	11 (1)	[2,3]*Pinus* L.*	Pacini et al., 1999
Cupressaceae	30 (1)	[2,3]*Metasequoia* Hu & W. C. Cheng	Xi, 1988; Xi & Wang, 1989; Liu & Basinger, 2009
Podocarpaceae	16 (1)	[2,3]*Podocarpus* L'Hér. ex Pers.	Del Fueyo, 1996

Appendix 1. Continued.

Taxonomic classification	Total # of genera per family (total # of genera sampled)	Genus name, with species voucher	References and voucher information
CYCADALES			
Zamiaceae	9 or 10 (1)	[2,3]*Zamia* L.	Zavada, 1983; Dehgan & Dehgan, 1988; Tekleva et al., 2007.
Cycadaceae	1 (1)	[2,3]*Cycas* L.	Tang et al., 1995; Tekleva et al., 2007
GINKGOALES			
Ginkgoaceae	1 (1)	[2,3]*Ginkgo* L.*	Pacini et al., 1999; Zhang et al., 2000; Tekleva et al., 2007

* Additional data were taken from PalDat – Palynological Database, an Online Publication on fossil and recent Pollen and Spores, at <http://www.paldat.org/>.

[1] A representative species was selected to show pollen morphological diversity (cf. Figs. 1–6).

[2] The genus is represented in analyses using the comprehensive method with Fitch parsimony (CFP) on 12 different phylogenetic trees (Table 1 and Figs. 7–9).

[3] The genus is represented in analyses using the democratic method with Fitch parsimony (DFP), maximum likelihood (DML), and hierarchical Bayesian inference (DHB) on the maximum likelihood tree based on data from Soltis et al., 2011; Tables 2, 3, and Fig. 10).

APPENDIX 2. Pollen characters and their states as defined in this study.

1. Dispersal unit: 0, monads; 1, tetrads.
2. Polarity: 0, apolar; 1, heteropolar; 2, isopolar (or subisopolar).
3. Symmetry (in polar view): 0, bilateral; 1, radial.
4. Basic shape: 0, boat-shaped; 1, globose.
5. Shape class: 0, oblate; 1, spheroidal; 2, prolate.
6. Outline in polar view (amb): 0, circular; 1, elliptic.
7. Size (diameter of largest axis), states defined by Walker and Doyle (1975): 0, small (10–24 μm); 1, medium (25–49 μm); 2, large (50–99 μm); 3, very large (100–199 μm); 4, gigantic (> 200 μm).
8. Aperture number: 0, zero; 1, one; 2, two; 3, three or multiples of three; 4, more than three.
9. Aperture position: 0, polar: distal; 1, polar: proximal; 2, equatorial; 3, global.
10. Aperture membrane: 0, smooth; 1, sculptured.
11. Ectoaperture shape: 0, colpate; 1, porate; 2, zonate; 3, spiral; 4, syncolpate.
12. Supratectal element: 0, absent; 1, present.
13. Supratectal element shape: 0, verrucate; 1, spinulate.
14. Supratectal element size: 0, < 0.5 μm; 1, ≥ 0.5 μm.
15. Tectum sculpture: 0, imperforate (psilate); 1, perforate/fossulate (micro- and macro-); 2, rugulate; 3, reticulate (including striato-reticulate); 4, areolate; 5, striate; 6, *Amborella*-type ("a gemmate [*sic*]-like appearance but consisting of small cupules constructed of coiled cylindrical strands"), described by Sampson (1993: 1); 7, rudimentary, i.e., partly fused hemispherical processes supported by columellae, terminology from Sampson and Foreman (1988).
16. Infratectum: 0, absent; 1, present.
17. Infratectum structure: 0, alveolate; 1, granulate; 2, intermediate (between granulate and columellate), terminology from Doyle (2005); 3, columellate.
18. Foot layer: 0, absent; 1, present.