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Angiosperm phylogeny inferred from sequences of four mitochondrial genes

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Abstract An angiosperm phylogeny was reconstructed in a maximum likelihood analysis of sequences of four mitochondrial genes, *atp1, matR, nad5*, and *rps3,* from 380 species that represent 376 genera and 296 families of seed plants. It is largely congruent with the phylogeny of angiosperms reconstructed from chloroplast genes *atpB, matK,* and *rbcL*, and nuclear 18S rDNA. The basalmost lineage consists of *Amborella* and Nymphaeales (including Hydatellaceae). Austrobaileyales follow this clade and are sister to the mesangiosperms, which include Chloranthaceae, *Ceratophyllum*, magnoliids, monocots, and eudicots. With the exception of Chloranthaceae being sister to *Ceratophyllum,* relationships among these five lineages are not well supported. In eudicots, Ranunculales, Sabiales, Proteales, Trochodendrales, Buxales, Gunnerales, Saxifragales, Vitales, Berberidopsidales, and Dilleniales form a basal grade of lines that diverged before the diversification of rosids and asterids. Within rosids, the COM (Celastrales–Oxalidales–Malpighiales) clade is sister to malvids (or rosid II), instead of to the nitrogen-fixing clade as found in all previous large-scale molecular analyses of angiosperms. Santalales and Caryophyllales are members of an expanded asterid clade. This study shows that the mitochondrial genes are informative markers for resolving relationships among genera, families, or higher rank taxa across angiosperms. The low substitution rates and low homoplasy levels of the mitochondrial genes relative to the chloroplast genes, as found in this study, make them particularly useful for reconstructing ancient phylogenetic relationships. A mitochondrial gene-based angiosperm phylogeny provides an independent and essential reference for comparison with hypotheses of angiosperm phylogeny based on chloroplast genes, nuclear genes, and non-molecular data to reconstruct the underlying organismal phylogeny.

Key words angiosperm, homoplasy, mitochondrial gene, molecular evolutionary rate, phylogeny, rosids.

Angiosperms are the main primary producers in most modern terrestrial ecosystems, and their evolution has had a major impact on the environment of the earth and the evolution of animals, fungi, and other plants (Friis et al., 1987; Dilcher, 2000; Algeo et al., 2001; Berner, 2001; Schneider et al., 2004; Moreau et al., 2006; Heinrichs et al., 2007; Newton et al., 2007; Hibbett & Matheny, 2009). Knowledge of their phylogeny is essential in the study of structure, function, and evolution of this important group of plants, and hence, has always been an important goal of research in botany and evolutionary biology (Takhtajan, 1969; Cronquist, 1988). Over the last two decades, unprecedented progress has been made in reconstructing angiosperm phylogeny, thanks to a large number of phylogenetic studies analyzing molecular and non-molecular data. Several large-scale analyses of chloroplast (*atpB, matK,* and *rbcL*) and nuclear (18S rDNA) gene sequences from all major angiosperm lineages have played an especially significant role in establishing the main framework of angiosperm phylogeny (Chase et al., 1993; Soltis et al., 1997, 2000; Savolainen et al., 2000a; Hilu et al., 2003). These analyses and many others focusing on specific groups of angiosperms together have clarified the following major issues. First, *Amborella*, Nymphaeales (including Hydatellaceae), and Austrobaileyales (the so-called ANITA grade) are established as the earliest divergent lineages of extant angiosperms (Mathews & Donoghue, 1999; Parkinson et al., 1999; Qiu et al., 1999, 2001, 2005; Soltis et al., 1999, 2000; Barkman et al., 2000; Graham & Olmstead, 2000; Savolainen et al., 2000a; Zanis et al., 2002; Borsch et al., 2003; Hilu et al., 2003; Stefanovic et al., 2004; Leebens-Mack et al., 2005; Jansen et al., 2007; Moore et al., 2007; Saarela et al., 2007; Goremykin et al., 2009). In retrospect, several pre-molecular systematic studies and pioneering molecular phylogenetic

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analyses had identified some members of ANITA as potentially the basalmost living angiosperms before the 1999–2000 wave of discoveries (Upchurch, 1984; Endress, 1986; Donoghue & Doyle, 1989; Martin & Dowd, 1991; Hamby & Zimmer, 1992; Qiu et al., 1993; Soltis et al., 1997). Second, eudicots are recognized as a monophyletic group (Chase et al., 1993; Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003). Again, comparative analyses of palynological data from extant and fossil plants had suggested such a hypothesis earlier (Walker & Doyle, 1975; Wolfe et al., 1975; Brenner, 1976; Doyle et al., 1977), and recognition of the deep division between angiosperms with monosulcate pollen and those with tricolpate pollen dated to an even earlier time (Wodehouse, 1935, 1936; Bailey & Nast, 1943; Hu, 1950). In fact, three phylogenetic analyses of mostly morphological data (Dahlgren & Bremer, 1985; Donoghue & Doyle, 1989; Loconte & Stevenson, 1991) recovered the monophyly of eudicots several years before the first large scale analysis of molecular data (Chase et al., 1993). Third, the general phylogenetic outlines of rosids and asterids are now well circumscribed (Chase et al., 1993; Soltis et al., 1997, 2000; Nandi et al., 1998; Savolainen et al., 2000a; Hilu et al., 2003). Fourth, a deep split within monocots is identified between Alismatales (including Araceae) and Petrosaviidae (sensu Cantino et al., 2007) (Chase et al., 1993, 2006; Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003; Qiu & Palmer, 2004), with *Acorus* placed as the sister to all other monocots (Chase et al., 1993; Duvall et al., 1993). Finally, Caryophyllales are placed as a close relative of asterids (Soltis et al., 1997, 2000; Hilu et al., 2003; Burleigh et al., 2009).

Despite this tremendous progress, some important problems remain, particularly in regard to relationships among major lineages within some large groups such as rosids and asterids, as well as the composition and placement of several key lineages related to diversification of the following large clades: angiosperms overall; eudicots; rosids; and asterids. Further, as indicated above, only three chloroplast genes and one nuclear gene have been used in the large-scale analyses, that is, of hundreds of taxa. The plant cell contains a third DNA-containing organelle, the mitochondrion, and several mitochondrial genes have been used at different levels for plant phylogenetic studies (Malek et al., 1996; Beckert et al., 1999, 2001; Parkinson et al., 1999; Qiu et al., 1999, 2005, 2006a, 2006b, 2007; Vangerow et al., 1999; Barkman et al., 2000, 2007; Bowe et al., 2000; Chaw et al., 2000; Anderberg et al., 2002; Davis et al., 2004; Dombrovska & Qiu, 2004; Wikstrom & Pryer, 2005; Forrest et al., 2006; Petersen et al., 2006; Zhu et al., 2007; Jian et al., 2008; Ran et al., 2009; Wurdack & Davis, 2009). Hence, it is important to explore the potential of mitochondrial genes for a full-scale angiosperm phylogeny reconstruction, so that an accurate organismal phylogeny, with information from all three genomes and non-molecular data, can be reconstructed (Doyle, 1992; Qiu & Palmer, 1999; Delsuc et al., 2005).

An angiosperm-wide phylogenetic study using mitochondrial gene sequences can serve several purposes. First, it can help to assess how accurately the phylogeny reconstructed with the chloroplast and nuclear genes represents the underlying organismal phylogeny. Theoretically, it can be argued that the true organismal phylogeny can never be known. In practice, this phylogeny can be inferred using multiple sources of information from organisms, such as gene sequences from all three genomes in the plant cell, morphology, and other non-molecular data. The more similar the phylogenetic hypotheses inferred from different data sources, the more likely that the true underlying plant phylogeny has been reconstructed. Even though the studies using the three chloroplast genes and one nuclear gene have established the main framework of angiosperm phylogeny, it is always desirable to assess the results with data from the mitochondrial genome, which represents a under-utilized and independent source of information. The recent short history of molecular systematics has provided some examples on gene- or genome-specific biases in phylogenetic reconstruction. The enigmatic genus *Ceratophyllum* has been shown to have different positions in analyses of different genes (Les et al., 1991; Chase et al., 1993; Soltis et al., 1997; Savolainen et al., 2000a; Hilu et al., 2003; Qiu et al., 2006a) or different portions of the same set of chloroplast genes (Goremykin et al., 2009). An analysis of animal mitochondrial genes reveals that there can be genome-wide noise in phylogenetic reconstruction, which is likely generated by over-representation of proteins with hydrophobic domains (Naylor & Brown, 1997). Chloroplast and mitochondrial genomes in plants both encode a disproportionately large set of *trans*-membrane domain-rich proteins (Jobson & Qiu, 2008), and are thus likely to be under selection of special evolutionary forces and contain certain genome-wide phylogenetic noise. Second, it is possible that mitochondrial genes may offer some insights for problems that have not been solved with chloroplast and nuclear genes. The generally lower point mutation rates of mitochondrial genes compared to chloroplast genes (Wolfe et al., 1987; Palmer & Herbon, 1988) should make them more suitable for unraveling more ancient diversification patterns. Finally, two interesting molecular evolutionary phenomena, namely, horizontal transfer (Bergthorsson et al., 2003; Won & Renner, 2003; Davis & Wurdack, 2004; Mower et al.,

2004) and dramatic evolutionary rate acceleration (Cho et al., 2004; Parkinson et al., 2005) have been reported for some mitochondrial genes and lineages over the last few years. These phenomena can potentially affect the usefulness of mitochondrial genes in phylogenetic studies, and a broad survey like this one is likely to provide a realistic estimate as to how widespread these phenomena are across angiosperms.

1 Material and methods

A total of 380 species level operational taxonomic units (OTUs) were included in this study, which represented 376 genera and 296 families of all APG III (Angiosperm Phylogeny Group, 2009) orders and non-ordinal families except Petrosaviales, Picramniales, and Dasypogonaceae (Table 1). Eight diverse gymnosperms were included as the outgroup. This taxon sampling scheme was designed to reconstruct an overall angiosperm phylogeny, to resolve relationships among major clades of angiosperms, and to identify the composition and placement of some key clades involved in the origins of angiosperms, eudicots, rosids, and asterids.

Four mitochondrial genes, *atp1* (ATPase subunit 1), *matR* (a group II intron-encoded maturase), *nad5* (NADH dehydrogenase subunit 5), and *rps3* (ribosomal protein S3), were selected for sequencing, with approximately 1.0, 1.6, 1.1, 1.4 kb sequenced, respectively. Among these genes, *atp1* and *matR* have been widely used over the last 10 years, but *nad5* and *rps3* have only recently received attention from plant systematists (Qiu et al., 2006a; Jian et al., 2008; Ran et al., 2009; Wurdack & Davis, 2009). Analyses on how these four genes performed in an angiosperm-wide phylogenetic study will be described below.

The methods of DNA extraction, gene amplification, and sequencing are the same as reported before (Qiu et al., 2006a), the only modification being that nested PCR was used in some cases to improve amplification success rate. All primers were newly designed (Table 2) except those for *nad5*, which were published in Qiu et al., 2006a.

A total of 900 new sequences were generated in this study; the rest were retrieved from GenBank. Their accession numbers and voucher information are provided in Table 1. Sequences were aligned using ClustalX (Thompson et al., 1997) followed by manual adjustment. Because point substitution rate was low in these genes (see below), it is relatively easy to locate mis-aligned regions and bring them to proper positions by aligning neighboring regions that share high levels of sequence identity. For each of the four genes, a single gene analysis was carried out using the parsimony method implemented in PAUP*4.0b10 (Swofford, 2003), to ensure that no contaminated or fundamentally incongruent sequences due to horizontal gene transfer were present in the dataset. The data were then combined to construct two matrices. The first contained 380 OTUs, and most OTUs had all four genes except for a small number with only one, two, or three genes. Because the OTUs with a significant amount of missing data or highly divergent sequences could artificially lower bootstrap (BS) values of the clades to which these OTUs belonged (Felsenstein, 2004), they were removed and a second matrix was constructed (see Table 1 for removed taxa). This matrix contained 356 OTUs, with each OTU having at least three genes.

Maximum likelihood analyses were carried out using a web version of RAxML 7.0.4 (Stamatakis et al., 2008) on the CIPRES cluster at the San Diego Supercomputer Center (Miller et al., 2009). The matrices were analyzed as a single partition under the GTR+G model of nucleotide evolution. Maximum likelihood BS analyses were carried out with 500 replicates of character resampling. The automatic estimation of BS replicate number in RAxML showed that for both matrices 150 replicates were sufficient. Comparison of the 150 and 500 replicate BS analysis trees showed that BS values were indeed similar.

To understand how evolutionary rates and homoplasy levels of these four mitochondrial genes may have influenced their performance in reconstructing angiosperm phylogeny, especially relative to those of the four genes (chloroplast *atpB, matK*, and *rbcL*, and nuclear 18S rDNA) that have been used in the previous large-scale angiosperm phylogenetic analyses, two more analyses were carried out after the phylogenetic analyses. To make the results comparable, a total of 272 OTUs that have sequence for each of the eight genes were selected. For the four mitochondrial genes, the sequence accession numbers are shown in Table 1. For chloroplast *atpB, matK*, and *rbcL*, and nuclear 18S rDNA, the data were retrieved from GenBank and are available from the corresponding author upon request. Eight single gene matrices were assembled accordingly. A consensus angiosperm phylogeny was drawn based on the results of this study and the previous large-scale analyses of chloroplast *atpB, matK*, and *rbcL*, and nuclear 18S rDNA (Chase et al., 1993; Soltis et al., 1997, 2000; Savolainen et al., 2000a; Hilu et al., 2003), and this tree is shown in Fig. S1.

The first analysis was to calculate the evolutionary rate of each gene. Enforcing the consensus topology of angiosperms, a phylogram was inferred from each gene matrix assuming a GTR+I+G model of nucleotide

Table 1 Taxa and sequences used in this study

Species	Family	*atp1*	*matR*	*nad5*	*rps3*	Voucher/DNA number
Acanthus mollis L.	Acanthaceae	GU350950	AY289667, *A. ebracteatus* Vahl	GU351329	GU351549	Qiu 05019
†*Acorus calamus* L.	Acoraceae	AF197621	ND	DQ406951	ND	No new data
†*Acorus gramineus* Soland.	Acoraceae	AF197622	ND	DQ406994	GU351550	Qiu 97131
Actinidia arguta Miq.	Actinidiaceae	GU350951	AY163745, *A. rubricaulis* Dunn	GU351330	GU351551	Qiu 95102
Aextoxicon punctatum Ruiz & Pav.	Aextoxicaceae	GU350952	GU351143	GU351331	GU351552	Th Borsch 3459 (BONN)
Agave attenuata Salm-Dyck	Agavaceae	AY299703, *A. ghiesbreghtii* K. Koch	DQ401408	DQ407009	GU351553	Qiu 96067
Ailanthus altissima Swingle	Simaroubaceae	GU350953	GU351144	ND	GU351554	Qiu 96141
Albizia julibrissin Durazz.	Fabaceae	GU350954	GU351145	GU351332	GU351555	A.A. Reznicek 11735/(Qiu 05026)
Alisma plantago-aquatica L.	Alismataceae	AF197717	AF197815	DQ406947	GU351556	Qiu 96177
Allium cepa L.	Alliaceae	DQ401321	DQ401400	DQ407007	GU351557	Qiu 94060
Alluaudia ascendens Drake	Didiereaceae	GU350955	AF520129, *A. humbertii* Choux	AF520129, *A. humbertii* Choux	ND	Qiu 97036
Alnus rugosa Spreng.	Betulaceae	GU350956	GU351146	GU351333	GU351558	Qiu 05007
Alseuosmia macrophylla A. Cunn.	Alseuosmiaceae	GU350957	GU351147	GU351334	GU351559	Morgan 2141 (WS)
Altingia excelsa Noronha	Altingiaceae	EF370686	EF370708	EF370726	EF370747	Qiu 93006
Amborella trichopoda Baill.	Amborellaceae	DQ007412	AF197813	AY832180	GU351560	Qiu 97123
Ancistrocladus tectorius Merr.	Ancistrocladaceae	GU350958	GU351148	GU351335	GU351561	Y.P. Hong 99394 (PE)
Androsace samentosa Wall.	Primulaceae	GU350959	GU351149	GU351336	GU351562	A.A. Reznicek 11751/(Qiu 05029)
Anisophyllea sp.	Anisophylleaceae	GU350960	GU351150	GU351337	GU351563	P. Boyce 758 (K)
Anisoptera marginata Korth.	Dipterocarpaceae	GU350961	GU351151	GU351338	GU351564	Chase 2486 (K)
Annona muricata L.	Annonaceae	AF197695	AF197766	DQ406917	GU351565	Qiu 90031
Antirrhinum majus L.	Plantaginaceae	GU350962	GU351152	GU351339	GU351566	Qiu 05011
Aphanopetalum resinosum Endl.	Aphanopetalaceae	EF370687	EF370709	EF370727	GU351567	Bradford 845
Aphloia theiformis Benn.	Aphloiaceae	GU350963	GU351153	GU351340	GU351568	REU 10012 (REU)
Apium graveolens L.	Apiaceae	GU350964	GU351154	GU351341	GU351569	Qiu 05032
Arabidopsis thaliana Schur.	Brassicaceae	NC_001284	Y08501	NC_001284	NC_001284	No new data
Arbutus canariensis Duhamel	Ericaceae	GU350965	GU351155	GU351342	GU351570	Albach 241 (K)
Aristolochia macrophylla Lam.	Aristolochiaceae	AF197669	AF197732	GU351343	GU351571	Qiu 91019
Ascarina sp.	Chloranthaceae	AF197667	AF197755	DQ406865	GU351572	Thien 500 (NO)
Asparagus officinalis L.	Asparagaceae	AF197713	AF197736	DQ407000	GU351573	Qiu 94063
Atherosperma moschatum Labill	Atherospermaceae	AF197683	AF197799	DQ406929	GU351574	Qiu 92007
Austrobaileya scandens C.T. White	Austrobaileyaceae	AF197664	AF197742	DQ406986	GU351575	Qiu 90030
Barbeuia madagascariensis Steud.	Barbeuiaceae	GU350966	GU351156	GU351344	GU351576	J.L. Zarucetti 7407 (K)
Basella alba L.	Basellaceae	GU350967	GU351157	GU351345	GU351577	Qiu 02055
Batis maritima L.	Bataceae	GU350968	GU351158	GU351346	GU351578	(Qiu 96206)
Begonia sp.	Begoniaceae	GU350969	GU351159	GU351347	GU351580	Qiu 05009
Berberidopsis beckleri Veldkamp	Berberidopsidaceae	DQ401303	DQ401394	DQ406898	GU351581	Qiu 98040
Berzelia lanuginose	Bruniaceae	GU350970	GU351160	GU351348	GU351582	Kirstenbosch 75–89
Bistorta sp.	Polygonaceae	ND	GU351161	GU351349	GU351583	A.A. Reznicek 11752/(Qiu 06003)
†*Bixa orellana* L.	Bixaceae	ND	GU351162	ND	ND	Qiu 97032
Blandfordia grandiflora R. Br.	Blandfordiaceae	AY299727	DQ401412	DQ406966	GU351584	Qiu 97016
Borago officinalis L.	Boraginaceae	GU350971	GU351163	GU351350	GU351585	Chase 2746 (K)
Bougainvillea alba	Nyctaginaceae	AY818932, *B. glabra* Choisy	ND	GU351351	GU351586	(Qiu M67)
Brasenia schreberi J. Gmelin	Nymphaeaceae	AF197640	AF197728	DQ406956	GU351587	Qiu 91031
Brexia madagascariensis	Celastraceae	GU350972	GU351164	GU351352	GU351588	Chase 17719 (K)
Bruguiera gymnorhiza Sav.	Rhizophoraceae	GU350973	GU351165	GU351353	GU351589	(Qiu 96188)

Continued.

Table 1 Continued

Species	Family	*atp1*	*matR*	*nad5*	*rps3*	Voucher/DNA number
Brunellia sp.	Brunelliaceae	GU350974	DQ110330, *B. acutangula* Humb. & Bonpl.	GU351354	GU351590	G.P. Lewis 3366 (K)
Bursera sp.	Burseraceae	GU350975	GU351166	GU351355	GU351591	Qiu 94206
Buxus sempervirens L.	Buxaceae	AF197636	AF197786	DQ406879	GU351592	Qiu 97057
Byblis liniflora Salisb.	Byblidaceae	GU350976	GU351167	GU351356	GU351593	Qiu 95128–2
Cabomba sp.	Nymphaeaceae	AF197641	AF197729	DQ406957	GU351594	Qiu 97027
Calceolaria integrifolia Murr.	Calceolariaceae	GU350977	GU351168	GU351357	GU351595	Chase 2850 (K)
Calycanthus floridus L.	Calycanthaceae	AF197678	AF197777	DQ406922	GU351596	Qiu 94155
Calyptrotheca somalensis Gilg	Portulacaceae	GU350978	GU351169	GU351358	GU351597	(Qiu M257), no voucher
Camellia japonica L.	Theaceae	AF420952, *C. sinensis* Kuntze	AF421034, *C. sinensis* Kuntze	DQ406870	GU351598	Qiu 90999
Campanula rotundidolia	Campanulaceae	AY741815, *C. garganica* Ten.	GU351170	GU351359	GU351599	A.A. Reznicek 11819/(Qiu 05033)
Cananga odorata Hook. F. & Thomson	Annonaceae	AF197700	AF197763	GU351360	GU351600	Chase 219 (NCU)
Canella winteriana Gaertn.	Canellaceae	AF197676	AF197757	DQ406920	GU351601	Qiu 90017
Cannabis sativa L.	Cannabaceae	GU350979	GU351171	GU351361	GU351602	Chase 2992 (K)
Capparis cynophallophora L.	Brassicaceae	GU350980	GU351172	GU351362	GU351603	(Qiu 96210)
Carica papaya L.	Caricaceae	GU350982	GU351173	GU351363	GU351604	Qiu 94050
Carludovica palmata Ruiz & Pavon	Cyclanthaceae	AF197707	AF197734	DQ406948	GU351605	Qiu 97021
Catalpa fargeseii Bur.	Bignoniaceae	AY741840, *C. bignonioides* Walter	GU351174	GU351364	GU351606	Qiu 95099
Caulophyllum thalictroides Regel	Berberidaceae	GU350983	GU351175	GU351365	ND	A.A. Reznicek 11733/(Qiu 05023)
Ceanothus sp.	Rhamnaceae	GU350984	GU351176	GU351366	GU351607	Qiu 96098
Celosia cristata L.	Amaranthaceae	GU350985	GU351177	GU351367	GU351608	Qiu 94153
Celtis yunnanensis C.K. Schneid.	Cannabaceae	GU350986	GU351178	GU351368	GU351609	Qiu P90002
Cephalotus follicularis Labill.	Cephalotaceae	GU350987	GU351179	GU351369	ND	R.W. Jobson CU-1023
Ceratophyllum demersum L.	Ceratophyllaceae	AF197627	AF197730	DQ406988	GU351610	Qiu 95003
Ceratophyllum submersum L.	Ceratophyllaceae	AF197628	ND	DQ406989	GU351611	Qiu 98088
Cercidiphyllum japonicum Siebold & Zucc.	Cercidiphyllaceae	EF370688	EF370710	EF370728	EF370748	Qiu 93013
Chamaedorea tenella H. Wendl.	Arecaceae	DQ401295	DQ401392	DQ407003	GU351612	Qiu 95075
Chloranthus multistachys Pei	Chloranthaceae	AF197665	AF197753	DQ406864	GU351613	K. Wurdack 92–0010
Choristylis rhamnoides Harv.	Iteaceae	GU350988	GU351180	GU351370	GU351614	Chase 9646 (K)
Chrysolepis sempervirens Hjelmq.	Fagaceae	GU350990	GU351182	GU351372	GU351616	Qiu P90007
Cinnamodendron ekmanii Sleum.	Canellaceae	AF197677	AF197758	DQ406921	GU351617	T. Zanoni & F. Jimenez 47067
Citrus limon Burm.f.	Rutaceae	GU350991	GU351183	GU351373	GU351618	Qiu 94085
Clavija eggersiana Mez	Theophrastaceae	AF420918, *C. domingensis* Urb. & Ekman	AF420995, *C. domingensis* Urb. & Ekman	GU351374	GU351619	Chase 216
Claytonia virginica L.	Portulacaceae	GU350992	GU351184	GU351375	GU351620	Qiu 06001
Clethra barbinervis Siebold & Zucc.	Clethraceae	GU350993	AF520204, *C. alnifolia* L.	GU351376	GU351621	Qiu 95103
Clidemia petiolaris Triana	Melastomaceae	GU350994	GU351185	GU351377	GU351622	Chase 2534 (K)
Clusia rosea Jacq.	Clusiaceae	GU350995	GU351186	GU351378	GU351623	Qiu 05042
Connarus championii Thwaites	Connaraceae	GU350996	GU351187	GU351379	GU351624	Chase 15937 (K)
Coriaria myrtifolia L.	Coriariaceae	GU350997	GU351188	GU351380	GU351625	Chase 245 (NCU)
Cornus florida Hook.	Cornaceae	AF420915, *C. suecica* L.	AF420990, *C. suecica* L.	DQ407012	GU351626	Qiu 96142
Corokia cotoneaster Raoul	Argophyllaceae	GU350998	GU351189	GU351381	GU351627	Chase 2752 (K)
Corylopsis glabrescens Franch. & Sav.	Hamamelidaceae	EF370689	EF370711	EF370729	EF370749	Qiu 94158

Continued.

Table 1 Continued

Species	Family	*atp1*	*matR*	*nad5*	*rps3*	Voucher/DNA number
Couroupita guianensis Aubl.	Lecythidaceae	AY725907	GU351190	GU351382	GU351628	Qiu 97025
Croomia pauciflora Miq.	Stemonaceae	AF197708	AF197735	DQ406939	GU351629	Qiu 97096
Crossosoma californicum Nutt.	Crossosomataceae	GU350999	GU351191	GU351383	GU351630	Beier (UPS)
Crypteronia paniculata Blume	Crypteroniaceae	GU351000	GU351192	GU351384	GU351631	Y.P. Hong 99203/(Qiu 05071) (PE)
Cryptocarya meissneriana Frodin	Lauraceae	AF197702	AF197804	DQ406932	GU351632	Qiu 98048
Cucurbita pepo Lour.	Cucurbitaceae	GU351001	GU351193	GU351385	GU351633	Qiu 05018
Cupaniopsis anacardioides Radlk.	Sapindaceae	GU351002	GU351194	GU351386	GU351634	Qiu 98053
Curtisia dentata C.A. Sm.	Curtisiaceae	GU351003	GU351195	GU351387	GU351635	(Qiu M299), no voucher
Cycas revoluta Thunb.	Cycadaceae	AF197623	AF197720	AJ130743	AY345867	No new data
Cyrilla racemiflora L.	Cyrillaceae	AF420922	AY725892	GU351388	GU351636	Qiu 95109
†*Dampiera diversifolia* de Vriese	Goodeniaceae	ND	GU351196	GU351389	ND	Qiu 97144
Daphnandra micrantha Benth.	Atherospermaceae	AF197684	AF197800	DQ406977	GU351637	Qiu 97015
Daphniphyllum sp.	Daphniphyllaceae	EF370691	EF370712	EF370730	EF370750	Qiu 94162
Datisca cannabina L.	Datiscaceae	GU351004	GU351197	GU351390	GU351638	Qiu 97102
Decaisnea fargesii Franch.	Lardizabalaceae	GU351005	GU351198	GU351391	GU351639	Qiu 02094
Degeneria vitiensis I.W. Bailey & A.C. Sm.	Degeneriaceae	AF293752	AF197771	DQ406991	*GU351640*	John J. Miller 1189–63
Desfontainia spinosa Ruiz & Pav.	Columelliaceae	GU351006	GU351199	GU351392	GU351641	Chase 6419 (K)
Dicentra sp.	Fumariaceae	AF197649	AF197796	DQ406890	GU351642	Qiu 95026
Didymeles perrieri Olivier	Buxaceae	AF197637	AF197811	DQ406993	GU351643	O. Andrianantoanina 387 (MO)
Dillenia indica L.	Dilleniaceae	DQ401306	AY163747	DQ406882	GU351644	Qiu 95129
Dioncophyllum tholloni Baill.	Dioncophyllaceae	GU351007	AF520129, *A. humbertii* Choux	GU351393	GU351645	A.F. Bradley et al. 1107 (MO)
Dioscorea sp.	Dioscoreaceae	AF197709	AF197737	DQ406959	GU351646	Qiu 94044
Diospyros virginiana L.	Ebenaceae	GU351008	AF520202, *D. mollifolia* Rehder & Wilson	GU351394	GU351647	Qiu 94106
Dipentodon sinicus Dunn	Dipentodontaceae	GU351009	AY121494	GU351395	ND	Forrest 26561 (K)
Dipsacus sp.	Dipsacaceae	AY741814, *D. fullonum* S.G. Gmel.	AY453093, *D. fullonum* S.G. Gmel.	GU351396	GU351648	Qiu 95111
Donatia fascicularis Forst.	Stylidiaceae	GU351010	GU351200	GU351397	ND	Morgan 2142 (WS)
Drimys winteri J.R. Forster & G. Forster	Winteraceae	AF197673	AF197781	DQ406919	GU351649	Qiu 90016
Drosera regia Stephens	Droseraceae	GU351011	GU351201	GU351398	GU351650	Steve Williams D18
Drosophyllum lusitanicum Link.	Drosophyllaceae	GU351012	GU351202	GU351399	GU351651	Steve Williams D100
Drypetes perreticulata Gagnep.	Putranjivaceae	GU351013	GU351203	GU351400	ND	Y.P. Hong 99310/(Qiu 05058) (PE)
Ehretia anacua I.M. Johnst.	Boraginaceae	GU351014	GU351204	ND	GU351652	(Qiu 96209)
Elaeagnus sp.	Elaeagnaceae	GU351015	ND	GU351401	GU351653	Qiu 95028
Elaeocarpus obovatus G. Don	Elaeocarpaceae	GU351016	GU351205	GU351402	GU351654	Qiu 98054
†*Elatine hexandra* DC.	Elatinaceae	ND	AY674507, *E. triandra* Schkuhr	ND	GU351655	Qiu 99051
Eremosyne pectinata Endl.	Escalloniaceae	GU351017	GU351206	GU351403	ND	Annels & Hearn 4795 (PERTH)
Escallonia rubra spp. *macrantha*	Escalloniaceae	GU351018	GU351207	GU351404	GU351656	Qiu 02081
Eschscholzia californica Cham.	Papaveraceae	GU351019	GU351208	GU351405	GU351657	Qiu 05049
Eucommia ulmoides Oliver	Eucommiaceae	DQ401311	DQ401387	DQ406872	GU351658	Qiu 91024
Euonymus sp.	Celastraceae	GU351020	GU351209	GU351406	GU351659	Qiu 94190

Continued.

Table 1 Continued

Species	Family	*atp1*	*matR*	*nad5*	*rps3*	Voucher/DNA number
Euphorbia milii var. *splendens* Desmoul.	Euphorbiaceae	DQ401317	AY674512, *E. polychroma* Kern.	DQ406908	GU351660	Qiu 94056
Eupomatia bennettii F. Muell.	Eupomatiaceae	AF197692	AF197772	DQ406927	GU351661	Qiu 90022
Euptelea polyandra Sieb. & Zucc.	Eupteleaceae	AF197650	AF197787	DQ406873	GU351662	Qiu 95098
Exbucklandia longipetala H.T. Chang	Hamamelidaceae	EF370692	EF370713	EF370731	EF370751	Qiu 93004
Floerkea proserpinicoidese	Limnanthaceae	GU351021	GU351210	GU351407	GU351663	A.A. Reznicek 11750/(Qiu 06004)
Frankenia pulverulenta L.	Frankeniaceae	GU351022	GU351211	GU351408	ND	Collenette 6/93 (K)
Gaiadendron sp.	Loranthaceae	DQ110147, *G. punctatum* G.Don	GU351212	GU351409	GU351664	N. Munoz et al. 102 (MO)
Galax urceolata Brummitt	Diapensiaceae	AF420929	AF421007	GU351410	GU351665	Qiu 02069
Galbulimima belgraveana Sprague	Himantandraceae	AF197693	AF197773	GU351411	GU351666	Qiu 90034
Galium sp.	Rubiaceae	GU351023	GU351213	GU351412	GU351667	Qiu 95025
Garrya elliptica Lindl.	Garryaceae	GU351024	AY453095	GU351413	GU351668	Chase 1098 (K)
Geissois biagiana F. Muell.	Cunoniaceae	GU351025	GU351214	GU351414	GU351669	Qiu 97011
†*Geissoloma marginata* A. Juss.	Geissolomataceae	GU351026	ND	GU351415	ND	Savolainen GMA1 (G)
Gelsemium sp.	Gelsemiaceae	AY741816, *G. sempervirens* J.St.-Hil.	GU351215	GU351416	GU351671	Qiu 95096
Gentiana macrophylla Pall.	Gentianaceae	GU351027	GU351216	ND	GU351672	Qiu 96090
†*Geranium sanguinieum* L.	Geraniaceae	ND	AY121488, *G. wilfordii*	GU351417	ND	A.A. Reznicek 11731/(Qiu 05036)
Ginkgo biloba L.	Ginkgoaceae	AF197625	AF197722	AJ409109	GU351673	Qiu 94015
Glaucidium palmatum Siebold & Zucc.	Ranunculaceae	GU351028	GU351217	GU351418	GU351674	A.A. Reznicek 10719/(Qiu 05025)
Gnetum gnemon L.	Gnetaceae	AF197617	AF197718	AJ409110	ND	no new data
†*Gomortega keule* Baill.	Gomortegaceae	ND	ND	GU351419	GU351675	M.F. Doyle III-6–1986/(Qiu 05048)
Gossypium arboreum Vell.	Malvaceae	GU351029	GU351218	GU351420	GU351676	Qiu 05015
Griselinia littoralis Raoul	Griseliniaceae	GU351030	AY453096, *G. racemosa* Taub.	GU351421	GU351677	Strybing Arboretum xy-2609
Guaiacum officinale L.	Zygophyllaceae	DQ401291	AY674517, *G. sanctum* L.	DQ406954	GU351678	Qiu 97035
Gunnera monoica Raoul	Gunneraceae	DQ401302	DQ401383	DQ406897	GU351679	Qiu 98071
Gyrocarpus sp.	Hernandiaceae	AF197701	AF197805	DQ406931, *G. americanus* Jacq.	GU351680	Chase 317 (NCU)
Halophytum ameghinoi Speg.	Halophytaceae	GU351031	GU351219	GU351422	ND	Qiu (M244), Tortosa, Bartoli, Chubut, nv
Haloragis erecta Schindl.	Haloragaceae	EF370693	EF370714	EF370732	EF370752	No new data
Hamamelis mollis Forb. & Hemsl.	Hamamelidaceae	DQ401289	AY453082, *H. vernalis* Sarg.	DQ407011	EF370753	Qiu 91035
Hedera helix L.	Araliaceae	DQ401310	DQ401390	DQ406955	GU351681	Qiu 98085
Hedycarya arborea J. R. & G. Forst.	Monimiaceae	AF197689	AF197806	DQ406909	GU351682	Qiu 90028
Hedyosmum arborescens Sw.	Chloranthaceae	AF197668	AF197756	DQ406863	GU351683	Chase 338 (NCU)
Heisteria parvifolia Sm.	Olacaceae	GU351032	GU351220	GU351423	GU351684	Qiu 99018
Helianthemum grandiflorum DC.	Cistaceae	GU351033	GU351221	GU351424	ND	A.A. Reznicek 11775/(Qiu 05028)
Helianthus annuus L.	Asteraceae	X55963	GU351222	GU351425	AF319170	(Qiu MD34), no voucher
Helwingia japonica C. Morren & Decne.	Helwingiaceae	GU351034	GU351223	GU351426	GU351685	Qiu 99031
Hernandia ovigera L.	Hernandiaceae	DQ007413	DQ007424	DQ406930	GU351686	Qiu 01007
Heuchera sp.	Saxifragaceae	DQ401290	DQ401398	DQ406953	EF370754, *H. micrantha* Lindl.	Qiu 95076

Continued.

Table 1 Continued

Species	Family	*atp1*	*matR*	*nad5*	*rps3*	Voucher/DNA number
Hibbertia cuneiformis Gilg	Dilleniaceae	GU351035	GU351224	GU351427	GU351687	Qiu 97020
Hirtella jamaicensis Urb.	Chrysobalanaceae	GU350989	GU351181	GU351371	GU351615	Qiu 01010
Houttuynia cordata Thunb.	Saururaceae	AF197632	AF197749	DQ406980	GU351688	Qiu 92016
Hua gabonii De Wild.	Huaceae	GU351036	GU351225	GU351428	GU351689	J. J. Wiernga 3177 (WAG)
Humiria balsamifera Aubl.	Humiriaceae	GU351037	GU351226	GU351429	GU351690	W. R. Anderson 13654 (MICH)
Hydnocarpus anthelminthica Pierre & Gagnep.	Achariaceae	GU351038	GU351227	GU351430	GU351691	Y.P. Hong H001/(Qiu 05070) (PE)
Hydrangea arborescens L.	Hydrangeaceae	GU351039	AY453091, *H. macrophylla* Ser.	GU351431	GU351692	Qiu 95021
Hydrastis canadensis Poir.	Ranunculaceae	GU351040	GU351228	GU351432	GU351693	Z.D. Chen 2002016/(Qiu 05066) (PE)
†*Hydrolea ovata* Nutt. ex Choisy	Hydroleaceae	GU351041	GU351229	GU351433	GU351694	Olmstead 89–009 (COLO)
Hydrophyllum virginianum L.	Boraginaceae	GU351042	GU351230	GU351434	GU351695	A.A. Reznicek 7887/(Qiu 05031)
Hypecoum imberbe Sibth. & Sm.	Fumariaceae	GU351043	GU351231	GU351435	GU351696	Chase 528 (K)
Hypericum sp.	Hypericaceae	GU351044	GU351232	GU351436	GU351697	Qiu 95082
Icacina mannii Oliv.	Icacinaceae	GU351045	GU351233	GU351437	GU351698	Chase 2244 (K)
Idiospermum australiense S.T. Blake	Calycanthaceae	AF197680	AF197779	DQ406974	GU351699	Qiu 91042
Idria columnaria Kellogg	Fouquieriaceae	GU351046	GU351234	GU351438	GU351700	Qiu 95065
Ilex sp.	Aquifoliaceae	AY741812, *I. verticillata* A.Gray	AY453090, *I. aquifolium* Lour.	DQ406884	GU351701	Qiu 94038
Illicium floridanum Ellis	Schisandraceae	AF197663	AF197740	DQ406985	GU351702	Qiu 61
Impatiens pallida Nutt.	Balsaminaceae	AF420933, *I. parviflora* DC.	AF421011, *I. parviflora* DC.	DQ406952	GU351703	Qiu 95124
Ipomoea batatas Poir.	Convolvulaceae	AY596672	GU351235	GU351439	GU351704	Qiu 96152
Iris sp.	Iridaceae	DQ401300	DQ401386	DQ407006	GU351705	Qiu 95091
Itea virginica L.	Iteaceae	EF370696	EF370716	EF370735	EF370755	No new data
Ixerba brexioides A. Cunn	Strasburgeriaceae	GU351047	GU351236	GU351440	GU351706	P.J. de Lange 5809 (AK285208)
Ixonanthes icosandra var. *cuneata*	Ixonanthaceae	GU351048	GU351237	GU351441	GU351707	Chase 1301 (K)
Juglans cinerea L.	Juglandaceae	GU351049	GU351238	GU351442	GU351708	Qiu 96022
Kadsura japonica Dunal	Schisandraceae	AF197661	AF197738	DQ406971	GU351709	Qiu 94159
Kalanchoe pinnata Pers.	Crassulaceae	EF370697	EF370717	EF370736	EF370756	Qiu 94118
Krameria lanceolata Torr.	Krameriaceae	GU351050	GU351239	GU351443	GU351710	Simpson 88–05-1–1 (MICH)
Lactoris fernandeziana Phil.	Lactoridaceae	AF197710	AF197812	DQ406910	ND	Chase 1014 (K)
Lamium sp.	Lamiaceae	DQ401312	DQ401385	DQ406871	GU351711	Qiu 95019
†*Lampranthus emarginatus* N.E. Br.	Aizoaceae	ND	GU351240	GU351444	ND	Qiu 94115
Lardizabala biternata Ruiz & Pavon	Lardizabalaceae	AF197643	Qiu97135	DQ406867	GU351712	Qiu 97135
Laurus nobilis L.	Lauraceae	AF197682	AF197798	DQ406923	GU351713	Qiu 94209
Leea guineensis G. Don.	Vitaceae	DQ401304	AY674530	DQ406899	GU351714	Qiu 97034
Lilium sp.	Liliaceae	AY394729, *L. tigrinum* Ker Gawl.	DQ401403	DQ407002	GU351715	Qiu 96072
Limeum africanum Moq.	Limeaceae	GU351051	GU351241	GU351445	GU351716	Goldblatt et al. 11512
Limonium tartaricum	Plumbaginaceae	GU351052	GU351242	GU351446	GU351717	Qiu 96151
Linnaea borealis L.	Linnaeaceae	GU351053	GU351243	GU351447	GU351718	Qiu 05035
Liquidambar styraciflua L.	Hamamelidaceae	EF370698	EF370718	EF370737	EF370757	Qiu 95089
Liriodendron chinense Sarg.	Magnoliaceae	AF197690	AF197774	DQ406926	GU351719	Qiu 28
Lomandra obliqua J.F. Macbr.	Laxmanniaceae	DQ401296	DQ401380	DQ406942	GU351720	Qiu 98016
Lonicera sp.	Caprifoliaceae	GU351054	AY453088	GU351448	GU351721	Qiu 05010
†*Luculia intermedia* Hutch.	Rubiaceae	GU351055	ND	ND	ND	Howick, Lord, & McNamara HOMC1524 (K)

Continued.

Table 1 Continued

Species	Family	*atp1*	*matR*	*nad5*	*rps3*	Voucher/DNA number
†*Lythrum salicaria* L.	Lythraceae	ND	GU351244	GU351449	ND	A.A. Reznicek 11741/(Qiu 05040)
Maesa japonica Zoll.	Maesaceae	AF420937, *M. tenera* Mez	GU351245	GU351450	GU351722	A.M. Lu 2073/(Qiu 05060) (PE)
Magnolia tripetala L.	Magnoliaceae	AF197691	AF197770	DQ406916	GU351723	Qiu 3
Malpighia glabra L.	Malpighiaceae	GU351056	AF520187	GU351451	GU351724	Qiu 95044–1
Manilkara zapota P.Royen	Sapotaceae	AF420938	AF421016	GU351452	GU351725	Chase 129 (NCU)
Maranta leuconeura E. Morr.	Marantaceae	AY299801	DQ401410	DQ406943	GU351726	Qiu 95081
Marcgravia rectiflora Triana & Planch.	Marcgraviaceae	AF420939, *M.* sp.	AF421017, *M.* sp.	GU351453	GU351727	Qiu 01014
Mauloutchia chapelieri Warb.	Myristicaceae	AF197699	AF197769	DQ406960	GU351728	Qiu 99019
Medicago sativa L.	Fabaceae	GU351057	GU351246	GU351454	GU351729	(Qiu M61), no voucher
Melianthus major L.	Melianthaceae	GU351058	GU351247	GU351455	GU351730	Qiu 97029
Meliosma squmulata Hance.	Sabiaceae	AF197656	DQ007426	DQ406896	GU351731	Qiu 99002
Menispermum canadense Pall.	Menispermaceae	GU351059	GU351248	GU351456	GU351732	A.A. Reznicek 11732/(Qiu 05024)
Mentzelia floridana Torr. & A.Gray	Loasaceae	GU351060	GU351249	GU351457	ND	Qiu 96179
Menyanthes trifoliata L.	Menyanthaceae	GU351061	GU351250	GU351458	GU351733	A.A. Reznicek 11748/(Qiu 05034)
†*Metasequoia glyptostroboides* Hu & Cheng	Taxodiaceae	AF197619	ND	DQ406973	ND	Qiu 95084
Mirabilis jalapa L.	Nyctaginaceae	EU280980	GU351251	GU351459	GU351734	Qiu 05022
Mollugo verticillata L.	Molluginaceae	GU351062	GU351252	GU351460	ND	(Qiu M111), no voucher
Montinia caryophyllacea Thunb.	Montiniaceae	AY596706	GU351253	GU351461	GU351735	Williams 2833 (MO)
Morina longifolia Wall.	Morinaceae	GU351063	GU351254	GU351462	GU351736	Qiu 97121
Morus alba L.	Moraceae	GU351064	GU351255	GU351463	GU351737	Qiu 96020
Myodocarpus involucratus Dubard & R.Vig.	Myodocarpaceae	GU351065	GU351256	GU351464	ND	P. Lowry 4710 (MO)
Myrica cerifera L.	Myricaceae	GU351066	GU351257	GU351465	GU351738	Qiu 91036
Myriophyllum sp.	Haloragaceae	EF370699	EF370719	EF370738	EF370758	Qiu 95020
Myristica maingayi Hook. f.	Myristicaceae	AF197698, *M. fragrans* Houtt.	AF197768, *M. fragrans* Houtt.	DQ406967	GU351739	A.R. Khalit 15762/(Qiu M142) (Z)
Myrothamnus flabellifolia Welw.	Myrothamnaceae	GU351067	GU351258	GU351466	GU351740	P. Winter 72 (RAU, JHB)
Myrtus communis L.	Myrtaceae	GU351068	GU351259	GU351467	GU351741	Qiu 05043
Nandina domestica Thunb.	Berberidaceae	GU351069	GU351260	GU351468	GU351742	Qiu 05014
Nelumbo nucifera Gaertner	Nelumbonaceae	AF197654	AF197795	DQ406894	GU351743	Qiu 91028
Nepenthes × kosobe	Nepenthaceae	DQ401307	DQ401379	DQ406900	GU351744	Qiu 94164
Nerium oleander L.	Apocynaceae	GU351070	GU351261	GU351469	GU351745	Qiu 95048
Nicotiana tabacum L.	Solanaceae	AY596704	AY453113, *N. sylvestris* Speg.	NC_006581	BA000042	No new data
Nitraria retusa Asch.	Nitrariaceae	GU351071	GU351262	GU351470	GU351746	Chase 597 (K)
Nolina recurvata Hemsl.	Asparagaceae	DQ401301	DQ401405	DQ407008	GU351579	Qiu 96043
Nothofagus moorei Maiden	Nothofagaceae	DQ401292	DQ401401	DQ406905	GU351747	Qiu 98036
Nuphar sp.	Nymphaeaceae	AF197638	AF197726	DQ406982	GU351748	Qiu M114, no voucher
Nymphaea sp.	Nymphaeaceae	AF197639	AF197727	DQ406981	GU351749	Qiu 91029
Nyssa sylvatica Marshall	Cornaceae	GU351072	GU351263	GU351471	GU351750	Qiu 94156
Ochna serrulata Walp.	Ochnaceae	GU351073	GU351264	GU351472	GU351751	Qiu 97059
Oenothera berteroana Spach	Onagraceae	X04023, *O. biennis* L.	AY453083, *O. biennis* L.	X07566	X69140	No new data
Olinia emarginata Burtt Davy	Penaeaceae	GU351074	GU351265	GU351473	GU351752	Chase 6413 (K)
Oncidium sphacelatum Lindl.	Orchidaceae	DQ401299	DQ401393	DQ407005	GU351753	Qiu 94134
Oncotheca balansae Baill.	Oncothecaceae	GU351075	GU351266	GU351474	GU351754	Chase 2392 (K)
†*Opilia amentacea* Roxb.	Opiliaceae	ND	ND	GU351475	GU351755	Chase 1902 (K)
Opuntia sp.	Cactaceae	GU351076	GU351267	GU351476	ND	Qiu 05020
Orontium aquaticum L.	Araceae	AF197705	AF197745	DQ406996	GU351756	Qiu 97112
Oryza sativa L.	Poaceae	NC_007886	DQ401382	BA000029	BA000029	Qiu 01094
Oxalis sp.	Oxalidaceae	DQ401314	AY453111, *O. corniculata* L.	DQ406907	GU351757	Qiu 94028

Continued.

Table 1 Continued

Species	Family	*atp1*	*matR*	*nad5*	*rps3*	Voucher/DNA number
Pachysandra terminalis Siebold & Zucc.	Buxaceae	AF197634, *P. procumbens* Michx.	AF197784, *P. procumbens* Michx.	DQ406887, *P. procumbens* Michx.	GU351758	Qiu QL99028
Paeonia sp.	Paeoniaceae	GU351077	GU351268	GU351477	ND	Qiu 95090
Paeonia tenuifolia L.	Paeoniaceae	EF370700	EF370720	EF370739	ND	K. Kron 447 (NCU)
Paracryphia alticola Steenis	Paracryphiaceae	GU351078	GU351269	GU351478	GU351759	J.C. Pintaud 561 (K)
Parnassia grandiflora Raf.	Celastraceae	GU351079	GU351270	GU351479	GU351760	A.A. Reznicek 11734/(Qiu 05027)
Passiflora suberosa L.	Passifloraceae	DQ401315	AY453071, *P. edulis* Sims	DQ406902	GU351761	Qiu 95030
Peltanthera floribunda Benth.	Gesneriaceae	GU351080	GU351271	GU351480	GU351762	L.D. Vargas et al. 329 (MO)
Pennantia corymbosa J.R. Forst. & G. Forst.	Pennantiaceae	GU351081	GU351272	GU351481	GU351763	C. Gemmill s.n.
Pentaphragma sp.	Pentaphragmataceae	GU351082	GU351273	GU351482	GU351764	Duangjai 049 (BRUN)
Penthorum sedoides L.	Penthoraceae	EF370701	EF370721	EF370740	EF370760	Qiu 97114
Peperomia obtusifolia A. Dietr.	Piperaceae	AF197629	AF197814	DQ406924	GU351765	Qiu 94135
Pereskia grandifolia Haw.	Cactaceae	GU351083	GU351274	GU351483	ND	Qiu 94203
Peridiscus lucidus Benth.	Peridiscaceae	EF370702	AY674550	AY674550	EF370761	Soares 205/(Qiu 05069)
Petrophile canescens R. Br.	Proteaceae	AF197653	AF197807	DQ406983	GU351766	Qiu 98018
Peumus boldus Molina	Monimiaceae	AF197686	AF197803	DQ406990	GU351767	Royal Bot. Gard. Edinburgh 19870707
Phelline comosa Labill.	Phellinaceae	GU351084	GU351275	GU351484	GU351768	P. D. Ziesing 289 (CBG)
Philydrum lanuginosum Gaertn.	Philydraceae	AY299824	DQ401406	GU351485	GU351769	Qiu 98102
Phyllanthus angustifolius Sw.	Phyllanthaceae	GU351085	GU351276	GU351486	GU351770	Qiu 05041
Phyllonoma laticuspus Engl.	Phyllonomaceae	GU351086	ND	GU351487	GU351771	Morgan 2124 (WS)
†*Physena madagascariensis* Steud.	Physenaceae	GU351087	ND	ND	ND	Miller et al. 8817 (MO)
Phytolacca americana L.	Phytolaccaceae	GU351088	GU351277	GU351488	GU351772	Qiu 94109
†*Pilea fontana* Rydb.	Urticaceae	GU351089	GU351278	ND	ND	Qiu 96119
Pinguicula vulgaris L.	Lentibulariaceae	GU351090	GU351279	GU351489	GU351773	Qiu 96115
Pinus sp.	Pinaceae	AF197626	AF197723	AY832181, *P. thunbergii* Parl.	GU351774	Qiu 94013
Piper betle L.	Piperaceae	AF197630	AF197750	DQ406925	GU351775	Qiu 91048
Pittosporum tobira Dryand.	Pittosporaceae	GU351091	AF520127, *P. glabratum* Lindl.	GU351490	GU351776	Qiu 95031
Platanus occidentalis L.	Platanaceae	AF197655	AF197793	AY832177	GU351777	Qiu 94152
Pleea tenuifolia Michaux	Tofieldiaceae	AF197703	AF197743	DQ406995	GU351778	(Qiu 96128)
†*Podocarpus macrophyllus* Sweet	Podocarpaceae	AF197620	DQ007425	DQ406962	GU351779	Qiu 95006
Polygala cruciata L.	Polygalaceae	GU351092	GU351280	GU351491	GU351780	Chase 155 (NCU)
Polygonum sp.	Polygonaceae	GU351093	GU351281	GU351492	GU351781	Qiu 94110
Polyosma sp.	Polyosmaceae	GU351094	GU351282	GU351493	GU351782	Johns 9558 (BO, FREE, K, MAN)
†*Populus* sp.	Salicaceae	ND	GU351283	GU351494	ND	Qiu 05021
Portulaca oleracea L.	Portulacaceae	GU351095	GU351284	GU351495	ND	Qiu 94111
Potamogeton berchtoldii Fieber	Potamogetonaceae	AF197715	AF197724	DQ406938	GU351783	Qiu 96063
Qualea sp.	Vochysiaceae	GU351096	GU351285	GU351496	GU351784	Chase 168 (NCU)
Quercus alba L.	Fagaceae	GU351097	GU351286	GU351497	GU351785	Qiu 95115
Quillaja saponaria Poir.	Quillajaceae	GU351098	GU351287	GU351498	GU351786	Chase 10931 (K)
Quintinia verdonii F. Muell.	Quintiniaceae	GU351099	GU351288	GU351499	GU351787	Y. Pillon et al. 379 (NOU)
Ranunculus sp.	Ranunculaceae	AF197714	AF197759	DQ406876	GU351788	Qiu 95024
Reinwardtia trigyna Dalzell & A. Gibson	Linaceae	GU351100	GU351289	GU351500	GU351789	Y.P. Hong H103/(Qiu 05056) (PE)
Reseda alba Delile.	Resedaceae	GU351101	GU351290	GU351501	GU351790	Qiu 97070
Rhabdodendron amazonicum Huber	Rhabdodendraceae	GU351102	GU351291	GU351502	GU351791	E. Ribeiro (K)

Continued.

Table 1 Continued

Species	Family	*atp1*	*matR*	*nad5*	*rps3*	Voucher/DNA number
Rhodoleia championii Hook.	Hamamelidaceae	EF370703	EF370722	EF370742	EF370762	Royal Bot. Gard. Edinburgh, no voucher
Ribes sp.	Grossulariaceae	EF370704	EF370723	EF370743	EF370763	Qiu 95022
Rivina humulis L.	Phytolaccaceae	GU351103	GU351292	GU351503	GU351792	D. Soltis 2643 (FLAS)
Roupala macrophylla Phol	Proteaceae	GU351104	GU351293	GU351504	GU351793	Douglas 131 (MEL)
Roussea simplex Sm.	Rousseaceae	GU351105	GU351294	GU351505	GU351794	Mauritius Sugar Res. Inst.
Ruptiliocarpon caracolito Hammel & N. Zamora	Lepidobotryaceae	GU351106	GU351295	GU351506	GU351795	Pennington 631 (K)
Sabia sp.	Sabiaceae	AF197657	AF197780	DQ406895	GU351796	Qiu 91025
Saintpaulia magungensis E.P. Roberts	Gesneriaceae	GU351107	GU351296	GU351507	GU351797	Chase 696 (K)
Santalum album L.	Santalaceae	GU351108	GU351297	GU351508	GU351798	Chase 1349 (K)
Sarcandra chloranthoides Gardner	Chloranthaceae	AF197666	AF197754	DQ406866	GU351799	Qiu 92002
Sarcobatus vermiculatus Torr.	Sarcobataceae	GU351109	GU351298	GU351509	GU351800	King and Garvey 13892 (MO)
Sargentodoxa cuneata Rehder & Wilson	Lardizabalaceae	AF197644	AF197790	DQ406875	GU351801	X. Pan 93001 (Qiu M178) (NCU)
Sarracenia flava L.	Sarraceniaceae	AF420947	AF421028	GU351510	GU351802	Qiu 94141
Saruma henryi Oliv.	Aristolochiaceae	AF197672	AF197752	DQ406912	GU351803	Qiu 91018
Saururus cernuus L.	Saururaceae	AF197633	AF197748	DQ406934	GU351804	Qiu 97098
Saxifraga sarmentosa L.f.	Saxifragaceae	EF370705	EF370724	EF370744	EF370764	Qiu 95074
†*Scaevola aemula* R.Br.	Goodeniaceae	GU351110	AY453118	ND	ND	Qiu 97058
Schinus molle L.	Anacardiaceae	GU351111	GU351299	GU351511	GU351805	Z.D. Chen KEN073/(Qiu 05065) (PE)
Schisandra sphenanthera Rehder & Wilson	Schisandraceae	AF197662	AF197739	DQ406972	GU351806	Qiu 94165
Schoepfia schreberi J.F. Gmel.	Schoepfiaceae	GU351112	GU351300	GU351512	GU351807	Nickrent 2599 (ILL)
Scrophularia marilandica L.	Scrophulariaceae	GU351113	GU351301	ND	GU351808	A.A. Reznicek 11737/(Qiu 05037)
Sedum humifusum Rose	Crassulaceae	EF370706	AF520100	EF370745	EF370765	Qiu 05017
Sesamum triphyllum Asch.	Pedaliaceae	GU351114	GU351302	GU351513	GU351809	Chase 5710 (K)
Simmondsia chinensis C.K. Schneid.	Simmondsiaceae	DQ401309	DQ401397	DQ406903	GU351810	Qiu 96120
Siparuna brasiliensis A. DC.	Siparunaceae	AF197687	AF197809	DQ406976	GU351811	Qiu 02003
Smilax sp.	Smilacaceae	AF039251	DQ401391	DQ406940	GU351812	Qiu 95117
Sparganium americanum Nutt.	Sparganiaceae	AY124509, *S. eurycarpum* Engelm.	DQ401396	DQ407010	GU351813	Qiu 96108
Spathiphyllum clevelandii	Araceae	AF197706	AF197746	DQ406997, *S. wallisii* Hort.	GU351814	Qiu 94140
Sphenoclea zeylanica Gaertn.	Sphenocleaceae	GU351115	GU351303	GU351514	GU351815	(Qiu M253), Bot. Gard. Bonn, no voucher
Sphenostemon lobosporus L.S. Sm.	Sphenostemonaceae	GU351116	GU351304	GU351515	GU351816	Chase 1900 (K)
Spinacia oleracea L.	Amaranthaceae	DQ401287	AY453110	DQ406883	GU351817	Qiu 94059
Spiraea sp.	Rosaceae	GU351117	GU351305	GU351516	GU351818	Qiu 05008
Stachyurus chinensis Franch.	Stachyuraceae	GU351118	GU351306	GU351517	GU351819	Z.D. Chen JGS 005/(Qiu 05062) (PE)
Staphylea trifolia L.	Staphyleaceae	DQ401294	AY453105	DQ406906	GU351820	Qiu 95106
Stegnosperma halimifolium Benth.	Stegnospermataceae	GU351119	GU351307	GU351518	GU351821	Martin et al. s.n. (MO)
Stegolepis sp.	Rapateaceae	AY124535, *S. parvipetala* Steyerm.	DQ401411	DQ407004	GU351822	Qiu 97132
†*Stellaria* sp.	Caryophyllaceae	GU351120	ND	GU351519	ND	Qiu 95015
Sterculia balanghas L.	Malvaceae	DQ401316	DQ401402	DQ406869	GU351823	Qiu 97056
Strasburgeria robusta Guillaumin	Strasburgeriaceae	GU351121	GU351308	GU351520	GU351824	Y. Pillon et al 60 (NOU, K)

Continued.

Table 1 Continued

Species	Family	*atp1*	*matR*	*nad5*	*rps3*	Voucher/DNA number
Strelitzia reginae Aiton	Strelitziaceae	AY299843, *S. nicolai* Regel & K.Koch	AY453112	DQ406965	GU351825	Qiu 96045
Strychnos spinosa Lam.	Loganiaceae	AY741818	GU351309	GU351521	ND	(Qiu 96187)
Stylobasium spathulatum Desf.	Surianaceae	GU351122	GU351310	GU351522	GU351826	G. Brummitt et al 21242 (K)
Styrax americanus Lam.	Styracaceae	AF420950, *S. officinalis* L.	AF520205, *S. grandiflora* Griff.	GU351523	GU351827	K. Kron 521 (NCU)
Swietenia macrophylla King	Meliaceae	GU351123	GU351311	GU351524	GU351828	(Chris W. Dick 646)/(Qiu M294), no voucher
Syringa sp.	Oleaceae	AY741821, *S. vulgaris* L.	GU351312	GU351525	GU351829	Qiu 95037
Tacca chantrieri Andre	Dioscoreaceae	AF039252, *T. pinnatifida* J.R. Forst & G. Forst	DQ401377	DQ406941	HM357127	Qiu 01015
Takhtajania perrieri M. Baranova & J. Leroy	Winteraceae	DQ007416	DQ007427	DQ406913	GU351830	J. Rabenantoandro 219 (MO)
Talinum patens Juss.	Talinaceae	GU351124	GU351313	GU351526	ND	K.X. Xu 015/(Qiu 05064) (PE)
Tamarix sp.	Tamaricaceae	GU351125	GU351314	GU351527	ND	Qiu 95034
Tapiscia sinensis Oliv.	Tapisciaceae	GU351126	GU351315	GU351528	GU351831	Chase 1021 (K)
Tasmannia insipida DC.	Winteraceae	AF197674	AF197782	DQ406970	GU351832	Qiu 90032
Terminalia catappa L.	Combretaceae	GU351127	ND	GU351529	GU351833	US Natl Trop Bot Gard # 731222022
Ternstroemia stahlii Krug & Urb.	Pentaphylaceae	AY725909	AY163754, *T. gymnanthera* Sprague	GU351530	GU351834	Chase 360 (K)
Tetracarpaea tasmanica Hook.f.	Tetracarpaeaceae	EF370707	EF370725	EF370746	EF370766	No new data
Tetracentron sinense Oliv.	Trochodendraceae	AF197647	AF197791	DQ406874	GU351835	Qiu 90009
Tetracera asiatica Hoogland	Dilleniaceae	GU351128	AF520094	GU351531	GU351836	Chase 1238 (K)
Tetramerista sp.	Tetrameristaceae	AF420958	GU351316	GU351532	GU351837	M. Coode 7925 (K)
Thottea tomentosa Ding Hou	Aristolochiaceae	AF197670	AF197733	DQ406914	GU351838	Chase 2086 (K)
Thymelaea hirsuta Endl.	Thymelaeaceae	GU351129	GU351317	GU351533	GU351839	(Qiu M284)
Tinospora sagittata Gagnep.	Menispermaceae	GU351130	GU351318	GU351534	GU351840	Y.P. Hong 99258/(Qiu 05061) (PE)
Tofieldia calyculata Wahlenb.	Tofieldiaceae	AF197704	AF197744	DQ406935	*GU351841*	Qiu 97041
Tradescantia sp.	Commelinaceae	DQ401320	AY453108, *T. ohiensis* Raf	DQ406950	GU351842	Qiu 96059
Triglochin maritima L.	Juncaginaceae	AF197716	AF197725	DQ406998	ND	Qiu 97106
Trillium sp.	Melanthiaceae	AF039253, *T. grandiflorum* Salisb.	DQ401407	DQ406949	GU351843	Qiu 95016
Trimenia moorei W.R. Philipson	Trimeniaceae	AY009428, *Trimenia* sp.	AF197741	DQ406987	GU351844	Australia Natl. Bot. Gard. 701680
Triphyophyllum peltatum Airy Shaw	Dioncophyllaceae	GU351131	GU351319	GU351535	GU351845	Chase 663 (K)
†*Trithuria inconspicua* Cheeseman	Hydatellaceae	GU351132	ND	GU351536	ND	P.D. Chapman s.n., NSW 428712
†*Trithuria lanterna* D.A. Cooke	Hydatellaceae	GU351133	ND	GU351537	ND	T.D. Macfarlane et al. 4321
Trochodendron aralioides Sieb. & Zucc.	Trochodendraceae	AF197648	AF197792	DQ406880	GU351846	Qiu 90026
Tropaeolum peltophorum Benth.	Tropaeolaceae	GU351134	GU351320	GU351538	GU351847	Peter Kuhlman s.n./(Qiu 96150)
†*Urtica dioica* L.	Urticaceae	GU351135	ND	GU351539	ND	A.A. Reznicek 11740/(Qiu 05039)
Vahlia capenis Thunb.	Vahliaceae	GU351136	GU351321	GU351540	GU351848	Van Wyk 10–579 (PUR)
Valeriana officinalis L.	Valerianaceae	GU351137	GU351322	GU351541	ND	Peter Kuhlman s.n./(Qiu 96013)
Verbena bonariensis L.	Verbenaceae	AY741828	GU351323	GU351542	GU351849	Qiu 05012
Viburnum sp.	Adoxaceae	GU351138	GU351324	GU351543	GU351850	Qiu 95083

Continued.

Table 1 Continued

Species	Family	*atp1*	*matR*	*nad5*	*rps3*	Voucher/DNA number
Viola sp.	Violaceae	GU351139	GU351325	GU351544	GU351851	Qiu 95018
Vitis sp.	Vitaceae	DQ401305	AY453123, *V. riparia* Michx.	DQ406881	GU351852	Qiu 94046
Viviania marifolia Cav.	Vivianiaceae	GU351140	GU351326	GU351545	ND	M. Ackermann 543 (B)
Vriesea splendens Lem.	Bromeliaceae	DQ401298	DQ401378	DQ406945	GU351853	Qiu 96073
Welwitschia mirabilis Hook.f.	Welwitschiaceae	AF197618	AF197719	DQ406958	ND	No new data
†*Wendtia gracilis* Meyen	Ledocarpaceae	ND	ND	GU351546	ND	Kubitzki & Feuerer 990–68 (HBG)
Xanthorrhoea quadrangulata F. Muell.	Xanthorrhoeaceae	AF039250, *X. australis* R.Br.	DQ401384	DQ406946	GU351854	Qiu 97039
Xanthosoma mafaffa Schott	Araceae	DQ401318	DQ401376	DQ406936	GU351855	Qiu 95063
Ximenia americana L.	Olacaceae	GU351141	GU351327	GU351547	GU351856	(Qiu 96204)
Zamia floridana A. DC.	Zamiaceae	AF197624	AF197721	DQ406961, *Z. integrifolia* Rich.	GU351857	Qiu 95035
Zelkova serrata Makino	Ulmaceae	GU351142	GU351328	GU351548	GU351858	A.A. Reznicek 11739/(Qiu 05038)

Vouchers with numbers Qiu 1–Qiu 93999 are deposited in NCU, Qiu 94001–Qiu 97999 in IND, Qiu 98001–Qiu 99999 in Z, and Qiu 00001–Qiu 10999 in MICH. Numbers in parentheses are DNA numbers (no voucher or a voucher by someone without a number). Vouchers by collectors other than Y.-L. Qiu are indicated with the herbaria where they have been deposited. All vouchers by A.A. Reznicek are deposited in MICH. All sequences with GenBank accession numbers GUxxxxxx and HM357127 were generated in this study. All others were retrieved from GenBank. †Taxa removed from the 380 taxon analysis because of highly divergent sequences or lacking data for two or three genes. ND, no data.

Table 2 Primer sequences of *atp1*, *matR*, and *rps3* used in this study

Primers for *atp1*	
Aatp1-F1 (64–85)	Aatp1-R1 (1369–1388)
TAC RCG AAW TTK CAA GTG GAT G (62 °C)	CT GTC TAG KGG CAT TYG RTC (60 °C)
Aatp1-F2 (461–479)	Aatp1-R2 (1001–1018)
CG GTR GAT AGC CTN GTT CC (60 °C)	A GGC CGA YAC GTC TCC NG (58 °C)
Aatp1-F3 (978–997)	Aatp1-R3 (619–638)
S TTA CCC GTS ATT GAA ACA C (58 °C)	CG TTT CTG TCC AAT YGC NAC (60 °C)
Primers for *matR*	
AmatR-F1 (61–80)	AmatR-R1 (1835–1852)
ATC AGA AYG GTA CYC GAA TC (56 °C)	T GTG CTT KTG GGC WRG GG (58 °C)
AmatR-F2 (598–616)	AmatR-R2 (1395–1411)
TCC CTT GTT TYG TCR TKG C (60 °C)	G CCG GAT GTG CTK KAC G (60 °C)
AmatR-F3 (1087–1105)	AmatR-R3 (957–975)
RTA RYT GCA CGG AGT ACG G (60 °C)	TRA GTC RTC GGC RTA TCG C (58 °C)
AmatR-F4 (1395–1411)	AmatR-R4 (716–733)
C GTC AAG CAC ATM HGG C (56 °C)	C GGC GMA AAG RAR GCT CG (60 °C)
	AmatR-R5 (353–372)
	TAG GGC CRA TAG TAR TAC AC (60 °C)
MmatR-F2 (388–406)	MmatR-R2 (1398–1415)
CTA MRC AAG CTC GAT CAG G (58 °C)	YCT TGC CGG ATG TGC TTG (58 °C)
MmatR-F3 (850–866)	MmatR-R3 (1093–1111)
CHK ATA GAG CTG GGC GG (56 °C)	ATT CTA CCG TAC TCC GTG C (58 °C)
MmatR-F4 (1182–1200)	MmatR-R4 (716–733)
GCG TCT ACG GGT AAA GCA C (60 °C)	CGC MGC AAA RGA RGC TCG (62 °C)
MmatR-F5 (1470–1487)	MmatR-R5 (353–371)
CGT TCA ACA GRC AGT CTC (56 °C)	AGS GCC GAT AGT AGT ACA C (58 °C)
Primers for *rps3*	
rps3-F1 (128–146)	rps3-R1 (1643–1662)
GT TCG ATA CGT CCA CCT AC (58 °C)	GTA CGT TTC GGA TAT RGC AC (58 °C)
rps3-F12 (161–179)	rps3-R12 (1640–1658)
GC TTT CGY CTC GGT AGG TG (60 °C)	GT TTC GGA TAT RGC ACG TC (56 °C)
rps3-F2 (382–401)	rps3-R2 (1270–1289)
GCA GGG AAA ASW GTC RAG TC (60 °C)	CT ATT AGA CAA NAA AGA TCG (54 °C)
rps3-F3 (528–545)	rps3-R3 (910–928)
C GKG GCC TWC AAG CAT CC (60 °C)	A CCT CTT TTT GKC TYS GGC (56 °C)
rps3-F4 (997–1016)	rps3-R4 (469–488)
TTT CCW TTC TTC GGT GCT AC (58 °C)	GG TGA TCG GTC ATG GTA TCC (60 °C)
rps3-F5 (1343–1360)	
GT GCT TCT CYR ATT GCT C (58 °C)	

The primer position is indicated by the coordinate number in the corresponding gene of *Arabidopsis thaliana* (NC_001284) given in parentheses. The primer melting temperature, estimated in Oligo v. 6 (Molecular Biology Insights, Cascade, CO, USA), is given in parentheses after the sequence.

substitution in PAUP*v4.0b10 (Swofford, 2003). The phylogram was then fit to a molecular clock using the Langley–Fitch method in r8s v1.7.1 (Sanderson, 2003). Because taxon complements are identical across genes, fixing the root age to 1.0 in each of these analyses ensures that molecular rate estimates are directly comparable.

The other analysis aimed to estimate the homoplasy level in each of the eight genes. A parsimony search was carried out on each matrix under the constraint consensus angiosperm phylogeny using PAUP*v4.0b10. These searches were not run to completion because: (i) they took too long to complete; and (ii) consistency and homoplasy index values varied little on trees that differed by a small percentage of parsimony length. Consistency and homoplasy indexes were then output from the tree.

Finally, we examined the effect of RNA editing on reconstructing angiosperm phylogeny using mitochondrial gene sequences. Previously, RNA editing was shown to have some effect on phylogenetic analysis when the editing level was high in a slowly evolving gene, such as *nad5* (Qiu et al., 2006a). However, this effect can be minimized through combined analyses with less edited genes (Petersen et al., 2006; Qiu et al., 2006a). In this study, we removed all RNA editing sites according to the information available in GenBank for these four genes in *Arabidopsis thaliana* (NC_001284) (Giege & Brennicke, 1999), *Brassica napus* (NC_008285) (Handa, 2003), *Beta vulgaris* (NC_002511) (Mower & Palmer, 2006), and *Oryza sativa* Japonica Group (NC_011033) (Notsu et al., 2002). As a result, 2, 8, 17, and 17 sites were deleted from the 356 OTU matrix in *atp1, matR, nad5*, and *rps3*, respectively. The matrix was then analyzed using RAxML 7.0.4 in the same way as the regular 356 OTU matrix except that only 100 BS replicates were run.

2 Results and discussion

2.1 Mitochondrial gene-based angiosperm phylogeny

A generally well resolved angiosperm phylogeny, with many major nodes moderately to strongly supported, was reconstructed from the 356 OTU matrix ($-ln$ L = 150192.44). A schematic version of this tree is presented in Fig. 1 and a detailed version is shown in Fig. 2. A topologically highly similar phylogenetic tree, with BS support on some nodes slightly to significantly lower, was obtained from the 380 OTU matrix ($-ln$ L = 164322.98), and it is shown in Fig. S2.1, 2.2. A topologically similar phylogenetic tree, with similar BS support values on most nodes, was obtained from the 356 OTU matrix with RNA editing sites removed ($-ln$ L = 146408.591300), and it is shown in Fig. S3.

2.1.1 Basalmost angiosperms The first diverging lineage of angiosperms consists of Amborellales and Nymphaeales (Figs. 1, 2-1). Hydatellaceae, a recently identified member of this lineage based on chloroplast and nuclear phytochrome gene sequences as well as morphology (Saarela et al., 2007), also fall in this group and are sister to Nymphaeaceae–Cabombaceae (Fig. S2.1). This family is not included in the 356 OTU matrix, but is included in the 380 OTU matrix because both sampled species (*Trithuria inconspicua* and *T. lanterna*) have only two of the four genes amplified, and they are rather divergent (Table 1, Fig. S2.2). The monophyly of this group has moderate BS support (77 or 79% when Hydatellaceae are included). The sister relationship of this clade to the rest of the angiosperms has 99–100% BS support in the two analyses.

The lineage that follows Amborellales + Nymphaeales in diversification of extant angiosperms is Austrobaileyales. Both the monophyly of this order and its relationship to the remaining angiosperms are strongly supported (Figs. 1, 2-1).

With the exception of all *rbcL* analyses and some chloroplast genome analyses, all other analyses of molecular data have identified Amborellales, Nymphaeales, and Austrobaileyales as the basalmost extant angiosperms (Mathews & Donoghue, 1999; Parkinson et al., 1999; Qiu et al., 1999, 2005, 2006a; Barkman et al., 2000; Graham & Olmstead, 2000; Soltis et al., 2000; Zanis et al., 2002; Borsch et al., 2003; Hilu et al., 2003; Stefanovic et al., 2004; Leebens-Mack et al., 2005; Jansen et al., 2007; Moore et al., 2007; Saarela et al., 2007; Goremykin et al., 2009). In all analyses of *rbcL* sequences (Les et al., 1991; Chase et al., 1993; Qiu et al., 1993; Savolainen et al., 2000a) and the first and related chloroplast genome analyses (Goremykin et al., 2003), *Ceratophyllum* was placed as the sister to all other angiosperms, albeit with low to moderate support in cases where support values were obtained. Despite this consensus, there has been considerable controversy regarding whether the first diverging lineage of angiosperms consists of *Amborella* alone or *Amborella* and Nymphaeales (now also including Hydatellaceae) together. In fact, this controversy emerged almost as soon as the ANITA lineages were identified as the basalmost extant angiosperms. An alternative topology test using a combined dataset of chloroplast *atpB* and *rbcL*, mitochondrial *atp1* and *matR*, and nuclear 18S showed that the topologies of *Amborella* alone, Nymphaeales alone, or *Amborella* and Nymphaeales together being sister to all other angiosperms were

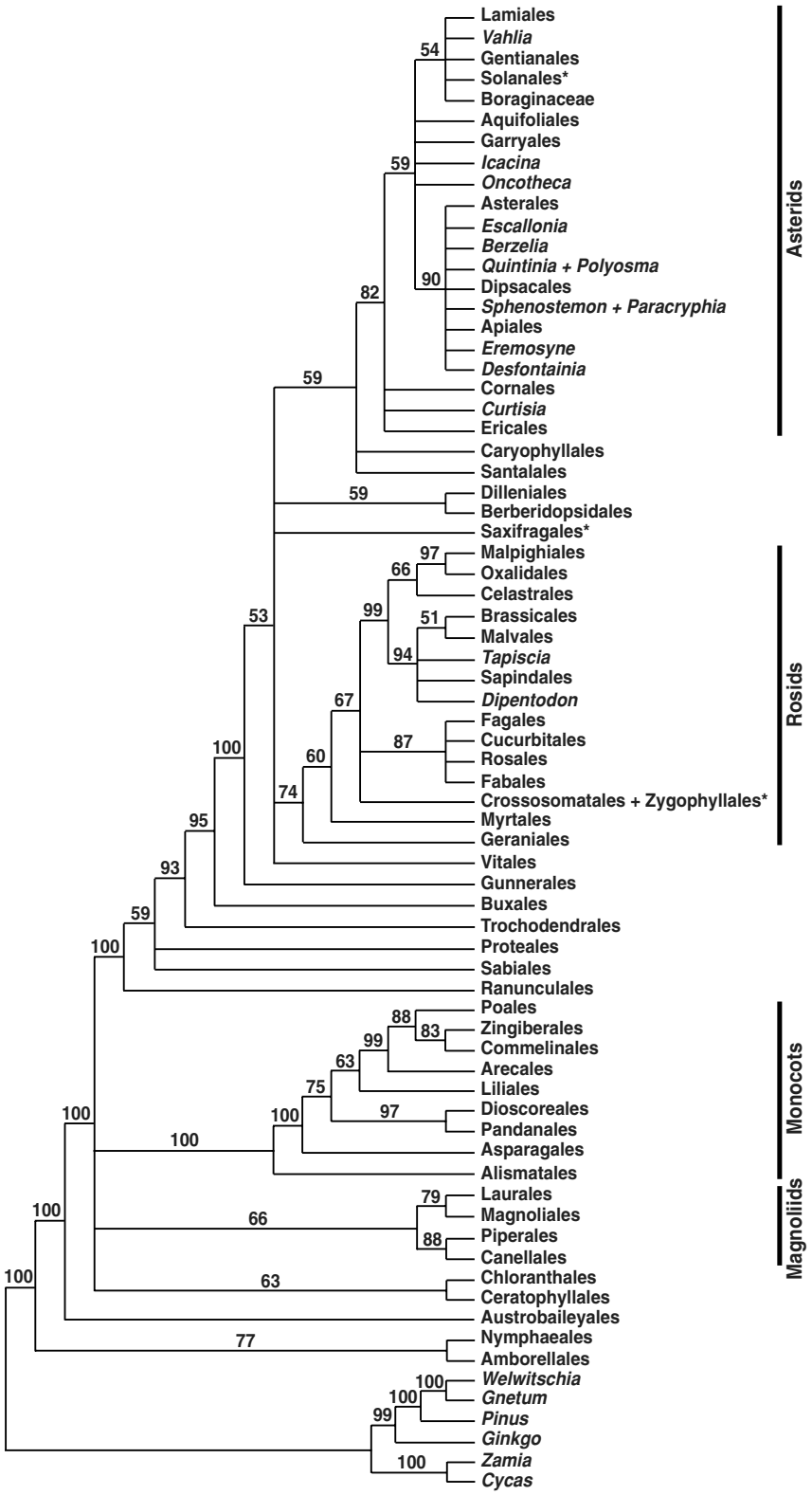

Fig. 1. Schematic cladogram of angiosperm phylogeny inferred from four mitochondrial genes *atp1, matR, nad5,* and *rps3* from 356 seed plants, and a detailed version is shown in Fig. 2. Bootstrap values >50% are shown above branches. All angiosperm orders have >50% bootstrap support except the three labeled with asterisks (Saxifragales has 97% bootstrap support if *Peridiscus* is not included).

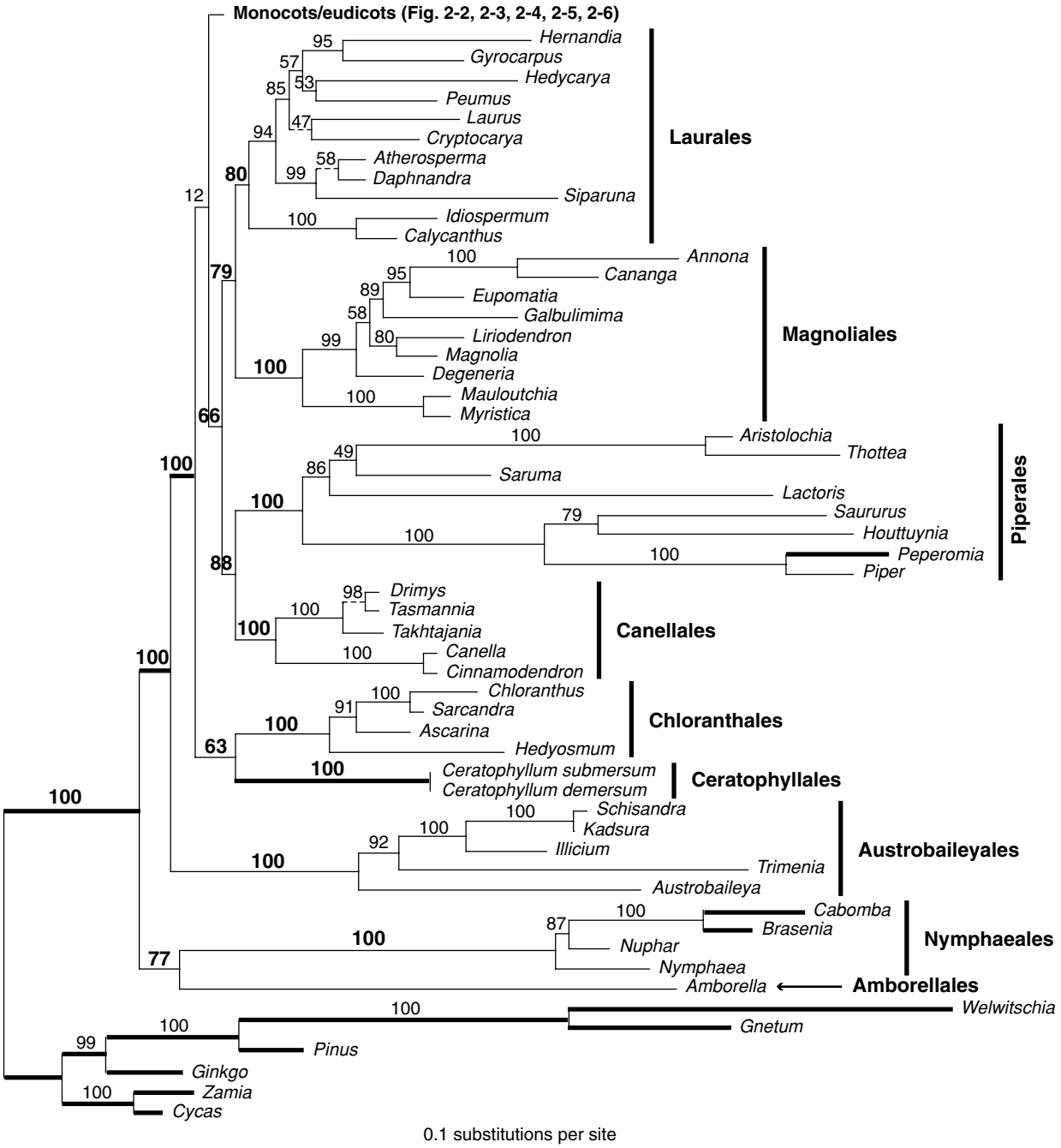

Fig. 2. Maximum likelihood tree of 356 seed plants inferred from nucleotide sequences of mitochondrial genes *atp1, matR, nad5,* and *rps3*. Bootstrap values are shown above the branches, with those for key nodes shown in a larger font size and boldface. The branch length is indicative of the divergence level among taxa except those in thick or dashed lines, with the scale bar shown at the bottom of the first part of the tree. Thick lines represent one-sixth of the real length of the branches. Dashed lines are near-zero length branches, and are expanded for the presentation purpose. *(Fig. 2-1)*

statistically indistinguishable (Qiu et al., 2000). An analysis of 17 chloroplast genes obtained similar results through alternative topology testing (Graham & Olmstead, 2000). However, an analysis of a somewhat different dataset than that of Qiu et al. (2000), using a different method and an orthologous copy of *atp1,* suggested that the *Amborella* + Nymphaeales basal topology was the preferred hypothesis (Barkman et al., 2000). In a recent analysis of mitochondrial *atp1, matR,* and *nad5* from 162 of mostly basal angiosperms, at least 91% BS

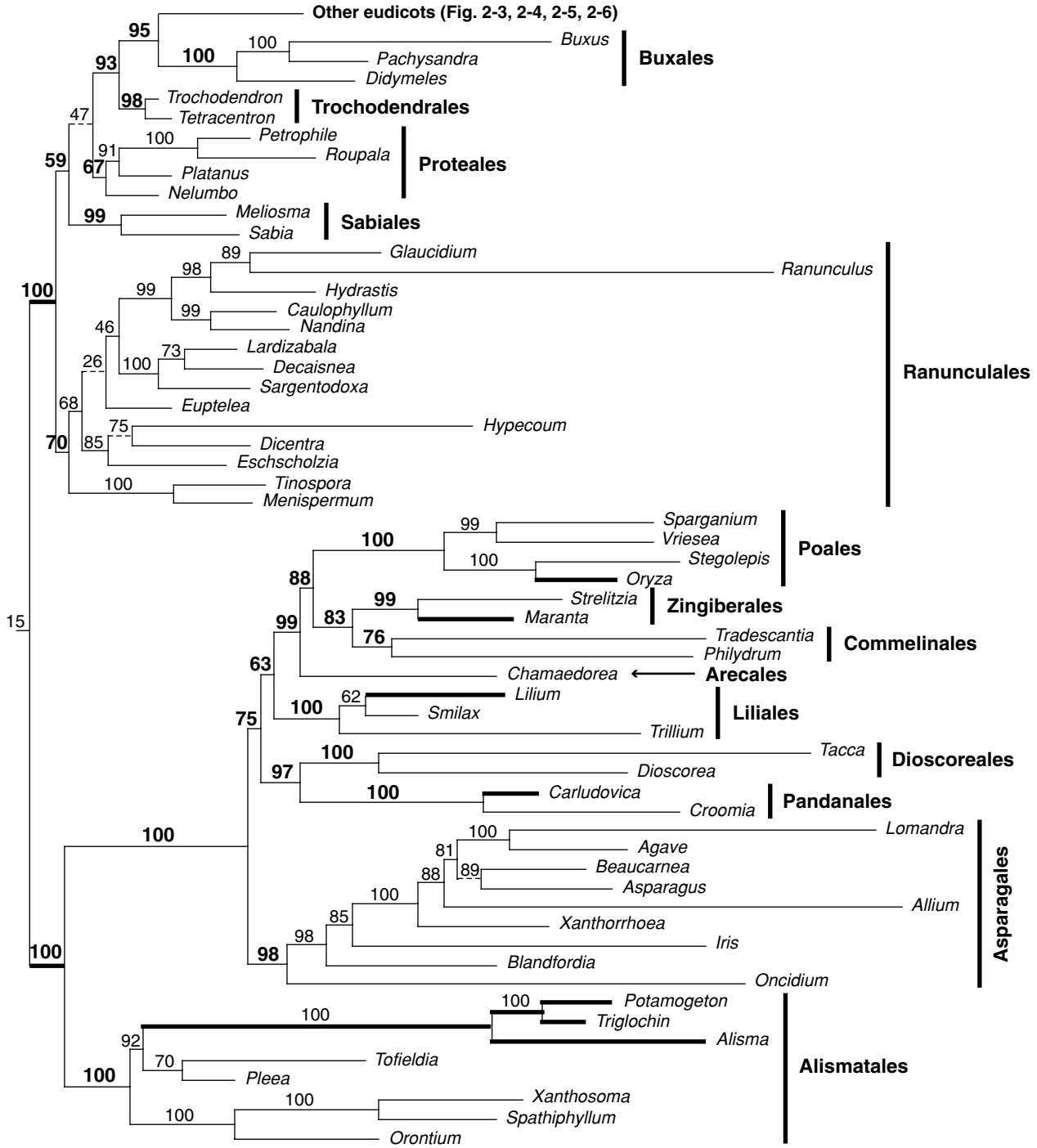

Fig. 2. Continued. *(Fig. 2-2)*

support was found to support the topology with *Amborella* and Nymphaeales together being sister to all other angiosperms (Qiu et al., 2006a). Most recently, in an analysis of a chloroplast genomic dataset, it was shown that removal of a significant percentage of highly variable sites changed the placement of *Amborella* to *Amborella* + Nymphaeales as the first diverging lineage of angiosperms (Goremykin et al., 2009), although the issue of removing fast-evolving sites needs to be explored further (Graham & Iles, 2009). The facts that the

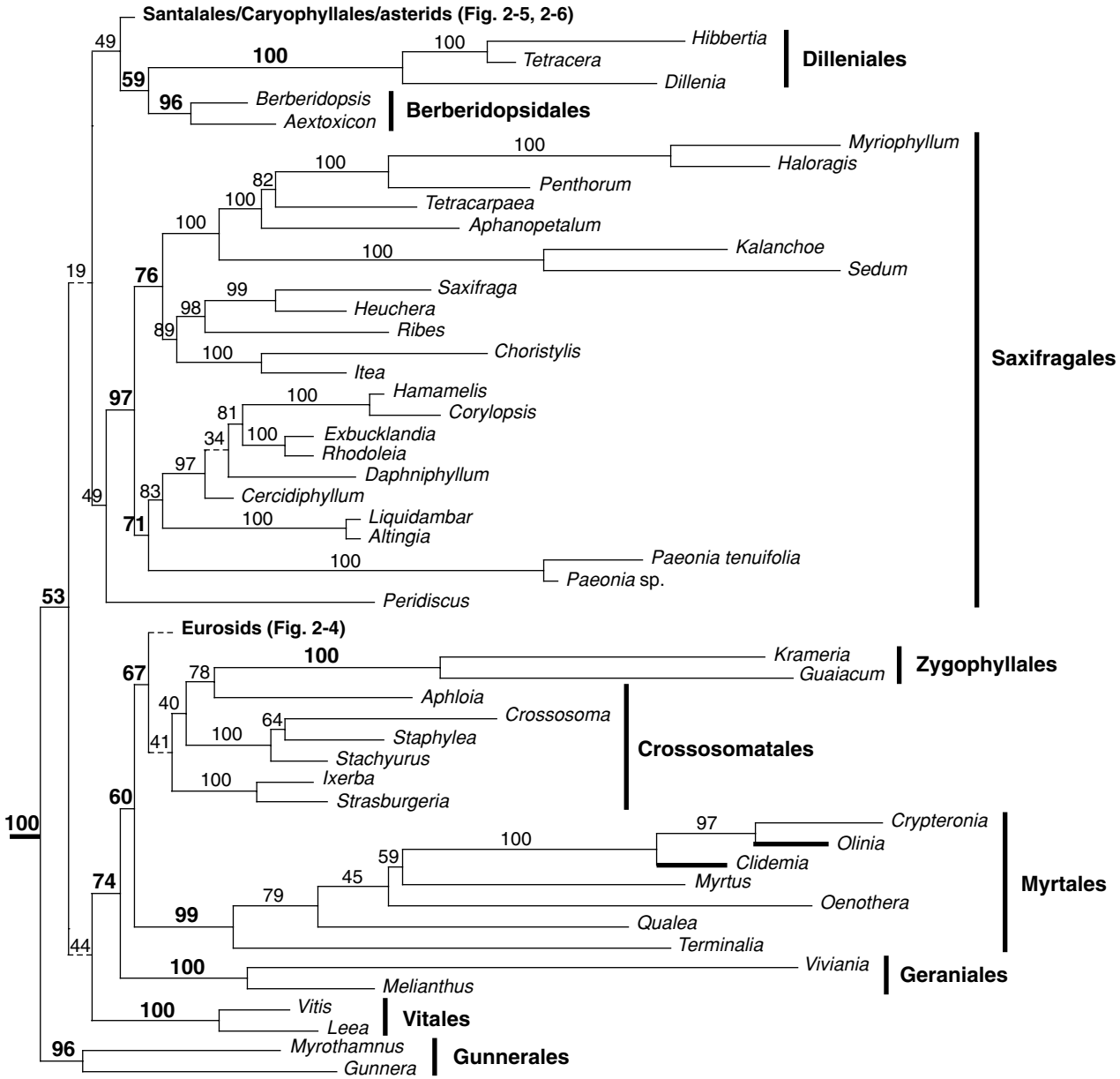

Fig. 2. Continued. *(Fig. 2-3)*

mitochondrial genes used in all the mentioned studies (*atp1, cox1, matR,* and *nad5* [Barkman et al., 2000; Qiu et al., 2000, 2006a]) have lower or significantly lower substitution rates than the commonly used chloroplast genes (*atpB, matK, rbcL*) (Table 3), and that the divergence gap between gymnosperms and angiosperms is large, increase the likelihood that the *Amborella*-basal topology seen in the studies that used fast-evolving chloroplast genes is an artifact. In Qiu et al., 2006a and this study, analyses of three or four mitochondrial genes from 162 or 356 seed plants with two different maximum likelihood methods both recovered the topology with *Amborella* and Nymphaeales together as the sister group of all other angiosperms with moderate to strong BS support. This level of consistency in the results of the two studies, as well as those cited above, supports the hypothesis that the basalmost extant angiosperm lineage includes *Amborella*, Hydatellaceae, and Nymphaeales.

2.1.2 Major lineages of mesangiosperms Mesangiosperms, as defined by Cantino et al. (2007), include five groups: Chloranthaceae, *Ceratophyllum,*

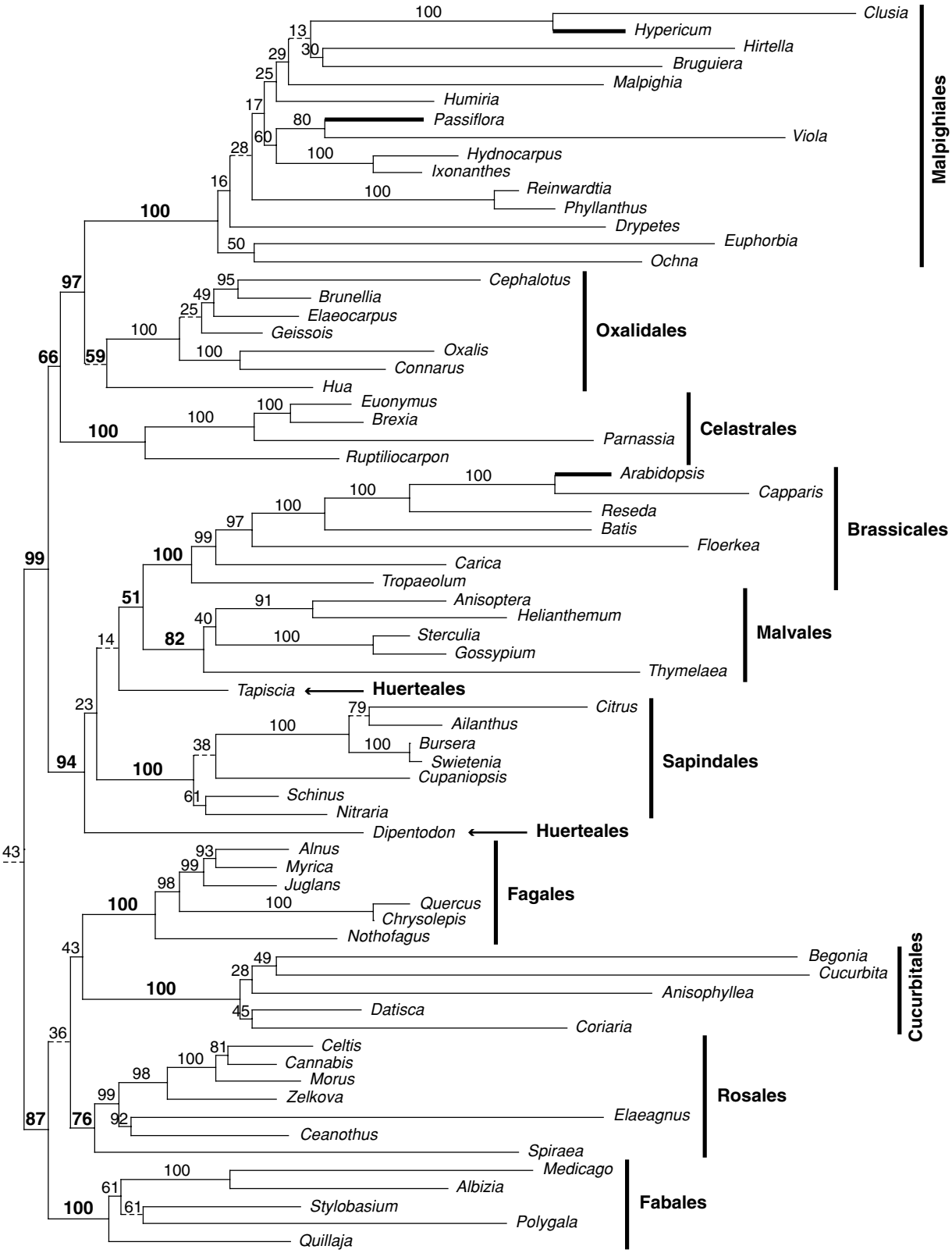

Fig. 2. Continued. *(Fig. 2-4)*

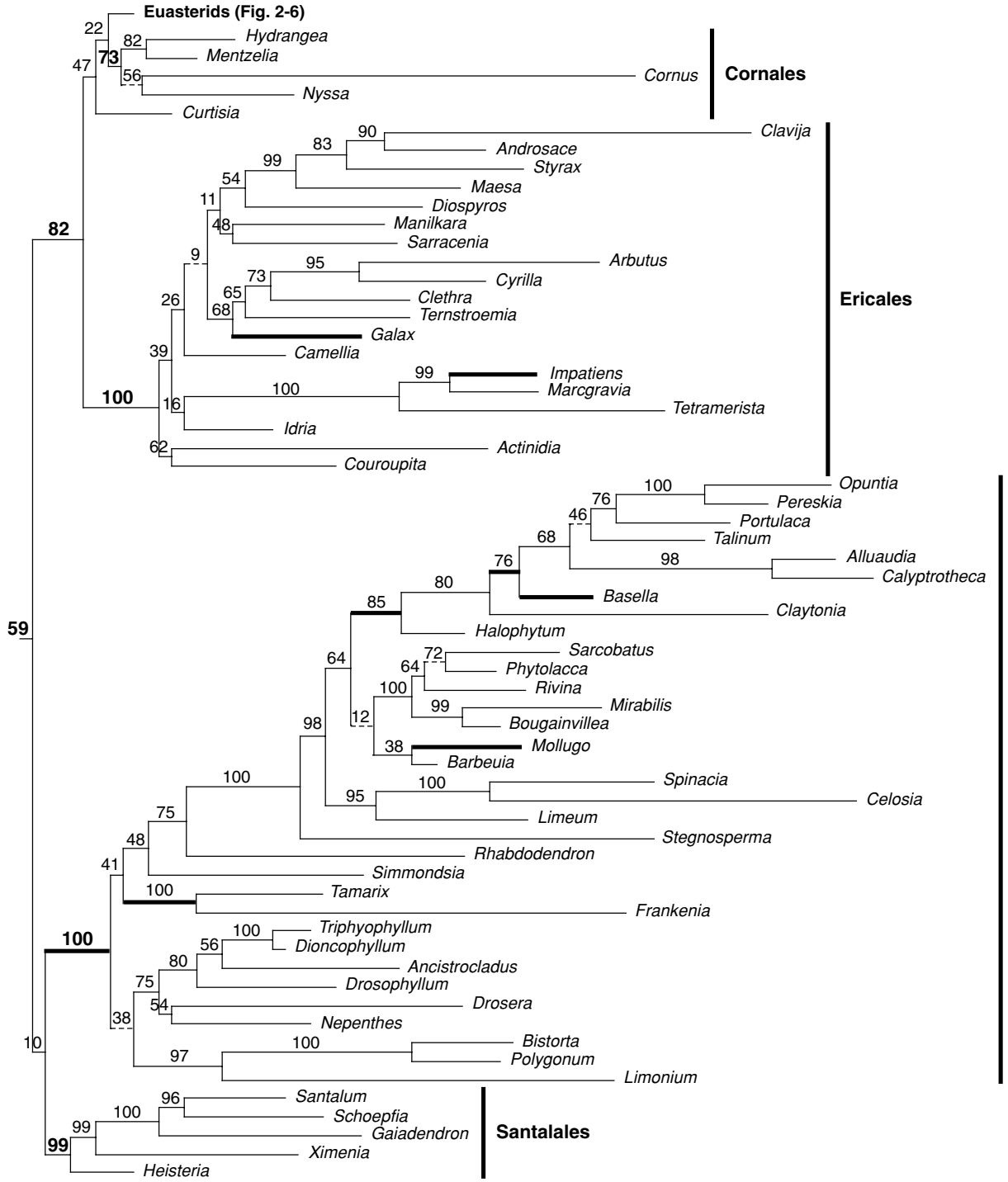

Fig. 2. Continued. *(Fig. 2-5)*

magnoliids, monocots, and eudicots. In this study, Chloranthaceae and *Ceratophyllum* form a clade with 63% BS support. All magnoliid taxa form a monophyletic group with 66% BS support. Both monocots and eudicots receive 100% BS support. The relationships among these four clades have virtually no BS support (Figs. 1, 2-1, 2-2).

Upon identification of the ANITA lines as the basalmost extant angiosperms, it was realized that resolving relationships among five mesangiosperm

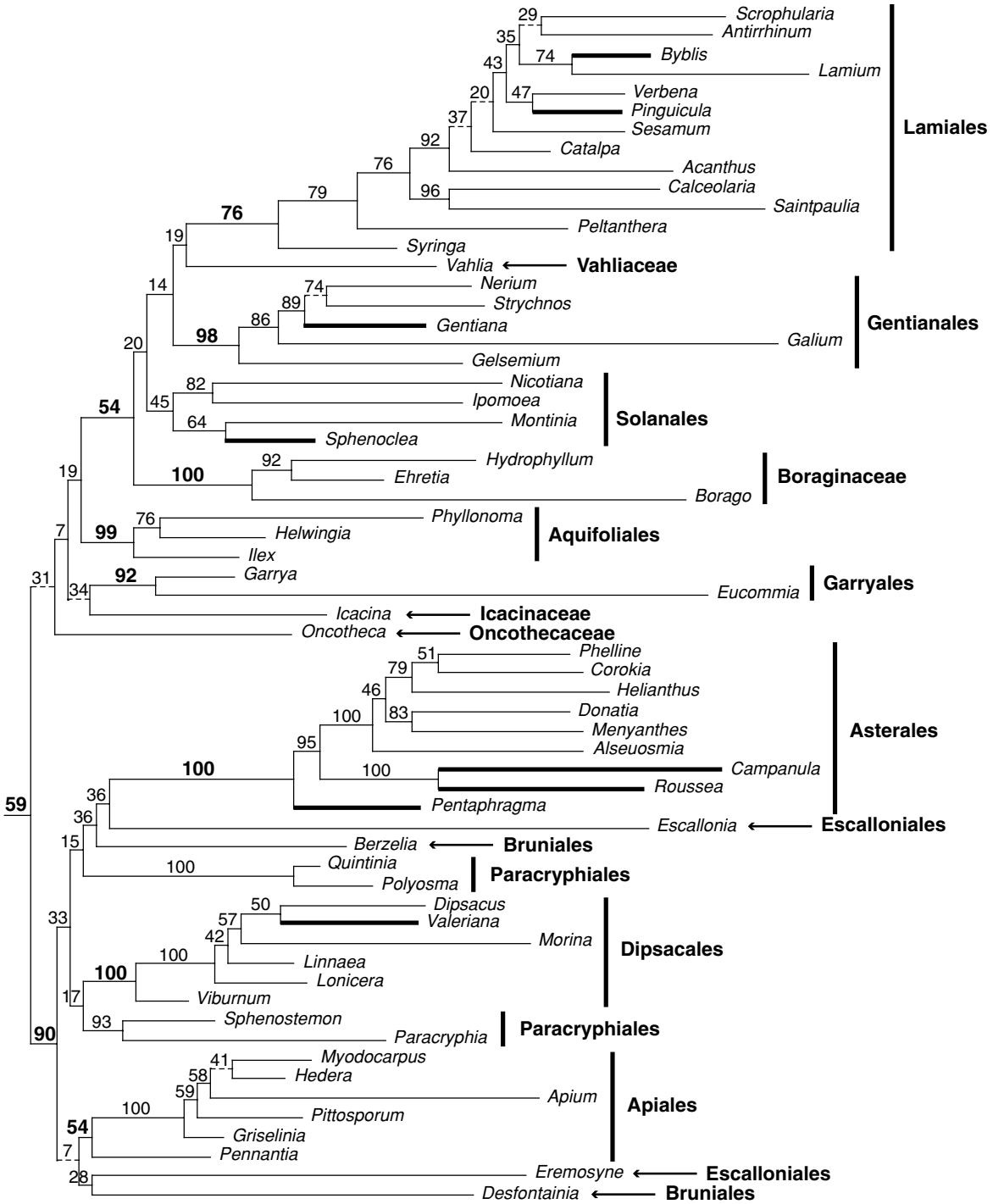

Fig. 2. Continued. *(Fig. 2-6)*

lineages was the next major challenge in the study of phylogenetic patterns among basal angiosperms (Doyle & Endress, 2000; Qiu et al., 2000). Four types of analyses can be categorized among all studies that have attempted to resolve these relationships or have them as part of their study questions, and some promising results have been obtained. First, in three large-scale analyses, which sampled one, three, and five genes, respectively, from a large number of angiosperms, especially monocots and eudicots, only the sister

Table 3 Molecular clock rates of the eight genes estimated from the matrix of 272 seed plants under a constraint tree shown in Fig. S1

Gene	Length (bp)	Molecular clock rate (substitution/site/unit time)
cp-*matK*	1876	0.1430
cp-*rbcL*	1406	0.1080
mt-*rps3*	2380	0.0838
nu-18S rDNA	1732	0.0774
cp-*atpB*	1482	0.0675
mt-*matR*	3673	0.0441
mt-*atp1*	1169	0.0405
mt-*nad5*	1133	0.0292

cp, chloroplast; mt, mitochondrial; nu, nuclear.

relationship between *Ceratophyllum* and eudicots received >50% BS support (Soltis et al., 2000; Hilu et al., 2003; Burleigh et al., 2009). Second, a number of medium-scale analyses were carried out, in which several genes from two or three plant genomes were analyzed from a densely sampled set of taxa. In these analyses, Chloranthaceae were sister to magnoliids (Barkman et al., 2000; Saarela et al., 2007) or magnoliids + eudicots (Zanis et al., 2002, 2003); *Ceratophyllum* was sister to monocots (Zanis et al., 2002, 2003; Qiu et al., 2005), eudicots (Qiu et al., 2005, 2006a; Saarela et al., 2007; Qiu & Estabrook, 2008), or Chloranthaceae (Antonov et al., 2000; Duvall et al., 2006, 2008; Qiu et al., 2006a); magnoliids were sister to eudicots (Zanis et al., 2002, 2003), or *Ceratophyllum* + eudicots (Qiu & Estabrook, 2008); and monocots were sister to *Ceratophyllum* + eudicots (Saarela et al., 2007). Perhaps because different phylogenetic methods were used to analyze highly diverse sets of data in these studies, the results were also very variable. Third, a series of analyses of largely morphological data with topological constraints derived from some molecular studies placed *Ceratophyllum* as the sister to Chloranthaceae (Doyle et al., 2008; Endress & Doyle, 2009; Doyle & Endress, 2010). Finally, four recent chloroplast phylogenomic analyses resolved relationships among the five mesangiosperm lineages with moderate to strong BS support, with monocots being sister to eudicots, and magnoliids being sister to monocots + eudicots (Jansen et al., 2007; Moore et al., 2007, 2010; Goremykin et al., 2009). *Ceratophyllum*, when included in the analyses, changed its position between being sister to eudicots or magnoliids, depending on the portion of the genome sequences analyzed (Moore et al., 2007, 2010; Goremykin et al., 2009). Chloranthaceae were consistently sister to magnoliids when they were included in analyses (Jansen et al., 2007; Moore et al., 2007, 2010).

Despite the heterogeneity of these analyses and the diverse results obtained, there seems to be an emerging consensus on the relationship of *Ceratophyllum*, as a sister to either eudicots or Chloranthaceae. Its placement as the sister to monocots was only seen in the studies that sampled insufficient number of monocots, and further, several monocots such as *Acorus* and alismatids had highly divergent mitochondrial genes in those studies (Zanis et al., 2002, 2003; Qiu et al., 2005). Hence, this result may be an artifact. The placement of *Ceratophyllum* as the sister to Chloranthaceae emerged relatively late in the studies of basal angiosperm phylogeny, but deserves some consideration. Two mitochondrial gene-based analyses, with extensive sampling of all five mesangiosperm lineages, consistently identified this relationship, even though the BS values were only over 60% (Qiu et al., 2006a) (this study, Figs. 2-1, S2.1, S3.1). Placement of *Ceratophyllum* and Chloranthaceae together in the morphological cladistic analyses was mostly due to their simple flowers (Doyle et al., 2008; Endress & Doyle, 2009; Doyle & Endress, 2010). At present, it is difficult to determine whether these simple flowers reflect a common ancestry or independent reduction due to adaptation to anemophily and hydrophily. Excavation of chloranthoid and ceratophyllaceous flowers or fruits from the Lower Cretaceous indicates that these simple flowers have had a long history (Friis et al., 1986; Dilcher & Wang, 2009). Given that the sister relationship between *Ceratophyllum* and Chloranthaceae has been recovered in two well-designed mitochondrial gene phylogenetic studies and a series of analyses of carefully constructed morphological matrices, it may be premature to dismiss this result, as was done in the recently published APG III (Angiosperm Phylogeny Group, 2009).

All other relationships among the major lineages of mesangiosperms have been resolved only in three recent chloroplast phylogenomic analyses (Jansen et al., 2007; Moore et al., 2007, 2010). While it is encouraging to see the stable results from these studies, some caution is needed in interpreting the phylogenetic meaning of the reported high BS values, as this type of analysis tends to produce high support values for whatever relationships the particular taxon sampling scheme leads to, probably because of character over-sampling and taxon under-sampling (Delsuc et al., 2005; Hedtke et al., 2006; Heath et al., 2008). Three examples published over the last decade on phylogenetic analyses of some key green alga and land plant lineages serve as a sober reminder of this effect: *Amborella* (Goremykin et al., 2003, 2009; Soltis et al., 2004; Stefanovic et al., 2004; Leebens-Mack et al., 2005; Qiu et al., 2005; Jansen et al., 2007; Moore et al., 2007, 2010); liverworts, mosses, and hornworts (Nishiyama et al., 2004; Goremykin & Hellwig, 2005; Qiu et al., 2006b, 2007); and *Mesostigma* (Lemieux et al., 2000, 2007; Qiu & Lee, 2000). In a

recent chloroplast phylogenomic study, it was shown that the relationships among the five mesangiosperm lineages, despite having moderate BS support, were statistically indistinguishable in an alternative topology test (Moore et al., 2007). One should also bear in mind that BS values only measure the fit between the data and the resulting tree, and that if the data contain any bias that undercuts representativeness of the data for the whole data space of the investigated group, phylogenetic informativeness of BS values, high or low, may be compromised (Sanderson, 1995). In the case of chloroplast phylogenomic analyses, because taxon sampling is usually sparse relative to the taxonomic scope covered, any amount of bias can be amplified through sampling of a large number of characters. Compact organellar genomes, because they have coded for highly specialized functions during a long period of evolution, are known for molecular evolutionary oddities such as hydrophobicity bias, RNA editing, and GC content skew (Naylor & Brown, 1997; Jobson & Qiu, 2008). They can lead phylogenetic algorithms astray if improper attention is paid to these complicating and potentially confounding factors.

2.1.3 Magnoliids The monophyly of magnoliids, and the sister relationships between their two pairs of member lineages, Magnoliales/Laurales and Canellales/Piperales, are weakly to moderately supported (Fig. 2-1). This is the second large-scale angiosperm phylogenetic analysis that has recovered these results; the first was the *matK* analysis by Hilu et al. (2003). Most medium-scale analyses focusing on basal angiosperms have also recovered these relationships with various degrees of support (Mathews & Donoghue, 1999; Qiu et al., 1999, 2000, 2005, 2006a; Barkman et al., 2000, 2007; Graham & Olmstead, 2000; Nickrent et al., 2002; Zanis et al., 2002, 2003; Borsch et al., 2003; Lohne & Borsch, 2005; Qiu & Estabrook, 2008). This is one of the cases where moderate support in many studies that sample a wide variety of genes from chloroplast, mitochondrial, and nuclear genomes has led to a consensus that the true underlying plant phylogeny has probably been reconstructed.

2.1.4 Monocots The monophyly of monocots is strongly supported, regardless of inclusion or exclusion of two *Acorus* species, which have divergent sequences for the three mitochondrial genes used in this study (Figs. 2-2, S2.1, Table 1; the *matR* sequences of *Acorus* were even more divergent and thus were not used). Within monocots other than *Acorus*, a deep split between Alismatales and all the remaining groups (Petrosaviidae of Cantino et al., 2007) is strongly supported. This deep split was previously identified with moderate to strong support in two large-scale analyses of angiosperms (Soltis et al., 2000; Hilu et al., 2003). Three medium-scale analyses, which had relatively dense taxon sampling and included seven genes (chloroplast *atpB, matK, ndhF,* and *rbcL*; mitochondrial *atp1*; nuclear 18S and 26S rDNA) (Chase et al., 2006), four genes (chloroplast *matK* and *rbcL*; mitochondrial *atp1* and *cob*) (Davis et al., 2006), or 16 kb of chloroplast DNA sequences (Graham et al., 2006), also identified this deep split with strong support. This result is further corroborated by the discovery of a *trans*-spliced group II intron in the mitochondrial gene *nad1* in 94 genera of petrosaviid monocots, which occurs rarely in the land plant mitochondrial genome (Qiu & Palmer, 2004).

The relationships among four strongly supported clades within petrosaviid monocots (Asparagales, Pandanales + Dioscoreales, Liliales, and commelinids) have only weak to moderate support. These results are comparable to or better than those obtained in previous large-scale angiosperm analyses (Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003). In three medium-scale analyses focusing on monocots (Chase et al., 2006; Davis et al., 2006; Graham et al., 2006), relationships among Asparagales, Pandanales + Dioscoreales, Liliales, and commelinids were resolved differently, with generally moderate support. However, Asparagales and commelinids were sister to each other in the analyses of Chase et al. (2006) and Graham et al. (2006), with moderate to strong BS support. This relationship seems to be supported by distribution of silica bodies and floral zygomorphy resulting from organ suppression in these two groups (Prychid et al., 2004; Rudall & Bateman, 2004), but some homoplasy in these characters suggest that they may not be synapomorphic. The placement of Asparagales as the sister to other petrosaviid monocots in this study may be an analytical artifact caused by long branches in several alismatalean taxa and insufficient taxon sampling (the critical Petrosaviales were not sampled here).

2.1.5 Basal eudicots The monophyly of eudicots is strongly supported (Fig. 1). The Ranunculales, Sabiales, Proteales, Trochodendrales, and Buxales form a series of diverging lineages at the base of eudicot phylogeny, with Buxales being sister to the group of eudicots that have been named Gunneridae (Cantino et al., 2007). All of these relationships, except those of Sabiales and Proteales, whose arrangement is effectively unresolved, have strong BS support (Figs. 1, 2-2). These results are similar to those obtained in a *matK* analysis of angiosperms (Hilu et al., 2003). The combined analyses of *atpB*/*rbcL* (Savolainen et al., 2000a), *atpB*/*rbcL*/18S rDNA (Soltis et al., 2000), and *atpB*/*matK*/*rbcL*/18S and 26S rDNAs (Burleigh et al., 2009) for angiosperms recovered similar, but less resolved relationships with

lower support. The most recently reported chloroplast phylogenomic analysis also showed similar resolution for these relationships, but again the arrangement of Sabiales and Proteales was resolved differently (Moore et al., 2010). Ancient losses of two ribosomal protein genes in the angiosperm mitochondrial genome support the relationships reconstructed here: the loss of *rps11* in the common ancestor of Buxales and Gunneridae, and the loss of *rps2* in the common ancestor of Trochodendrales, Buxales, and Gunneridae (note, however, that both genes have been lost a few times separately in angiosperms) (Adams et al., 2002).

The Ranunculales are the only group of basal eudicots with substantial living diversity. In this analysis (Fig. 2-2) and an earlier mitochondrial gene analysis with similar sampling of basal eudicots (Qiu et al., 2006a), Menispermaceae were shown to be sister to the rest of the order with moderate support. In contrast, most other molecular phylogenetic studies have shown that Papaveraceae or Eupteleaceae either singly or together were sister to the rest of Ranunculales (Hoot et al., 1999; Qiu et al., 1999, 2005, 2006a; Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003; Wang et al., 2009b). Gynoecium structure suggests that Menispermaceae and Lardizabalaceae are closely related (Endress & Igersheim, 1999). These families are distantly separated in the mitochondrial gene trees (Fig. 2-2; Qiu et al., 2006a), whereas the results of other molecular phylogenetic studies are more compatible with the relationship suggested by the gynoecium evidence. The long branch leading to Menispermaceae (Fig. 2-2), corroborated by observation of a number of unique mutations in the gene alignment, may have caused misplacement of the family in the mitochondrial gene trees shown here.

2.1.6 Basal gunnerids Five groups, Gunnerales, Saxifragales, Vitales, Berberidopsidales, and Dilleniales, all except Saxifragales having little taxonomic diversity, form another series of diverging lineages before eudicots differentiate into well supported rosids and asterids. The support for these relationships is generally low (Fig. 2-3). These taxa have also been difficult to place in previous large-scale analyses of angiosperms (Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003). The Gunnerales diverge first in this series, with low support. Previously, an analysis of *atpB, rbcL*, and 18S and 26S rDNA sequences from 201 eudicots obtained moderate support for the same placement (Soltis et al., 2003). The Saxifragales, often treated as a quasi-rosid member (Angiosperm Phylogeny Group, 2009), are essentially a member of a trichotomy that also includes an expanded rosid clade and an expanded asterid clade. The BS support for monophyly of Saxifragales is only 49% when the enigmatic genus *Peridiscus* (Davis & Chase, 2004) is included, but is high (97%) when *Peridiscus* is excluded. The relationships within this difficult order are fairly well resolved, and are in general agreement with the results of a recent study that sampled 16 chloroplast, mitochondrial, and nuclear genes (Jian et al., 2008) (the *rps3* sequence for *Paeonia tenuifolia* used in that study was found to be a contaminant during the course of this study).

The Vitales are sister to rosids, with only 44% BS support. This result is in agreement with the results of two previous large-scale analyses of angiosperms (Savolainen et al., 2000a; Soltis et al., 2000). The Berberidopsidales and Dilleniales form a weakly supported clade, which is sister to a monophyletic group consisting of Santalales, Caryophyllales, and asterids, but with only 49% BS support. The three previous large-scale analyses of angiosperms with dense taxon sampling have not been able to resolve these relationships (Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003).

2.1.7 Rosids The monophyly of rosids has a BS support of 74% (Figs. 1, 2-3). The current rosid concept was formed in the first large-scale analysis of angiosperms using molecular data (Chase et al., 1993). Other analyses of this kind have recovered the monophyly of rosids with various degrees of support, depending on the number and evolutionary rates of the genes sampled: 99% jackknife (JK) value in the *atpB*/*rbcL*/18S rDNA analysis (Soltis et al., 2000), 95% JK value in the *matK* analysis (Hilu et al., 2003), 99% BS value in the *atpB*/*matK*/*rbcL*/18S/26S rDNA analysis (Burleigh et al., 2009), and 61% BS value in the *atpB*/*rbcL* analysis (Savolainen et al., 2000a). In an analysis of non-molecular data, the rosids were also clustered together, although the group contained a few non-rosid taxa (Nandi et al., 1998).

Within rosids, Geraniales, Myrtales, and Crossosomatales/Zygophyllales are placed as a series of successively closer outgroups to the remaining rosids, or eurosids (Soltis et al., 2000) (Zygophyllales were among eurosids in that study), all with weak BS support (Fig. 2-3). The eurosids form a monophyletic group with only 43% BS support, and contain three large clades: (i) the nitrogen-fixing clade, as identified in a previous study (Soltis et al., 1995), which includes Fabales, Rosales, Cucurbitales, and Fagales; (ii) the malvid clade (Malvidae of Cantino et al., 2007), first recognized in Chase et al. (1993) as rosid II, which contains Huerteales, Sapindales, Malvales, and Brassicales; and (iii) the COM clade, as termed in two recent studies (Endress & Matthews, 2006; Zhu et al., 2007), which comprises Celastrales, Oxalidales, and Malpighiales (Fig. 2-4).

These relationships are in general agreement with the results from previous large-scale analyses of angiosperms or eudicots (Chase et al., 1993; Savolainen et al., 2000a, 2000b; Soltis et al., 2000; Hilu et al., 2003; Burleigh et al., 2009), but with one major exception.

The major result obtained in this study that differs from those of all previous large-scale analyses of angiosperms concerns the monophyly of fabids (Fabidae of Cantino et al., 2007; called rosid I in Chase et al., 1993). The weakly supported COM clade is sister to the strongly supported malvid clade, with 99% BS support (Fig. 2-4). Upon inclusion of *Bixa, Elatine*, and *Populus*, which had missing data for two or three of the four genes used in the analysis (Table 1), an 87% BS value was still recovered for this relationship (Fig. S2.1). In all previous large-scale analyses of angiosperms or eudicots, the COM clade was placed as a sister to the nitrogen-fixing clade (Chase et al., 1993; Savolainen et al., 2000a, 2000b; Soltis et al., 2000; Hilu et al., 2003; Burleigh et al., 2009), but never with strong support, the highest being 89% BS in Burleigh et al. (2009). It should be further pointed out that these analyses were based either entirely or mostly on chloroplast genes. Thus far, only one large-scale analysis of angiosperms has been carried out on nuclear gene data, 18S rDNA (Soltis et al., 1997), and in that study, no clearly defined fabids or malvids could be recognized.

Two medium-scale analyses focusing on rosids have been published recently, one using mitochondrial *matR*, chloroplast *atpB* and *rbcL*, and nuclear 18S rDNA (Zhu et al., 2007), and the other sampling 10 chloroplast genes (*atpB, matK, ndhF, psbBTNH* region, *rbcL, rpoC2,* and *rps4*) and two nuclear (18S and 26S) rDNAs (Wang et al., 2009a). In the former, a *matR* analysis generated 54% maximum likelihood BS support for the COM and malvid sister relationship, and a combined analysis of *atpB/rbcL/matR*/18S rDNA recovered fabid monophyly, with 70% or 85% BS support in parsimony or likelihood analyses, respectively. In the latter, 100% likelihood BS support was obtained for fabid monophyly. It is perhaps worth pointing out that most of the 10 chloroplast genes used in the latter study are fast-evolving. Although these chloroplast genes are certainly good for resolving rapid radiations, they are also susceptible to accumulation of homoplasious changes at deep nodes. These two medium-scale analyses indicate again that the support for fabid monophyly lies in chloroplast genes, as seen in the large-scale analyses.

One interesting piece of evidence that supports the newly identified sister relationship between the COM and the malvid clades comes from a recent broad survey of floral structural characters (Endress & Matthews, 2006). It was shown that 22 COM families and 18 malvid families share a type of ovule with a thicker inner integument than the outer one, which is otherwise very rare in eudicots (with only one other occurrence, in Trochodendrales). There are some other features that may indicate a close relationship between the COM and the malvid clades: contort petals, and a tendency towards polystemony and polycarpelly (Endress & Matthews, 2006). Notably, that survey did not find any feature supporting the monophyly of fabids.

We also examined our own data to see what type of mutations were behind the strong BS support of the sister relationship between the COM and the malvid clades. A total of six synapomorphic mutations were detected, two in *matR* (one synonymous and one non-synonymous) and four in *rps3* (one synonymous and three non-synonymous), but none in *atp1* or *nad5*. Two of these mutations (one a T→A change, the other T→C) were "perfect" synapomorphies, without any reversal in the identified monophyletic group (the COM–malvid clade) or independent evolution of the same apomorphic state in any other group. The third synapomorphy was a G→A change, which again did not have any reversal within the COM–malvid clade but had one independent evolution of the apomorphic state in *Potamogeton*, which showed accelerated evolution in *matR* and all other mitochondrial genes that had been examined (Y.-L. Qiu, unpublished observation, 2010). The other three synapomorphies involved G→A or C→T changes, and also had relatively low levels of homoplasy. The fact that six synapomorphies involved five types of substitutions, four being transitions and one being a transversion, indicated that there was not any special molecular evolution mechanism such as RNA editing or GC content skew that could generate these changes. Furthermore, the extremely low point mutation rates in the plant mitochondrial genome in general (Wolfe et al., 1987; Palmer & Herbon, 1988) and in the specific genes used in this study (Table 3) are most likely to have contributed to the low levels of homoplasy observed here. Indeed, the four mitochondrial genes show significantly (21% on the average) lower levels of homoplasy than chloroplast *atpB, matK*, and *rbcL* and nuclear 18S rDNA across angiosperms in our analyses (Table 4). We also examined alignment of the four genes to see if there were any types of mutations that supported the fabid monophyly hypothesis, and we did not find any.

Finally, as we assembled 272 OTU matrices for chloroplast *atpB, matK*, and *rbcL* and nuclear 18S rDNA in the rate and homoplasy analyses, we checked these matrices to see what type of mutations supported the fabid monophyly hypothesis, and if there was any evidence in these four genes that would support the sister

Table 4 Consistency index (CI) and homoplasy index (HI) values of the eight genes estimated from the matrix of 272 seed plants in parsimony searches under a constraint tree shown in Fig. S1

	mt-*atp1*	mt-*matR*	mt-*nad5*	mt-*rps3*
CI:	0.284	0.413	0.325	0.334
HI:	0.716	0.587	0.676	0.666
	cp-*atpB*	cp-*matK*	cp-*rbcL*	nu-18S
CI:	0.163	0.164	0.148	0.185
HI:	0.837	0.836	0.852	0.815

cp, chloroplast; mt, mitochondrial; nu, nuclear.

relationship between the COM and the malvid clades. As a result, we found that in *atpB, matK*, and *rbcL,* there were one (G→A), five (two A→G, three T→C), and five (three C→T, one T→C, one G→A) changes, respectively, in favor of the fabid monophyly hypothesis, and two (one T→C, one G→A), two (both C→T), and one (C→T) changes supporting the sister relationship between the COM and the malvid clades. We wish to point out that in comparison to synapomorphic changes in mitochondrial *matR* and *rps3*, these chloroplast synapomorphies contained a higher level of homoplasy, and several were extremely homoplasious. This observation is consistent with the results of our homoplasy analyses of these chloroplast genes across angiosperms (Table 4). The nuclear 18S rDNA contained no synapomorphy for either hypothesis.

2.1.8 Santalales and Caryophyllales Both Santalales and Caryophyllales are strongly supported (99 and 100% BS values, respectively) as monophyletic groups. They and asterids effectively form a trichotomy, as the support for the sister relationship between the two orders is negligibly low (Fig. 2-5). The expansion of Caryophyllales to include several traditionally non-caryophyllalean taxa, such as Nepenthaceae, Droseraceae, Dioncophyllaceae, Ancistrocladaceae, Frankeniaceae, Tamaricaceae, Simmondsiaceae, and Rhabdodendraceae (Albert et al., 1992; Fay et al., 1997), has been supported by previous large-scale analyses of angiosperms (Savolainen et al., 2000a, 2000b; Soltis et al., 2000; Hilu et al., 2003) as well as a cladistic analysis of morphological data (Nandi et al., 1998). In the previous large-scale analyses of angiosperms, Santalales and Caryophyllales have been placed as two successively closer outgroups to (or sometimes merely as close relatives of) asterids, usually with weak support (Soltis et al., 1997, 2000; Hilu et al., 2003; Burleigh et al., 2009). The most recent chloroplast phylogenomic analysis has reconstructed these relationships with strong BS support (Moore et al., 2010). Until recently, however, no clear morphological or other non-molecular data have been found to support these relationships. In a broad survey of floral structural and embryological characters across eudicots, it was noticed that Santalales and Caryophyllales tend to have a relatively thin nucellus in their ovules, thus conforming to asterids (Endress, in press). It has also been suggested that a shift from palmate leaf venation in basal eudicots (Ranunculales, Proteales, Trochodendrales) to pinnate venation seen in Berberidopsidales, Dilleniales, Santalales, Caryophyllales and asterids may represent a synapomorphy of the five latter groups (Doyle, 2007). Within Caryophyllales and Santalales, the relationships resolved here are in general agreement with those reconstructed in two medium-scale multigene analyses focusing on each of the two orders (Cuenoud et al., 2002; Malecot & Nickrent, 2008).

2.1.9 Asterids The asterids form a monophyletic group with 82% BS support (Figs. 2-5, 2-6). Although the subclass Asteridae was recognized in the pre-molecular systematics era (Cronquist, 1981), it was significantly expanded in the first wave of molecular phylogenetic studies (Downie & Palmer, 1992; Olmstead et al., 1992, 1993; Chase et al., 1993), to include a number of groups that were previously placed in the Rosidae and the now defunct Dilleniidae and Hamamelidae (Cronquist, 1981). All large-scale analyses of angiosperms have obtained strong support for the monophyly of asterids (Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003; Burleigh et al., 2009).

Within asterids, Ericales, *Curtisia,* and Cornales are placed as a series of successively closer outgroups to a weakly supported monophyletic group that has been called euasterids (Soltis et al., 2000). Among euasterids, Apiales, Dipsacales, Asterales and several seemingly poorly defined orders (Bruniales, Escalloniales, and Paracryphiales) form a strongly supported monophyletic group, the campanulids (Campanulidae of Cantino et al., 2007). Boraginaceae, Vahliaceae, Solanales, Gentianales, and Lamiales form another major clade, the lamiids (Lamiidae of Cantino et al., 2007), but with only weak support. Oncothecaceae, Icacinaceae, Garryales, and Aquifoliales represent basal euasterid lineages whose relationships to the two large clades are essentially unresolved. These relationships agree fairly well with what have been reconstructed in the previous phylogenetic studies of asterids (Olmstead et al., 1993, 2000; Albach et al., 2001; Bremer et al., 2002). Because three of the four mitochondrial genes (*atp1, matR*, and *nad5*) used in this study are slow- or very slow-evolving in comparison to most chloroplast genes used in angiosperm phylogenetic studies, and the fourth gene, *rps3*, is still not very fast (Table 3), relationships among the major clades within Ericales, campanulids, and lamiids are not well supported. Interestingly, in two studies that sampled several fast-evolving

chloroplast genes such as *ndhF, matK*, and *rps16* intron, lack of resolution among these clades was also observed (Bremer et al., 2002; Schonenberger et al., 2005). Hence, the asterids may well represent a case of rapid radiation of a major clade of angiosperms, and the late appearance of sympetalous flowers relative to other floral types in the Late Cretaceous (Friis, 1985; Martinez-Millan, 2010) is consistent with such a hypothesis.

2.2 Mitochondrial genes and angiosperm phylogeny

The rate analyses show that the eight genes are ranked in the following order, from the fastest to the slowest: *matK* > *rbcL* > *rps3* > 18S rDNA > *atpB* > *matR* > *atp1* > *nad5* (Table 3). The homoplasy analyses show that the four mitochondrial genes show significantly (on average 21%) lower levels of homoplasy than the three chloroplast genes and nuclear 18S rDNA, as measured by the homoplasy index (Table 4). The consistency indexes of the four mitochondrial genes were almost twice as high as those of the other four genes (Table 4). These data provide some theoretical underpinning to support the empirical results presented above and show that for angiosperm-wide phylogeny reconstruction, these mitochondrial genes are at least as good as, if not better than, those four genes that have been used widely. It is not surprising to see the correlation between the evolutionary rate and the homoplasy level, because homoplasy is after all determined by at least four factors: (i) rate (if no variation, no homoplasy); (ii) factors that cause character state changes in similar directions since common ancestry; (iii) limited character evolution space such as that of DNA with only four possible nucleotides (states); and (iv) rate heterogeneity.

Several general results can be summarized here. First, the mitochondrial gene-based angiosperm phylogeny agrees with the chloroplast and nuclear gene-based angiosperm phylogeny to a great extent. This indicates that the hypotheses developed for angiosperm phylogeny thus far are likely to be largely correct. The congruence of the results among these different studies is especially significant, as the several large-scale analyses of angiosperms have used different methods (parsimony or likelihood) for searching optimal trees and also used either BS or JK analyses for evaluating robustness of the topology. Until now, our knowledge of higher level relationships within angiosperms has been derived largely from three chloroplast genes, *atpB, matK*, and *rbcL* (Chase et al., 1993; Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003). The angiosperm phylogeny inferred from the sole nuclear gene, 18S rDNA, was inconclusive on some issues (Soltis et al., 1997). The other nuclear gene that has been used for reconstructing higher level relationships within angiosperms, 26S rDNA, had too much missing data to permit a critical evaluation (Burleigh et al., 2009). Use of non-molecular data for reconstructing an angiosperm-wide phylogeny has only been explored experimentally (Nandi et al., 1998), and much needs to be done after morphological and other non-molecular characters have been surveyed critically across all major groups, as has been done for basal angiosperms (Doyle & Endress, 2000, 2010; Endress & Igersheim, 2000; Endress & Doyle, 2009), basal eudicots (Endress & Igersheim, 1999), monocots (Prychid et al., 2004; Rudall & Bateman, 2004), rosids (Endress & Matthews, 2006), and eudicots (Endress, in press). Although the chloroplast gene-based or gene-dominated studies have significantly improved our understanding of angiosperm phylogeny, it is always desirable and perhaps necessary to obtain information from mitochondrial and nuclear genomes as well as non-molecular sources to reconstruct angiosperm phylogeny. Until such comparison and mutual corroboration is carried out, the phylogenetic hypothesis derived from the chloroplast genome remains a genome phylogeny, and cannot be equated to the organismal phylogeny (Doyle, 1992; Qiu & Palmer, 1999). The results discussed below provide some examples to illustrate this point.

Second, in certain areas where chloroplast gene-based or gene-dominated large-scale analyses of angiosperms failed to resolve or obtained lower support for relationships among member lineages, such as magnoliids, basal eudicots, Saxifragales, and the COM clade of rosids (Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003), this mitochondrial gene analysis appears to provide more resolution and/or better support. The agreement of these results with those obtained from other studies (Endress & Matthews, 2006; Qiu et al., 2006a; Jian et al., 2008; Burleigh et al., 2009) may be evidence that the results from this mitochondrial gene analysis are likely to be correct.

Third, this study produced different results in several critical areas than the previous chloroplast gene-based or gene-dominated large-scale analyses of angiosperms (Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003). The most notable example is the newly identified rosid clade that contains the COM and the malvid clades, which is independently supported by some morphological data (Endress & Matthews, 2006). In addition, this study also provided alternative hypotheses that deserve further investigation with respect to the basalmost angiosperm lineage and the placement of *Ceratophyllum*. This result and the second one

discussed above may be attributed to the facts that the mitochondrial genome has a lower overall substitution rate than the chloroplast genome (Wolfe et al., 1987; Palmer & Herbon, 1988), and that the mitochondrial genes used in this study are generally more slowly evolving and less homoplasious than the chloroplast genes that have been used in previous large-scale analyses of angiosperms (Savolainen et al., 2000a; Soltis et al., 2000; Hilu et al., 2003) (Tables 3, 4). These are also the parts of angiosperm phylogeny where reconstruction has been likely hampered by extinction. In this case, the less homoplasious information provided by the more slowly evolving genes may have contributed less conflicting signals within the dataset (Table 4), which is what a statistic resampling procedure like bootstrapping is designed to measure (Felsenstein, 2004).

Finally, several potential problems of mitochondrial genes for phylogenetic use, rate heterogeneity among lineages (Palmer et al., 2000; Cho et al., 2004; Parkinson et al., 2005), horizontal transfer (Bergthorsson et al., 2003; Won & Renner, 2003; Davis & Wurdack, 2004; Mower et al., 2004), and RNA editing (Bowe & dePamphilis, 1996; Petersen et al., 2006; Qiu et al., 2006a), turned out not to have serious negative effects on phylogenetic reconstruction of an angiosperm-wide phylogeny, probably for the following reasons. For rate heterogeneity, only a small number of lineages showed significant rate acceleration for the four genes sequenced here: *Metasequoia, Podocarpus, Acorus*, Alismatales, Geraniaceae, Urticaceae, *Viscum*, *Phaulothamnus, Asteropeia, Galenia, Phlox, Phryma,* Plantaginaceae, *Hydrolea*, and Goodeniaceae. Some of these were already detected in a previous large-scale Southern hybridization survey of mitochondrial genes and introns throughout land plants (Qiu et al., 1998; Adams et al., 2002; Qiu & Palmer, 2004). This problem was dealt with by either choosing a different member of the group or omitting the problematic taxa. The extent of horizontal gene transfer has probably been overstated, at least in non-parasitic plants, as only one such case was encountered in this study. The parasitic plant *Cynomorium songaricum*, depending on the genes analyzed, was placed with either Saxifragales (*matR* and *nad5*) or Sapindales (*atp1* and *rps3*). It was thus not included in the final analyses. Previously, this genus has been suggested to be related to Saxifragales in an analysis of *matR* (Nickrent et al., 2005) or Rosales in a study of the inverted repeat region of the chloroplast genome (Zhang et al., 2009). The level and distribution across lineages of RNA editing are clearly not high or widespread enough to have affected phylogenetic reconstruction in this analysis (Fig. S3), and this result is consistent with what was found in an earlier smaller-scale analysis of 162 seed plants (Qiu et al., 2006a). Other characteristics of plant mitochondrial DNA suggested previously that might affect its performance in phylogenetic analysis, such as slow rate and presence of introns (Palmer, 1992), can be exploited from different angles for phylogenetic uses, as plant molecular systematists become more skillful and knowledgeable of molecular techniques and the plant mitochondrial genome (Qiu et al., 1998, 2006b; Qiu & Palmer, 2004; Barkman et al., 2007; Ran et al., 2009; Wurdack & Davis, 2009). The slow rate, in fact, turns out to be a merit of plant mitochondrial DNA when it is used for resolving ancient phylogenetic relationships, as it contains less homoplasy. Therefore, the mitochondrial genome has great potential for investigating phylogenetic relationships of angiosperms as well as non-flowering land plants.

3 Conclusions and future prospects

An angiosperm phylogeny was reconstructed with information extracted from nucleotide sequence variation of four slowly evolving mitochondrial genes, *atp1, matR, nad5*, and *rps3*. It is largely congruent with the phylogeny of angiosperms that have been reconstructed from analyzing the chloroplast genes *atpB, matK, rbcL*, and nuclear 18S rDNA (Chase et al., 1993; Soltis et al., 1997, 2000; Savolainen et al., 2000a; Hilu et al., 2003). The most prominent difference is that the COM clade is sister to the malvid clade, instead of to the nitrogen-fixing clade. This relationship is not only supported by highly conservative mutations identified in this study, but also independently corroborated by one embryological character and perhaps a few other floral structural and embryological characters (Endress & Matthews, 2006). Hence, the long recognized monophyly of fabids (Chase et al., 1993) needs to be re-evaluated with evidence from all three plant genomes and non-molecular data. Other major differences between the results of this study and those of the earlier large-scale analyses of angiosperms include placement of *Amborella,* Hydatellaceae, and Nymphaeales together as the clade sister to all other angiosperms, and *Ceratophyllum* as the sister group of Chloranthaceae. This study shows that mitochondrial genes are informative markers for resolving relationships among genera, families, or higher rank taxa across angiosperms. Their slow evolutionary rates are particularly beneficial for reconstructing ancient phylogenetic relationships, as they have been shown to be less homoplasious than typically faster-evolving chloroplast genes (Table 4). Several potential problems of mitochondrial genes such as rate heterogeneity, horizontal transfer, and RNA editing have been

somewhat exaggerated, and can be effectively dealt with by selective taxon sampling and analysis of combined multigene datasets. Otherwise, one would not expect such a high level of congruence between the angiosperm phylogeny based on the mitochondrial genes and those based on the three chloroplast genes and nuclear 18S rDNA.

This study also shows that, despite the tremendous progress in our understanding of angiosperm phylogeny made through analyses of chloroplast genes, it is essential to develop independent hypotheses on angiosperm phylogeny using information from mitochondrial and nuclear genes as well as non-molecular data, so that the underlying organismal phylogeny, not just the phylogeny of a single genome, is reconstructed. The case of rosids uncovered in this study serves as a sober reminder that it is better not to rely on just a few genes to reconstruct phylogenetic relationships among major clades even in large-scale taxon dense analyses. Because nuclear genes often experience duplication over a long evolutionary time in large taxonomic groups, such as angiosperms and land plants, mitochondrial genes may be more suitable for developing hypotheses for large-scale phylogenies. The low substitution rates and low levels of homoplasy of the mitochondrial genes, relative to most chloroplast genes, make them particularly useful for reconstructing ancient phylogenetic relationships, and several studies have shown that the efforts of exploiting mitochondrial genes have produced insightful results (Beckert et al., 1999, 2001; Qiu et al., 1999, 2006a, 2006b, 2007; Vangerow et al., 1999; Bowe et al., 2000; Chaw et al., 2000; Wikstrom & Pryer, 2005; Barkman et al., 2007; Wurdack & Davis, 2009). More mitochondrial markers are thus needed in addition to the four genes used here. A preliminary analysis shows that there are at least four other mitochondrial genes that show variation at the levels of *atp1* and *matR*: *atp6* (ATPase subunit 6, 0.8 kb), *ccmFN* (cytochrome *c* biogenesis FN, 1.2 kb), *cox3* (cytochrome *c* oxidase subunit 3, 0.8 kb), and *mttB* (transport membrane protein, also called *tatC,* 0.8 kb) (Y.-L. Qiu, unpublished data, 2010). These genes are suitable for reconstructing phylogenies of both angiosperms and non-flowering land plants, as they are widely present throughout land plants (Li et al., 2009). Thus, it is entirely feasible that, in the near future, a well resolved and robustly supported mitochondrial gene-based phylogeny for angiosperms or land plants could be developed, and compared with a chloroplast gene-based phylogeny in order to reconstruct the underlying organismal phylogeny.

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References

Adams KL, Qiu YL, Stoutemyer M, Palmer JD. 2002. Punctuated evolution of mitochondrial gene content: High and variable rates of mitochondrial gene loss and transfer to the nucleus during angiosperm evolution. Proceedings of the National Academy of Sciences USA 99: 9905–9912.

Albach DC, Soltis PS, Soltis DE, Olmstead RG. 2001. Phylogenetic analysis of Asteridae based on sequences of four genes. Annals of the Missouri Botanical Garden 88: 163–212.

Albert VA, Williams SE, Chase MW. 1992. Carnivorous plants: phylogeny and structural evolution. Science 257: 1491–1495.

Algeo TJ, Scheckler SE, Maynard JB. 2001. Effects of the Middle to Late Devonian spread of vascular land plants on weathering regimes, marine biotas, and global climate. In: Gensel PG, Edwards D eds. Plants invade the land: Evolutionary & environmental perspectives. New York: Columbia University Press. 213–236.

Anderberg AA, Rydin C, Kallersjo M. 2002. Phylogenetic relationships in the order Ericales s.l.: Analyses of molcular data from five genes from the plastid and mitochondrial genomes. American Journal of Botany 89: 677–687.

Angiosperm Phylogeny Group. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Botanical Journal of the Linnean Society 161: 105–121.

Antonov AS, Troitsky AV, Samigullin TK, Bobrova V, Valiejo-Roman KM, Martin W. 2000. Early events in the evolution of angiosperms deduced from cp rDNA ITS 2–4 sequence comparisons. In: Liu YH, Fan HM, Chen ZY, Wu QG, Zeng QW eds. Proceedings of the International Symposium on the Family Magnoliaceae. Beijing: Science Press. 210–214.

Bailey IW, Nast CG. 1943. The comparative morphology of the Winteraceae I. Pollen and stamens. Journal of the Arnold Arboretum 24: 340–346.

Barkman TJ, Chenery G, McNeal JR, Lyons-Weiler J, Ellisens WJ, Moore G, Wolfe AD, dePamphilis CW. 2000. Independent and combined analyses of sequences from all three

genomic compartments converge on the root of flowering plant phylogeny. Proceedings of the National Academy of Sciences USA 97: 13166–13171.

Barkman TJ, McNeal JR, Lim S-H, Coat G, Croom HB, Young ND, dePamphilis CW. 2007. Mitochondrial DNA suggests at least 11 origins of parasitism in angiosperms and reveals genomic chimerism in parasitic plants. BMC Evolutionary Biology 7: 248.

Beckert S, Muhle H, Pruchner D, Knoop V. 2001. The mitochondrial *nad2* gene as a novel marker locus for phylogenetic analysis of early land plants: A comparative analysis in mosses. Molecular Phylogenetics and Evolution 18: 117–126.

Beckert S, Steinhauser S, Muhle H, Knoop V. 1999. A molecular phylogeny of bryophytes based on nucleotide sequences of the mitochondrial *nad5* gene. Plant Systematics and Evolution 218: 179–192.

Bergthorsson U, Adams KL, Thomason B, Palmer JD. 2003. Widespread horizontal transfer of mitochondrial genes in flowering plants. Nature 424: 197–201.

Berner RA. 2001. The effect of the rise of land plants on atmospheric CO_2 during the Paleozoic. In: Gensel PG, Edwards D eds. Plants invade the land: Evolutionary and environmental perspectives. New York: Columbia University Press. 173–178.

Borsch T, Hilu KW, Quandt D, Wilde V, Neinhuis C, Barthlott W. 2003. Noncoding plastid trnT-trnF sequences reveal a well resolved phylogeny of basal angiosperms. Journal of Evolutionary Biology 16: 558–576.

Bowe LM, Coat G, dePamphilis CW. 2000. Phylogeny of seed plants based on all three genomic compartments: Extant gymnosperms are monophyletic and Gnetales' closest relatives are conifers. Proceedings of the National Academy of Sciences USA 97: 4092–4097.

Bowe LM, dePamphilis CW. 1996. Effects of RNA editing and gene processing on phylogenetic reconstruction. Molecular Biology and Evolution 13: 1159–1166.

Bremer B, Bremer K, Heidari N, Erixon P, Olmstead RG, Anderberg AA, Kallersjo M, Barkhordarian E. 2002. Phylogenies of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. Molecular Phylogenetics and Evolution 24: 274–301.

Brenner GJ. 1976. Middle Cretaceous floral provinces and early migrations of angiosperms. In: Beck CB ed. Origin and early evolution of angiosperms. New York: Columbia University Press. 23–47.

Burleigh JG, Hilu KW, Soltis DE. 2009. Inferring phylogenies with incomplete data sets: A 5-gene, 567-taxon analysis of angiosperms. BMC Evolutionary Biology 9: 61.

Cantino PD, Doyle JA, Graham SW, Judd WS, Olmstead RG, Soltis DE, Soltis PS, Donoghue MJ. 2007. Towards a phylogenetic nomenclature of Tracheophyta. Taxon 56: 822–846.

Chase MW, Fay MF, Devey DS, Maurin O, Ronsted N, Davies TJ, Pillon Y, Petersen G, Seberg O, Tamura MN, Asmussen CB, Hilu K, Borsch T, Davis JI, Stvenson DW, Pires JC, Givnish TJ, Sytsma KJ, McPherson MA, Graham SW, Rai HS. 2006. Multigene analyses of monocot relationships: A summary. Aliso 22: 63–75.

Chase MW, Soltis DE, Olmstead RG, Morgan D, Les DH, Mishler BD, Duvall MR, Price RA, Hills HG, Qiu YL, Kron KA, Rettig JH, Conti E, Palmer JD, Manhart JR, Sytsma KJ, Michaels HJ, Kress WJ, Karol KG, Clark WD, Hedren M, Gaut BS, Jansen RK, Kim KJ, Wimpee CF, Smith JF, Furnier GR, Strauss SH, Xiang QY, Plunkett GM, Soltis PS, Swensen SM, Williams SE, Gadek PA, Quinn CJ, Eguiarte LE, Golenberg E, Learn GH, Graham SW, Barrett SCH, Dayanandan S, Albert VA. 1993. Phylogenetics of seed plants – an analysis of nucleotide-sequences from the plastid gene rbcL. Annals of the Missouri Botanical Garden 80: 528–580.

Chaw SM, Parkinson CL, Cheng YC, Vincent TM, Palmer JD. 2000. Seed plant phylogeny inferred from all three plant genomes: Monophyly of extant gymnosperms and origin of Gnetales from conifers. Proceedings of the National Academy of Sciences USA 97: 4086–4091.

Cho Y, Mower JP, Qiu Y-L, Palmer JD. 2004. Mitochondrial substitution rates are extraordinarily elevated and variable in a genus of flowering plants. Proceedings of the National Academy of Sciences USA 101: 17741–17746.

Cronquist A. 1981. An integrated system of classification of flowering plants. New York: Columbia University Press.

Cronquist A. 1988. The evolution and classification of flowering plants, 2nd ed. New York: The New York Botanical Garden.

Cuenoud P, Savolainen V, Chatrou LW, Powell M, Grayer RJ, Chase MW. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. American Journal of Botany 89: 132–144.

Dahlgren R, Bremer K. 1985. Major clades of the angiosperms. Cladistics 1: 349–368.

Davis CC, Chase MW. 2004. Elatinaceae are sister to Malpighiaceae; Peridiscaceae belong to Saxifragales. American Journal of Botany 91: 262–273.

Davis CC, Wurdack KJ. 2004. Host-to-parasite gene transfer in flowering plants: Phylogenetic evidence from Malpighiales. Science 305: 676–678.

Davis JI, Petersen G, Seberg O, Stevenson DW, Hardy CR, Simmons MP, Michelangeli FA, Goldman DH, Campbell LM, Specht CD, Cohen JI. 2006. Are mitochondrial genes useful for the analysis of monocot relationships? Taxon 55: 857–870.

Davis JI, Stevenson DW, Petersen G, Seberg O, Campbell LM, Freudenstein JV, Goldman DH, Hardy CR, Michelangeli FA, Simmons MP, Specht CD, Vergara-Silva F, Gandolfo M. 2004. Phylogeny of the monocots, as inferred from *rbcL* and *atpA* sequence variation, and a comparison of methods for calculating jackknife and bootstrap values. Systematic Botany 29: 467–510.

Delsuc F, Brinkmann H, Philippe H. 2005. Phylogenomics and the reconstruction of the tree of life. Nature Reviews Genetics 6: 361–375.

Dilcher D. 2000. Toward a new synthesis: Major evolutionary trends in the angiosperm fossil record. Proceedings of the National Academy of Sciences USA 97: 7030–7036.

Dilcher DL, Wang H. 2009. An Early Cretaceous fruit with affinities to Ceratophyllaceae. American Journal of Botany 96: 2256–2269.

Dombrovska O, Qiu Y-L. 2004. Distribution of introns in the mitochondrial gene *nad1* in land plants: Phylogenetic and

molecular evolutionary implications. Molecular Phylogenetics and Evolution 32: 246–263.
Donoghue MJ, Doyle JA. 1989. Phylogenetic analysis of angiosperms and the relationships of Hamamelidae. In: Crane PR, Blackmore S eds. Evolution, systematics, and fossil history of the Hamamelidae, vol. 1. Oxford: Clarendon Press. 17–45.
Downie SR, Palmer JD. 1992. Restriction site mapping of the chloroplast DNA inverted repeat: A molecular phylogeny of the Asteridae. Annals of the Missouri Botanical Garden 79: 266–283.
Doyle JA. 2007. Systematic value and evolution of leaf architecture across the angiosperms in light of molecular phylogenetic analyses. Courier Forschungsinstitut Senckenberg 258: 21–37.
Doyle JA, Biens P, Doerenkamp A, Jardine S. 1977. Angiosperm pollen from the pre-Albian Lower Cretaceous of Equatorial Africa. Bulletin des Centres de Recherches Exploration-Production Elf-Aquitaine 1: 451–473.
Doyle JA, Endress PK. 2000. Morphological phylogenetic analysis of basal angiosperms: Comparison and combination with molecular data. International Journal of Plant Sciences 161: S121–S153.
Doyle JA, Endress PK. 2010. Integrating Early Cretaceous fossils into the phylogeny of angiosperms: Magnoliidae and eudicots. Journal of Systematics and Evolution 48: 1–35.
Doyle JA, Endress PK, Upchurch GR. 2008. Early Cretaceous monocots: A phylogenetic evaluation. Acta Musei Nationalis Pragae, Series B, Historia Naturalis 64: 59–87.
Doyle JJ. 1992. Gene trees and species trees – molecular systematics as one-character taxonomy. Systematic Botany 17: 144–163.
Duvall MR, Learn GH, Eguiarte LE, Clegg MT. 1993. Phylogenetic analysis of *rbcL* sequences identifies *Acorus calamus* as the primal extant monocotyledon. Proceedings of the National Academy of Sciences USA 90: 4641–4644.
Duvall MR, Mathews S, Mohammad N, Russell T. 2006. Placing the monocots: Conflicting signal from trigenomic analyses. Aliso 22: 79–90.
Duvall MR, Robinson JW, Mattson JG, Moore A. 2008. Phylogenetic analyses of two mitochondrial metabolic genes sampled in parallel from angiosperms find fundamental interlocus incongruence. American Journal of Botany 95: 871–884.
Endress PK. 1986. Reproductive structures and phylogenetic significance of extant primitive angiosperms. Plant Systematics and Evolution 152: 1–28.
Endress PK. In press. Flower structure and trends of evolution in eudicots and their major subclades. Annals of the Missouri Botanical Garden.
Endress PK, Doyle JA. 2009. Reconstructing the ancestral angiosperm flower and its initial specializations. American Journal of Botany 96: 22–66.
Endress PK, Igersheim A. 1999. Gynoecium diversity and systematics of the basal eudicots. Botanical Journal of the Linnean Society 130: 305–393.
Endress PK, Igersheim A. 2000. Gynoecium structure and evolution in basal angiosperms. International Journal of Plant Sciences 161: S211–S223.
Endress PK, Matthews ML. 2006. First steps towards a floral structural characterization of the major rosids. Plant Systematics and Evolution 260: 223–251.
Fay MF, Cameron KM, Prance GT, Lledo MD, Chase MW. 1997. Familial relationships of *Rhabdodendron* (Rhabdodendraceae): Plastid *rbcL* sequences indicate a caryophyllid placement. Kew Bulletin 52: 923–932.
Felsenstein J. 2004. Inferring phylogenies. Sunderland: Sinauer.
Forrest LL, Davis EC, Long DG, Crandall-Stotler BJ, Clark A, Hollingsworth ML. 2006. Unraveling the evolutionary history of the liverworts (Marchantiophyta): Multiple taxa, genomes and analyses. The Bryologist 109: 303–334.
Friis EM. 1985. *Actinocalyx* gen. nov., sympetalous angiosperm flowers from the Upper Cretaceous of southern Sweden. Review of Palaeobotany and Palynology 45: 171–183.
Friis EM, Chaloner WG, Crane PR. 1987. The origins of angiosperms and their biological consequences. Cambridge: Cambridge University Press.
Friis EM, Crane PR, Pedersen KR. 1986. Floral evidence for Cretaceous chloranthoid angiosperms. Nature 320: 163–164.
Giege P, Brennicke A. 1999. RNA editing in *Arabidopsis* mitochondria effects 441 C to U changes in ORFs. Proceedings of the National Academy of Sciences USA 96: 15324–15329.
Goremykin VV, Hellwig FH. 2005. Evidence for the most basal split in land plants dividing bryophyte and tracheophyte lineages. Plant Systematics and Evolution 254: 93–103.
Goremykin VV, Hirsch-Ernst KI, Wolfl S, Hellwig FH. 2003. Analysis of the *Amborella trichopoda* chloroplast genome sequence suggests that *Amborella* is not a basal angiosperm. Molecular Biology and Evolution 20: 1499–1505.
Goremykin VV, Viola R, Hellwig FH. 2009. Removal of noisy characters from chloroplast genome-scale data suggests revision of phylogenetic placements of *Amborella* and *Ceratophyllum*. Journal of Molecular Evolution 68: 197–204.
Graham SW, Iles WJD. 2009. Different gymnosperm outgroups have (mostly) congruent signal regarding the root of flowering plant phylogeny. American Journal of Botany 96: 216–227.
Graham SW, Olmstead RG. 2000. Utility of 17 chloroplast genes for inferring the phylogeny of the basal angiosperms. American Journal of Botany 87: 1712–1730.
Graham SW, Zgurski JM, McPherson MA, Cherniawsky DM, Saarela JM, Horne ESC, Smith SY, Wong WA, O'Brien HE, Biron VI, Pires JC, Olmstead RG, Chase MW, Rai HS. 2006. Robust inference of monocot deep phylogeny using an expanded multigene plastid data set. Aliso 22: 3–20.
Hamby RK, Zimmer EA. 1992. Ribosomal RNA as a phylogenetic tool in plant systematics. In: Soltis PS, Soltis DE, Doyle JJ eds. Molecular systematics of plants. New York: Chapman and Hall.
Handa H. 2003. The complete nucleotide sequence and RNA editing content of the mitochondrial genome of rapeseed (*Brassica napus* L.): Comparative analysis of the mitochondrial genomes of rapeseed and *Arabidopsis thaliana*. Nucleic Acids Research 31: 5907–5916.
Heath TA, Hedtke SM, Hillis DM. 2008. Taxon sampling and the accuracy of phylogenetic analyses. Journal of Systematics and Evolution 46: 239–257.

Hedtke SM, Townsend TM, Hillis DM. 2006. Resolution of phylogenetic conflict in large data sets by increased taxon sampling. Systematic Biology 55: 522–529.

Heinrichs J, Hentschel J, Wilson R, Feldberg K, Schneider S. 2007. Evolution of leafy liverworts (Jungermanniidae, Marchantiophyta): Estimating divergence times from chloroplast DNA sequences using penalized likelihood with integrated fossil evidence. Taxon 56: 31–44.

Hibbett DS, Matheny PB. 2009. The relative ages of ectomycorrhizal mushrooms and their plant hosts estimated using Bayesian relaxed molecular clock analyses. BMC Biology 7: 13.

Hilu KW, Borsch T, Muller K, Soltis DE, Soltis PS, Savolainen V, Chase MW, Powell MP, Alice LA, Evans R, Sauquet H, Neinhuis C, Slotta TAB, Rohwer JG, Campbell CS, Chatrou LW. 2003. Angiosperm phylogeny based on *matK* sequence information. American Journal of Botany 90: 1758–1776.

Hoot SB, Magallon S, Crane PR. 1999. Phylogeny of basal eudicots based on three molecular data sets: atpB, rbcL, and 18S nuclear ribosomal DNA sequences. Annals of the Missouri Botanical Garden 86: 1–32.

Hu H-H. 1950. A polyphyletic system of classification of angiosperms. Science Record 3: 221–230.

Jansen RK, Cai Z, Raubeson LA, Daniell H, Depamphilis CW, Leebens-Mack J, Muller KF, Guisinger-Bellian M, Haberle RC, Hansen AK, Chumley TW, Lee SB, Peery R, McNeal JR, Kuehl JV, Boore JL. 2007. Analysis of 81 genes from 64 plastid genomes resolves relationships in angiosperms and identifies genome-scale evolutionary patterns. Proceedings of the National Academy of Sciences USA 104: 19369–19374.

Jian S, Soltis PS, Gitzendanner MA, Moore MJ, Li R, Hendry TA, Qiu Y-L, Dhingra A, Bell CD, Soltis DE. 2008. Resolving an ancient, rapid radiation in Saxifragales. Systematic Biology 57: 38–57.

Jobson RW, Qiu Y-L. 2008. Did RNA editing in plant organellar genomes originate under natural selection or through genetic drift? Biology Direct 3: 43.

Leebens-Mack J, Raubeson LA, Cui LY, Kuehl JV, Fourcade MH, Chumley TW, Boore JL, Jansen RK, dePamphilis CW. 2005. Identifying the basal angiosperm node in chloroplast genome phylogenies: Sampling one's way out of the Felsenstein zone. Molecular Biology and Evolution 22: 1948–1963.

Lemieux C, Otis C, Turmel M. 2000. Ancestral chloroplast genome in *Mesostigma viride* reveals an early branch of green plant evolution. Nature 403: 649–652.

Lemieux C, Otis C, Turmel M. 2007. A clade uniting the green algae *Mesostigma viride* and *Chlorokybus atmophyticus* represents the deepest branch of the Streptophyta in chloroplast genome-based phylogenies. BMC Biology 5: 2.

Les DH, Garvin DK, Wimpee CF. 1991. Molecular evolutionary history of ancient aquatic angiosperms. Proceedings of the National Academy of Sciences USA 88: 10119–10123.

Li L, Wang B, Liu Y, Qiu Y-L. 2009. The complete mitochondrial genome sequence of the hornwort *Megaceros aenigmaticus* shows a mixed mode of conservative yet dynamic evolution in early land plant mitochondrial genomes. Journal of Molecular Evolution 68: 665–678.

Loconte H, Stevenson DW. 1991. Cladistics of the Magnoliidae. Cladistics 7: 267–296.

Lohne C, Borsch T. 2005. Molecular evolution and phylogenetic utility of the *petD* group II intron: A case study in basal angiosperms. Molecular Biology and Evolution 22: 317–332.

Malecot V, Nickrent DL. 2008. Molecular phylogenetic relationships of Olacaceae and related Santalales. Systematic Botany 33: 97–106.

Malek O, Lattig K, Hiesel R, Brennicke A, Knoop V. 1996. RNA editing in bryophytes and a molecular phylogeny of land plants. EMBO Journal 15: 1403–1411.

Martin PG, Dowd JM. 1991. Studies of angiosperm phylogeny using protein sequences. Annals of the Missouri Botanical Garden 78: 296–337.

Martinez-Millan M. 2010. Fossil record and age of Asteridae. Botanical Review 76: 83–135.

Mathews S, Donoghue MJ. 1999. The root of angiosperm phylogeny inferred from duplicate phytochrome genes. Science 286: 947–950.

Miller MA, Holder MT, Vos R, Midford PE, Liebowitz T, Chan L, Hoover P, Warnow T. 2009. CIPRES [Cyberinfrasructure for Phylogenetic Research]: URL: http://www.phylo.org/sub_sections/portal. Archived by WebCite at http://www.webcitation.org/5imQlJeQa.

Moore MJ, Bell CD, Soltis PS, Soltis DE. 2007. Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. Proceedings of the National Academy of Sciences USA 104: 19363–19368.

Moore MJ, Soltis PS, Bell CD, Burleigh JG, Soltis DE. 2010. Phylogenetic analysis of 83 plastid genes further resolves the early diversification of eudicots. Proceedings of the National Academy of Sciences USA 107: 4623–4628.

Moreau CS, Bell CD, Vila R, Archibald SB, Pierce NE. 2006. Phylogeny of the ants: Diversification in the age of angiosperms. Science 312: 101–104.

Mower JP, Palmer JD. 2006. Patterns of partial RNA editing in mitochondrial genes of *Beta vulgaris*. Molecular Genetics and Genomics 276: 285–293.

Mower JP, Stefanovic S, Young GJ, Palmer JD. 2004. Gene transfer from parasitic to host plants. Nature 432: 165–166.

Nandi OI, Chase MW, Endress PK. 1998. A combined cladistic analysis of angiosperms using *rbc*L and non-molecular data. Annals of the Missouri Botanical Garden 85: 137–212.

Naylor GJP, Brown WM. 1997. Structural biology and phylogenetic estimation. Nature 388: 527–528.

Newton AE, Wikstrom N, Bell N, Forrest LL, Ignatov MS. 2007. Dating the diversification of the pleurocarpous mosses. Systematics Association Special Volume Series: Pleurocarpouse Mosses: Systematics and Evolution 71: 337–366.

Nickrent DL, Blarer A, Qiu YL, Soltis DE, Soltis PS, Zanis M. 2002. Molecular data place Hydnoraceae with Aristolochiaceae. American Journal of Botany 89: 1809–1817.

Nickrent DL, Der JP, Anderson FE. 2005. Discovery of the photosynthetic relatives of the "Maltese mushroom" *Cynomorium*. BMC Evolutionary Biology 5: 38.

Nishiyama T, Wolf PG, Kugita M, Sinclair RB, Sugita M, Sugiura C, Wakasugi T, Yamada K, Yoshinaga K, Yamaguchi K,

Ueda K, Hasebe M. 2004. Chloroplast phylogeny indicates that bryophytes are monophyletic. Molecular Biology and Evolution 21: 1813–1819.

Notsu Y, Masood S, Nishikawa T, Kubo N, Akiduki G, Nakazono M, Hirai A, Kadowaki K. 2002. The complete sequence of the rice (*Oryza sativa* L.) mitochondrial genome: Frequent DNA sequence acquisition and loss during the evolution of flowering plants. Molecular Genetics and Genomics 268: 434–445.

Olmstead RG, Bremer B, Scott KM, Palmer JD. 1993. A parsimony analysis of the Asteridae sensu lato based on *rbcL* sequences. Annals of the Missouri Botanical Garden 80: 700–722.

Olmstead RG, Kim K-J, Jansen RK, Wagstaff SJ. 2000. The phylogeny of the Asteridae sensu lato based on chloroplast *ndhF* gene sequences. Molecular Phylogenetics and Evolution 16: 96–112.

Olmstead RG, Michaels HJ, Scott KM, Palmer JD. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbcL*. Annals of the Missouri Botanical Garden 79: 249–265.

Palmer JD. 1992. Mitochondrial DNA in plant systematics: Applications and limitations. In: Soltis PS, Soltis DE, Doyle JJ eds. Molecular systematics of plants. New York: Chapman and Hall. 36–49.

Palmer JD, Herbon LA. 1988. Plant mitochondrial DNA evolves rapidly in structure, but slowly in sequence. Journal of Molecular Evolution 28: 87–97.

Palmer JD, Adams KL, Cho YR, Parkinson CL, Qiu YL, Song KM. 2000. Dynamic evolution of plant mitochondrial genomes: Mobile genes and introns and highly variable mutation rates. Proceedings of the National Academy of Sciences USA 97: 6960–6966.

Parkinson CL, Adams KL, Palmer JD. 1999. Multigene analyses identify the three earliest lineages of extant flowering plants. Current Biology 9: 1485–1488.

Parkinson CL, Mower JP, Qiu YL, Shirk AJ, Song KM, Young ND, dePamphilis CW, Palmer JD. 2005. Multiple major increases and decreases in mitochondrial substitution rates in the plant family Geraniaceae. BMC Evolutionary Biology 5: 73.

Petersen G, Seberg O, Davis JI, Stevenson DW. 2006. RNA editing and phylogenetic reconstruction in two monocot mitochondrial genes. Taxon 55: 871–886.

Prychid CJ, Rudall PJ, Gregory M. 2004. Systematics and biology of silica bodies in monocotyledons. Botanical Review 69: 377–440.

Qiu Y-L, Estabrook GF. 2008. Resolving phylogenetic relationships among key angiosperm lineages using a compatibility method on a molecular data set. Journal of Systematics and Evolution 46: 130–141.

Qiu Y-L, Lee J. 2000. Transition to a land flora: A molecular phylogenetic perspective. Journal of Phycology 36: 799–802.

Qiu Y-L, Palmer JD. 1999. Phylogeny of early land plants: insights from genes and genomes. Trends in Plant Science 4: 26–30.

Qiu Y-L, Palmer JD. 2004. Many independent origins of *trans* splicing of a plant mitochondrial group 2 intron. Journal of Molecular Evolution 59: 80–89.

Qiu Y-L, Chase MW, Les DH, Parks CR. 1993. Molecular phylogenetics of the Magnoliidae – cladistic analyses of nucleotide-sequences of the plastid gene rbcL. Annals of the Missouri Botanical Garden 80: 587–606.

Qiu Y-L, Cho YR, Cox JC, Palmer JD. 1998. The gain of three mitochondrial introns identifies liverworts as the earliest land plants. Nature 394: 671–674.

Qiu Y-L, Dombrovska O, Lee J, Li LB, Whitlock BA, Bernasconi-Quadroni F, Rest JS, Davis CC, Borsch T, Hilu KW, Renner SS, Soltis DE, Soltis PS, Zanis MJ, Cannone JJ, Gutell RR, Powell M, Savolainen V, Chatrou LW, Chase MW. 2005. Phylogenetic analyses of basal angiosperms based on nine plastid, mitochondrial, and nuclear genes. International Journal of Plant Sciences 166: 815–842.

Qiu Y-L, Lee J, Bernasconi-Quadroni F, Soltis DE, Soltis PS, Zanis M, Zimmer EA, Chen Z, Savolainen V, Chase MW. 2000. Phylogeny of basal angiosperms: Analyses of five genes from three genomes. International Journal of Plant Sciences 161: S3–S27.

Qiu Y-L, Lee J, Whitlock BA, Bernasconi-Quadroni F, Dombrovska O. 2001. Was the ANITA rooting of the angiosperm phylogeny affected by long-branch attraction? Molecular Biology and Evolution 18: 1745–1753.

Qiu Y-L, Lee JH, Bernasconi-Quadroni F, Soltis DE, Soltis PS, Zanis M, Zimmer EA, Chen ZD, Savolainen V, Chase MW. 1999. The earliest angiosperms: evidence from mitochondrial, plastid and nuclear genomes. Nature 402: 404–407.

Qiu Y-L, Li LB, Hendry TA, Li R, Taylor DW, Issa MJ, Ronen AJ, Vekaria ML, White AM. 2006a. Reconstructing the basal angiosperm phylogeny: evaluating information content of the mitochondrial genes. Taxon 55: 837–856.

Qiu Y-L, Li LB, Wang B, Chen ZD, Dombrovska O, Lee J, Kent L, Li RQ, Jobson RW, Hendry TA, Taylor DW, Testa CM, Ambros M. 2007. A nonflowering land plant phylogeny inferred from nucleotide sequences of seven chloroplast, mitochondrial, and nuclear genes. International Journal of Plant Sciences 168: 691–708.

Qiu Y-L, Li LB, Wang B, Chen ZD, Knoop V, Groth-Malonek M, Dombrovska O, Lee J, Kent L, Rest J, Estabrook GF, Hendry TA, Taylor DW, Testa CM, Ambros M, Crandall-Stotler B, Duff RJ, Stech M, Frey W, Quandt D, Davis CC. 2006b. The deepest divergences in land plants inferred from phylogenomic evidence. Proceedings of the National Academy of Sciences USA 103: 15511–15516.

Ran J-H, Gao H, Wang X-Q. 2009. Fast evolution of the retroprocessed mitochondrial *rps3* gene in Conifer II and further evidence for the phylogeny of gymnosperms. Molecular Phylogenetics and Evolution 54: 136–149.

Rudall PJ, Bateman RM. 2004. Evolution of zygomorphy in monocot flowers: iterative patterns and developmental constraints. New Phytologist 162: 25–44.

Saarela JM, Rai HS, Doyle JA, Endress PK, Mathews S, Marchant AD, Briggs BG, Graham SW. 2007. Hydatellaceae identified as a new branch near the base of the angiosperm phylogenetic tree. Nature 446: 312–315.

Sanderson MJ. 1995. Objections to bootstrapping phylogenies: A critique. Systematic Biology 44: 299–320.

Sanderson MJ. 2003. r8s: Inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. Bioinformatics 19: 301–302.

Savolainen V, Chase MW, Hoot SB, Morton CM, Soltis DE, Bayer C, Fay MF, De Bruijn AY, Sullivan S, Qiu YL. 2000a. Phylogenetics of flowering plants based on combined analysis of plastid *atpB* and *rbcL* gene sequences. Systematic Biology 49: 306–362.

Savolainen V, Fay MF, Albach DC, Backlund A, Van Der Bank M, Cameron KM, Johnson SA, Lledo MD, Pintaud J-C, Powell M, Sheahan MC, Soltis DE, Soltis PS, Weston P, Whitten WM, Wurdack KJ, Chase MW. 2000b. Phylogeny of the eudicots: A nearly complete familiial analysis based on *rbcL* gene sequences. Kew Bulletin 55: 257–309.

Schneider H, Schuettpelz E, Pryer KM, Cranfill R, Magallon S, Lupia R. 2004. Ferns diversified in the shadow of angiosperms. Nature 428: 553–557.

Schonenberger J, Anderberg AA, Sytsma KJ. 2005. Molecular phylogenetics and patterns of floral evolution in the Ericales. International Journal of Plant Sciences 166: 265–288.

Soltis DE, Albert VA, Savolainen V, Hilu K, Qiu Y-L, Chase MW, Farris JS, Palmer JD, Soltis JD. 2004. Angiosperm relationships, genome-scale data, and "ending incongruence": A cautionary tale in phylogenetics. Trends in Plant Science 9: 477–483.

Soltis DE, Senters A, Zanis MJ, Kim S, Thompson JD, Soltis PS, Ronse De Craene LP, Endress PK, Farris JS. 2003. Gunnerales are sister to other core eudicots: Implications for the evolution of pentamery. American Journal of Botany 90: 461–470.

Soltis DE, Soltis PS, Chase MW, Mort ME, Albach DC, Zanis M, Savolainen V, Hahn WH, Hoot SB, Fay MF, Axtell M, Swensen SM, Prince LM, Kress WJ, Nixon KC, Farris JS. 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. Botanical Journal of the Linnean Society 133: 381–461.

Soltis DE, Soltis PS, Morgan DR, Swensen SM, Mullin BC, Dowd JM, Martin PG. 1995. Chloroplast gene sequence data suggest a single origin of predisposition for symbiotic nitrogen fixation in angiosperms. Proceedings of the National Academy of Sciences USA 92: 2647–2651.

Soltis DE, Soltis PS, Nickrent DL, Johnson LA, Hahn WJ, Hoot SB, Sweere JA, Kuzoff RK, Kron KA, Chase MW, Swensen SM, Zimmer EA, Chaw SM, Gillespie LJ, Kress WJ, Sytsma KJ. 1997. Angiosperm phylogeny inferred from 18S ribosomal DNA sequences. Annals of the Missouri Botanical Garden 84: 1–49.

Soltis PS, Soltis DE, Chase MW. 1999. Angiosperm phylogeny inferred from multiple genes as a tool for comparative biology. Nature 402: 402–404.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web-servers. Systematic Biology 75: 758–771.

Stefanovic S, Rice DW, Palmer JD. 2004. Long branch attraction, taxon sampling, and the earliest angiosperms: *Amborella* or monocots? BMC Evolutionary Biology 4: 35.

Swofford DL. 2003. PAUP* 4.0b10: Phylogenetic Analysis Using Parsimony. Sunderland: Sinauer.

Takhtajan A. 1969. Flowering plants: Origin and dispersal. Edinburgh: Oliver and Boyd.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 25: 4876–4882.

Upchurch GR. 1984. Cuticle evolution in Early Cretaceous angiosperms from the Potomac Group of Virginia and Maryland. Annals of the Missouri Botanical Garden 71: 522–550.

Vangerow S, Teerkorn T, Knoop V. 1999. Phylogenetic information in the mitochondrial nad5 gene of pteridophytes: RNA editing and intron sequences. Plant Biology 1: 235–243.

Walker JW, Doyle JA. 1975. The bases of angiosperm phylogeny: palynology. Annals of the Missouri Botanical Garden 62: 664–723.

Wang H, Moore MJ, Soltis PS, Bell CD, Brockington SF, Alexandre R, Davis CC, Lavis M, Manchester SR, Soltis DE. 2009a. Rosid radiation and the rapid rise of angiosperm-dominated forests. Proceedings of the National Academy of Sciences USA 106: 3853–3858.

Wang W, Lu A-M, Ren Y, Endress ME, Chen Z-D. 2009b. Phylogeny and classification of Ranunculales: Evidence from four molecular loci and morphological data. Perspectives in Plant Ecology, Evolution and Systematics 11: 81–110.

Wikstrom N, Pryer KM. 2005. Incongruence between primary sequence data and the distribution of a mitochondrial atp1 group II intron among ferns and horsetails. Molecular Phylogenetics and Evolution 36: 484–493.

Wodehouse RP. 1935. Pollen grains: Their structure, identification and significance in science and medicine. New York: McGraw-Hill Book Co.

Wodehouse RP. 1936. Evolution of pollen grains. Botanical Review 2: 67–84.

Wolfe JA, Doyle JA, Page VM. 1975. The bases of angiosperm phylogeny: Paleobotany. Annals of the Missouri Botanical Garden 62: 801–824.

Wolfe KH, Li W-H, Sharp PM. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. Proceedings of the National Academy of Sciences USA 64: 9054–9058.

Won H, Renner SS. 2003. Horizontal gene transfer from flowering plants to *Gnetum*. Proceedings of the National Academy of Sciences USA 100: 10824–10829.

Wurdack KJ, Davis CC. 2009. Malpighiales phylogenetics: Gaining ground on one of the most recalcitrant clade in the angiosperm tree of life. American Journal of Botany 96: 1551–1570.

Zanis MJ, Soltis DE, Soltis PS, Mathews S, Donoghue MJ. 2002. The root of the angiosperms revisited. Proceedings of the National Academy of Sciences USA 99: 6848–6853.

Zanis MJ, Soltis PS, Qiu YL, Zimmer E, Soltis DE. 2003. Phylogenetic analyses and perianth evolution in basal angiosperms. Annals of the Missouri Botanical Garden 90: 129–150.

Zhang ZH, Li CQ, Li JH. 2009. Phylogenetic placement of *Cynomorium* in Rosales inferred from sequences of the inverted repeat region of the chloroplast genome. Journal of Systematics and Evolution 47: 297–304.

Zhu X-Y, Chase MW, Qiu Y-L, Kong H-Z, Dilcher DL, Li J-H, Chen ZD. 2007. Mitochondrial *matR* sequences help to resolve deep phylogenetic relationships in rosids. BMC Evolutionary Biology 7: 217.

Supplementary Material

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Consensus angiosperm phylogeny used as a constraint tree to estimate evolutionary rates and homoplasy levels in mitochondrial *atp1, matR, nad5,* and *rps3,* chloroplast *atpB, matK,* and *rbcL,* and nuclear 18S rDNA.

Fig. S2.1. Maximum likelihood tree (cladogram) of 380 seed plants inferred from nucleotide sequences of mitochondrial genes *atp1, matR, nad5,* and *rps3*. The difference between this tree and the 356-species tree is that the taxa with missing data for two or three of the four genes or highly divergent sequences were included here. Bootstrap values are shown above the branches in most cases, and around the nodes in some cases.

Fig. S2.2. Maximum likelihood tree (phylogram) of 380 seed plants inferred from nucleotide sequences of mitochondrial genes *atp1, matR, nad5,* and *rps3*. The difference between this tree and the 356-species tree is that the taxa with missing data for two or three of the four genes or highly divergent sequences were included here. This tree shows divergence levels of all lineages, and a scale bar is shown at the bottom of Fig. S2.2–1.

Fig. S3. A maximum likelihood tree (cladogram) of 356 seed plants inferred from nucleotide sequences of mitochondrial genes *atp1, matR, nad5,* and *rps3*, with all known RNA editing site removed. Bootstrap values are shown above the branches in most cases, and around the nodes in some cases.

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