

## Phylogeny and classification of Ranunculales: Evidence from four molecular loci and morphological data

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Received 30 June 2008; received in revised form 21 November 2008; accepted 5 January 2009

### Abstract

Previous phylogenetic analyses of Ranunculales, which have mostly been focused on an individual family and were based on molecular data alone, have recovered three main clades within the order. However, support for relationships among these three clades was weak. Earlier hypotheses were often hampered by limited taxon sampling; to date less than one-tenth of the genera in the order have been sampled. In this study, we used a greatly enlarged taxon sampling (105 species, representing 99 genera of all seven families in the order). Our study is, furthermore, the first to employ morphology (65 characters) in combination with sequence data from four genomic regions, including plastid *rbcL*, *matK* and *trnL-F*, and nuclear ribosomal 26S rDNA to reconstruct phylogenetic relationships within Ranunculales. Maximum parsimony and Bayesian inference were performed on the individual and combined data sets. Our analyses concur with those of previous studies, but in most cases provide stronger support and better resolution for relationships among the three main clades retrieved. The first, comprised solely of the monogeneric family Eupteleaceae, is the earliest-diverging lineage. The second clade is composed exclusively of taxa of Papaveraceae, which is sister to the third clade, the core Ranunculales, comprising the other five families of the order. Circaeasteraceae and Lardizabalaceae form a strongly supported clade. *Pteridophyllum* is supported as sister to *Hypecoum*, contradicting the viewpoint that the former is the earliest-diverging genus in Papaveraceae. *Glaucidium* is basalmost in Ranunculaceae. Within this phylogenetic framework, the evolution of selected characters is inferred and diagnostic morphological characters at different taxonomic levels are identified and discussed. Based on both morphological and molecular evidence, a classification outline for Ranunculales is presented, including the proposal of two new subfamilies, Menispermoideae and Tinosporoideae in Menispermaceae and a new tribe, Callianthemeae, for the genus *Callianthemum* (Ranunculaceae).

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**Keywords:** Bayesian inference; Combined molecular and morphological data; Diagnostic characters; Parsimony analysis; Phylogenetic relationships of Ranunculales

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## Introduction

The order Ranunculales is recognized in many classification systems (e.g., Hutchinson, 1973; Dahlgren, 1983; Cronquist, 1988; Kubitzki et al., 1993; Takhtajan, 1997; APG, 1998; Wu et al., 2002; APG II, 2003; Thorne, 2007); however, its circumscription has long been controversial. Dahlgren's (1983) Ranunculales included Ranunculaceae (excluding *Glaucidium*), Lardizabalaceae, Menispermaceae, Berberidaceae, Circaeasteraceae, and several segregate families, such as Kingdoniaceae, Sargentodoxaceae, Fumariaceae, and Glaucidiaceae. In addition to the nine families above, Cronquist (1988) added Sabiaceae and Coriariaceae, and Kubitzki et al. (1993) and Thorne (2007) merged Dahlgren's (1983) Papaverales into the order. In contrast, Takhtajan's (1997) and Wu et al.'s (2002) Ranunculales contained only the family Ranunculaceae (excluding *Hydrastis* and *Glaucidium*).

Molecular phylogenetics has contributed greatly to the delimitation of the Ranunculales over the past decade. Based on chloroplast *rbcL* sequences, Chase et al. (1993) first recovered a clade (labeled "Ranunculids"), which contained Berberidaceae, Eupteleaceae, Lardizabalaceae, Menispermaceae, Papaveraceae *sensu lato* (including Fumariaceae), and Ranunculaceae. The inclusion of *Euptelea* in the ranunculids was surprising, as it has traditionally been placed among the "lower" hamamelids. However, the same "Ranunculids" clade was recovered by 18S rDNA sequence analysis (Soltis et al., 1997), and this in turn was congruent with results from a study using three gene data by Hoot and Crane (1995), which also added Circaeasteraceae (including *Kingdonia*) to the clade. Subsequent analyses have supported seven families in Ranunculales: Berberidaceae (including Nandinaceae), Circaeasteraceae (including Kingdoniaceae), Eupteleaceae, Lardizabalaceae (including Sargentodoxaceae), Menispermaceae, Papaveraceae (including Fumariaceae and Pteridophyllaceae), and Ranunculaceae (including Glaucidiaceae and Hydrastidaceae) (APG, 1998; Hoot et al., 1999; Savolainen et al., 2000a; Soltis et al., 2000, 2003; APG II, 2003; Kim et al., 2004a).

The Ranunculales *sensu* APG II (2003) is a strongly supported monophyletic group within basal eudicots, which are sister to all other eudicots (Chase et al., 1993; Savolainen et al., 2000b; Soltis et al., 1997, 2000; Hilu et al., 2003). The consensus is that the order comprises three main constituent clades: the two earlier-diverging ranunculids Eupteleaceae and Papaveraceae on the one hand, and the well-supported clade of core Ranunculales, consisting of the other five families, which is more derived, on the other (see Kim et al., 2004a). The relationship among these three clades, however, remains uncertain, because previous analyses, employing different taxon and character sampling schemes and

phylogenetic methods, have produced conflicting topologies that are in part only weakly supported (e.g., Hoot and Crane, 1995; Hoot et al., 1999; Qiu et al., 2005, 2006; Savolainen et al., 2000a, b; Soltis et al., 2000, 2003; Nickrent et al., 2002; Hilu et al., 2003; Zanis et al., 2003; Kim et al., 2004a; Worberg et al., 2007). Furthermore, the weakly supported sister relationship identified between Circaeasteraceae and Lardizabalaceae (Hoot and Crane, 1995; Hoot et al., 1999; Savolainen et al., 2000a; Soltis et al., 2003; Kim et al., 2004a) needs reevaluation.

Several monotypic genera within the order, such as *Glaucidium*, *Hydrastis*, *Kingdonia*, *Nandina*, *Pteridophyllum*, and *Sargentodoxa*, differ in their position and taxonomic rank among the different classification systems. For example, *Glaucidium* has been placed in Ranunculaceae (e.g., Hutchinson, 1973; Dahlgren, 1980; Cronquist, 1988), in Hydrastidaceae (Tobe, 2002), or given familial rank and positioned close to Paeoniaceae or Paeoniales (e.g., Takhtajan, 1997; Wu et al., 2002; Thorne, 2007). The taxonomic contention involving these genera is the basis of the dispute over the delimitation of the order Ranunculales as well as that of some of its constituent families. Recent broad-based phylogenetic analyses in angiosperms or eudicots have placed each of these monotypic genera into a family; however, their positions have not been sufficiently clarified due to the relatively limited taxon sampling in each family. Analyses focusing on selected families in the Ranunculales, such as Berberidaceae (Kim and Jansen, 1996, 1998; Kim et al., 2004b; Wang et al., 2007a), Lardizabalaceae (Hoot et al., 1995; Wang et al., 2002), Papaveraceae (Hoot et al., 1997), and Ranunculaceae (Johansson and Jansen, 1993; Hoot, 1995; Johansson, 1995; Kosuge et al., 1995; Ro et al., 1997), resolved the positions of some of these monotypic genera, however, in most cases, did not follow through with the taxonomic consequences.

There exists a striking disproportion in species number among the families in the order. At one end of the spectrum are Circaeasteraceae and Eupteleaceae, each with only two species; Lardizabalaceae contains 50, Menispermaceae 450, Berberidaceae ca. 650 and Papaveraceae 750 species. At the other end of the spectrum are Ranunculaceae, with some 2000 species. Of the ca. 200 genera in Ranunculales, less than 20 genera have been sampled in previous studies (e.g., Chase et al., 1993; Hoot and Crane, 1995; Soltis et al., 1997, 2000, 2003; Hoot et al., 1999; Savolainen et al., 2000a, b; Hilu et al., 2003; Kim et al., 2004a; Worberg et al., 2007). Thus to date the patterns of morphological character evolution in Ranunculales have not been sufficiently well elucidated. In particular, the morphological synapomorphies that define Ranunculales are still unclear.

The objectives of this study are (1) to investigate the phylogenetic relationships at generic level within

Ranunculales using four molecular loci from the chloroplast and nuclear genomes and morphological data with a more extensive sampling than in any previous study, (2) to examine the diagnostic characters at different taxonomic levels, and (3) to update the classification system for Ranunculales so that it best reflects the evolutionary history of the group.

## Materials and methods

### Taxon sampling

A total of 105 taxa of Ranunculales were sampled in the present study, including 18 species and all 16 genera of Berberidaceae, one species each of the two monotypic genera of Circaeasteraceae, both species of the monogeneric Eupteleaceae, one species each of the 10 genera of Lardizabalaceae, 46 species from 43 of the 60 genera of Ranunculaceae, one species each from 17 of the 71 currently recognized genera of Menispermaceae (Kessler, 1993), including representatives of all major lineages based on the study that sampled most of the genera of this family (Ortiz et al., 2007), and one species each from 10 of the 41 genera of Papaveraceae, spanning all major lineages according to the analysis of Hoot et al. (1997). Our outgroups included nine species of 8 genera representing all lineages of other basal eudicots, one species each of 8 genera of core eudicots and 15 species of 10 genera representing all major lineages of basal angiosperms, following the results of Savolainen et al. (2000a, b), Soltis et al. (2000, 2003), Hilu et al. (2003), and Qiu et al. (2005, 2006). *Acorus* was used to represent monocots because it has been shown to be the earliest-diverging branch in monocots (see Chase, 2004; Li and Zhou, 2007). Voucher information and GenBank accession numbers are listed in Table 1.

### Morphological data

Sixty-five morphological characters were mainly obtained from several published morphological matrices (e.g., Loconte and Estes, 1989; Kadereit et al., 1994; Loconte et al., 1995; Nandi et al., 1998; Doyle and Endress, 2000; Wang and Li, 2002) or from the literature. Morphological characters and their states as well as the pertinent literature sources are given in Appendix A.

We analyzed the morphological data using the genus as a unit. In cases where different species of a genus have different characters states, the character was coded as polymorphic. Our morphological matrix contained 129 terminals (genera), 99 of them from Ranunculales. In cases in which a character is poorly known or for which information is currently unavailable, these were scored

as missing or inapplicable. The complete character matrix is provided in Appendix B.

### Molecular data

Forty-two new sequences of *rbcL*, 49 of *matK*, 42 of *trnL-F*, and 48 of 26S rDNA were generated in this study, including sequences for 3 genera and 6 species reported here for the first time, and have been deposited at GenBank. Most of the sequences used in this study were generated in our previous studies (Wang et al., 2007a, b, 2005; Wang and Chen, 2007); others were taken from GenBank. Taxa analyzed, voucher information and GenBank accession numbers are given in Table 1. We were unable to obtain material of a few taxa and for these sequences are missing as follows: *Achlys triphylla* DC. (lacking *trnL-F* and 26S), *Bongardia chrysogonum* Spach. (lacking *trnL-F*), *Helleborus foetidus* L. (lacking *rbcL*), *Hypecoum imberbe* Sm. (lacking *matK* and *trnL-F*), *Megaleranthis saniculifolia* Ohwi (lacking *rbcL* and *trnL-F*), *Ranzania japonica* Ito (lacking *trnL-F* and 26S), and *Sanguinaria canadensis* L. (lacking *trnL-F*). In addition, we encountered difficulties in obtaining some 26S rDNA sequences, and thus were unable to obtain sequences of the following taxa: *Albertisia laurifolia* Yamamoto, *Anamirta cocculus* (L.) Wight & Arn., *Archakebia apetala* (Xia et al.) Wu, Chen & Qin, *Boquila trifoliata* (DC.) Decaisne, *Hypserpa nitida* Miers, *Leptopyrum fumarioides* (L.) Reichb., *Macleaya microcarpa* (Maxim.) Fedde, *Naravelia zeylanica* (L.) DC., *Paropyrum anemonoides* (Kar. & Kir.) Ulbr., *Parvatia brunoniana* spp. *elliptica* Griffith, *Pycnarrhena lucida* (Teijsm. & Binn.) Miq. and *Stauntonia hexaphylla* (Thunb.) Decne. Consequently, the combined molecular data matrix (*rbcL*, *matK*, *trnL-F*, and 26S), including outgroups, comprised 137 exemplars (Table 1); lacking sequences were scored as missing.

Total genomic DNA was extracted from fresh silica gel-dried leaves or seeds or from herbarium specimens using the modified CTAB procedure of Doyle and Doyle (1987). The selected DNA regions were amplified with standard polymerase chain reaction (PCR). All regions were amplified following the PCR protocols used in Li et al. (2004) and Wang et al. (2005). The *rbcL* gene was amplified and sequenced using the 1F and 1494R primers as well as the 991R internal primer (Chen et al., 1998). The *matK* gene was amplified and sequenced using the *matK*-AF (Li et al., 2004) or *matK*-AF2 and *matK*-8R2 primers and the internal primers *matK*-mF2 and *matK*-mR2 (Wang et al., 2007a). The *trnL-F* region was sequenced using the *trnF* and *trnR* primers (Taberlet et al., 1991). The 26S rDNA was amplified and sequenced using F1 and R4 primers and R5 internal primer (Wang et al., 2005). The PCR

**Table 1.** GenBank accession numbers and vouchers/references for the sequences used in this study.

Taxon	GenBank accession numbers				Vouchers/References
	<i>rbcL</i>	<i>matK</i>	<i>trnL-F</i>	26S	
Ranunculaceae					
<i>Aconitum racemulosum</i> Franch.	*AY954488	FJ626484	FJ626533	*AY954473	Chongqing: Wang Wei 081 (PE)
<i>Actaea asiatica</i> Hara	FJ626575	FJ626485		FJ626436	Chongqing: Chen et al. 960060 (PE)
			<sup>1</sup> AJ222985		<sup>1</sup> Compton et al. (1998)
<i>Adonis amurensis</i> Regel & Radde	*AY954487	FJ626486	FJ626534	*AY954472	Jilin: Chen Z-D 003 (PE)
<i>Anemoclema glaucifolium</i> (Franch.) W. T. Wang	FJ626576	FJ626487	FJ626535	FJ626437	Yunnan: Wang Wei YN070 (PE)
<i>Anemone hupehensis</i> Lem.	FJ626577	FJ626488	FJ626536	FJ626438	Guizhou: Wang Wei 002 (PE)
<i>Anemonopsis macrophylla</i> Sieb. & Zucc.	FJ626578	FJ626489			provided by Hoot: Reznicek 9977 (MICH)
			<sup>1</sup> AJ222984	<sup>2</sup> AF131289	<sup>1</sup> Compton et al. (1998), <sup>2</sup> Ro et al. (2003)
<i>Aquilegia ecalcarata</i> Maxim.	*AY954495	*EF437127	*EF437096	*AY954481	Chongqing: Wang Wei 117 (PE)
<i>Aquilegia oxysepala</i> Trautv. & Kir.	*EF437140	*EF437128	*EF437097	FJ626439	Jilin: Chen Z-D 001 (PE)
<i>Asteropyrum cavaleriei</i> (Lévl. et Vant.) Drumm. & Hutch.	<sup>1</sup> AF079453				<sup>1</sup> Wang and Chen, unpubl.
<i>Batrachium bungei</i> (Steud.) L. Liou.	FJ626579	FJ626490	FJ626537	*AY954466	Chongqing: Wang Wei 110 (PE)
<i>Beesia calthifolia</i> (Maxim.) Ulbr.	<sup>1</sup> AF079452	FJ626491	FJ626538	FJ626440	Yunnan: Wang Wei YN092 (PE)
			<sup>2</sup> AJ496612		<sup>1</sup> Wang and Chen, unpubl.; <sup>2</sup> Compton and Culham (2002)
		FJ626492		*AY954468	Hubei: Chen 200010661 (PE)
<i>Callianthemum taipaicum</i> W. T. Wang	FJ626580	FJ626493	FJ626539	FJ626441	Shanxi: Wang Wei SX004 (PE)
					<sup>1</sup> Albert et al. (1992), <sup>2</sup> Adachi et al., unpubl.;
<i>Caltha palustris</i> L.	<sup>1</sup> L02431	<sup>2</sup> AB069845	<sup>3</sup> AJ496610	<sup>4</sup> U52632	<sup>3</sup> Compton and Culham (2002), <sup>4</sup> Ro et al. (1997)
<i>Caltha palustris</i> var. <i>membranacea</i> Turcz.	FJ626581	FJ626494	FJ626540	FJ626442	Jilin: Chen 2072 (PE)
<i>Cimicifuga simplex</i> Wormsk.	*AY954483			*AY954469	Jilin: Chen 2079 (PE)
		<sup>1</sup> AB044754	<sup>2</sup> AJ223008		Li et al., unpubl.; <sup>2</sup> Compton et al. (1998)
<i>Clematis ganpiniana</i> (Lévl. & Vant.) Tamura	*AY954491	FJ626495	FJ626541	*AY954476	Chongqing: Wang Wei 119 (PE)
<i>Consolida ajacis</i> (L.) Schur	FJ626582	FJ626496		FJ626443	Xi'an (cult.): Wang Wei Seed2 (PE)
			<sup>1</sup> AY150259		<sup>1</sup> Luo et al., unpubl.
<i>Coptis chinensis</i> Franch.	*AY954497	*DQ478614	*EF437110	*AY954482	Sichuan: Chen et al. 960105 (PE)
<i>Delphinium bonvalotii</i> Franch.	FJ626583	FJ626497	FJ626542	FJ626444	Guizhou: Wang Wei 030 (PE)
<i>Dichocarpum sutchuenense</i> W. T. Wang & Hsiao	*AY954493	*EF437130	*EF437099	*AY954479	Guizhou: Wang Wei 069 (PE)
<i>Enemion raddeanum</i> Regel	*AY954494	*EF437131	*EF437100	*AY954478	Jilin: Chen Z-D 2090 (PE)
<i>Eranthis stellata</i> Maxim.	*AY954484	FJ626498	FJ626543	*AY954467	Jilin: Chen Z-D 001 (PE)
<i>Glaucidium palmatum</i> Sieb. & Zucc.	<sup>1</sup> AF093723	<sup>2</sup> AB069850		<sup>3</sup> AF389267	<sup>1</sup> Hoot et al. (1999), <sup>2</sup> Adachi et al., unpubl.;
					<sup>3</sup> Kim et al. (2004a)
			*EF437113		Japan: Zhou J 001 (PE)
<i>Halerpestes cymbalaria</i> (Pursh) Green	*AY954490	FJ626499	FJ626544	*AY954475	Guizhou: Wang Wei 003 (PE)



<i>Helleborus foetidus</i> L.		<sup>1</sup> AJ414322	<sup>1</sup> AJ413283	<sup>2</sup> U52634	<sup>1</sup> Sun et al. (2001), <sup>2</sup> Ro et al. (1997)
<i>Helleborus thibetanus</i> Franch.	*AY954485	FJ626500		*AY954470	Shanxi: Wang Wei SX 032 (PE)
<i>Hepatica henryi</i> (Oliv.) Steward	FJ626584	FJ626501	<sup>1</sup> AJ413296	FJ626445	<sup>1</sup> Sun et al. (2001)
<i>Hydrastis canadensis</i> L.	<sup>1</sup> AF093725	<sup>2</sup> AB069849		<sup>3</sup> AF389268	Hubei: Chen 200010660 (PE)
<i>Isopyrum manshuricum</i> Kom.	*EF437143	*EF437133	*EF437112		<sup>1</sup> Hoot et al. (1999), <sup>2</sup> Adachi et al., unpubl.;
<i>Leptopyrum fumarioides</i> (L.) Reichb.	*EF437145	*EF437135	*EF437102	FJ626446	<sup>3</sup> Kim et al. (2004a)
<i>Megaleranthis saniculifolia</i> Ohwi		<sup>1</sup> AY515243	*EF437104		USA: Chen Z-D 2002016 (PE)
<i>Myosurus minimus</i> L.	<sup>1</sup> DQ099441		<sup>2</sup> AJ413305	<sup>2</sup> AF131285	Liaoning: Wang Wei LN004 (PE)
<i>Naravelia zeylanica</i> (L.) DC.	FJ626585	FJ626502		FJ626447	Xinjiang: Man Y-G T101 (PE)
<i>Nigella damascena</i> L.	FJ626586	FJ626503	FJ626546	FJ626448	<sup>1</sup> Lee and Heo, unpubl.; <sup>2</sup> Ro et al. (2003)
<i>Oxygraphis glacialis</i> (Fisch.) Bunge.	FJ626587	FJ626505	<sup>1</sup> AY150260	FJ626449	<sup>1</sup> Anderson et al. (2005), <sup>2</sup> Sun et al. (2001)
<i>Paraquilegia microphylla</i> (Royle) Drumm. & Hutch.	*EF437146	*EF437136	FJ626547	FJ626450	provided by Hoot: Hoot 98-2 (UWM)
<i>Paropyrum anemonoides</i> (Kar. & Kir.) Ulbr.	*EF437142	*EF437132	*EF437101		Yunnan: Wang Wei YN126 (PE)
<i>Pulsatilla cernua</i> (Thunb.) Bercht. & Opiz.	*AY954492		FJ626548	*AY954477	Yunnan: Wang Wei Seed1 (PE)
<i>Ranunculus cantoniensis</i> DC.	*AY954489	<sup>1</sup> AB110531			<sup>1</sup> Luo et al., unpubl.
<i>Semiaquilegia adoxoides</i> (DC.) Makino	*EF437147	FJ626506	FJ626549	*AY954474	Shanxi: Wang Wei SX006 (PE)
<i>Souliea vaginata</i> (Maxim.) Franch.	FJ626588	*EF437137	*EF437106	FJ626451	Chongqing: Li C-Y 001 (PE)
<i>Thalictrum javanicum</i> Bl.	*AY954496	*DQ478615	<sup>1</sup> AJ222983	FJ626452	Xinjiang: Wundish U. 177 (PE)
<i>Trautvetteria carolinensis</i> (Walt.) Vail.	FJ626589	FJ626508	*EF437107	*AY954480	Jilin: Chen 2048 (PE)
<i>Trollius laxus</i> Salisb.	*AY954486	FJ626509	FJ626550	<sup>1</sup> U52630	Miikeda et al. (2006)
<i>Urophyssa henryi</i> (Oliv.) Ulbr.	*EF437149	*EF437139	<sup>1</sup> AH012578	*AY954471	Guizhou: Wang Wei 031 (PE)
<i>Xanthorhiza simplicissima</i> Marshall	<sup>1</sup> L12669	<sup>2</sup> AB069848	*EF437109	FJ626453	Hunan: Shao Q 001 (PE)
			*EF437111	<sup>3</sup> AF389270	Shanxi: Wang Wei SX015 (PE)
					<sup>1</sup> Compton et al. (1998)
					Guizhou: Wang Wei 067 (PE)
					provided by Hoot: Hoot 92-18 (UWM)
					<sup>1</sup> Ro et al. (1997)
					USA: CL 108 (PE)
					<sup>1</sup> Despres et al. (2003)
					Guizhou: Wang Wei 096 (PE)
					<sup>1</sup> Qiu et al. (1993), <sup>2</sup> Adachi et al., unpubl.; <sup>3</sup> Kim et al. (2004a)
					Provided by Qiu: Qiu Y-L 91030 (UCN)
Berberidaceae					
<i>Achlys triphylla</i> DC.	<sup>1</sup> L75868	<sup>2</sup> AB069825			<sup>1</sup> Kim and Jansen (1996), <sup>2</sup> Adachi et al., unpubl.
<i>Berberis thunbergii</i> DC.	<sup>1</sup> AF139878	<sup>2</sup> AB069827			<sup>1</sup> Feng et al., unpubl.; <sup>2</sup> Adachi et al., unpubl.
<i>Bongardia chrysogonum</i> Spach.	<sup>1</sup> L75870	<sup>2</sup> AB069840	FJ626551	FJ626454	Beijing (cult.): Hong Y-P H464 (PE)
				<sup>3</sup> X83830	<sup>1</sup> Kim and Jansen (1996), <sup>2</sup> Adachi et al., unpubl.; <sup>3</sup> Oxelman and Lidén (1995)

Table 1. (continued)

Taxon	GenBank accession numbers				Vouchers/References
	<i>rbcL</i>	<i>matK</i>	<i>trnL-F</i>	26S	
<i>Caulophyllum robustum</i> Maxim.	<sup>1</sup> AF190441	<sup>2</sup> AB069832	<sup>3</sup> AF325911		<sup>1</sup> Xiang et al. (2000), <sup>2</sup> Adachi et al., unpubl.; <sup>3</sup> Liu et al. (2002)
<i>Caulophyllum thalictroides</i> (L.) Michx.	<sup>1</sup> AF190442	<sup>2</sup> AB069831		FJ626455	Chongqing: Chen et al. 960601 (PE)
<i>Diphylleia cymosa</i> Michx.	<sup>1</sup> L75866		FJ626552	<sup>3</sup> AF389240	<sup>1</sup> Xiang et al. (2000), <sup>2</sup> Adachi et al., unpubl.; <sup>3</sup> Kim et al. (2004a)
<i>Diphylleia sinensis</i> H. L. Li	*DQ478618	*DQ478620	FJ626553	FJ626456	provided by Qiu: Qiu Y-L 05023 (PE)
<i>Dysosma versipellis</i> (Hance) M. Cheng ex Ying	*EF173669	*DQ478618	FJ626554	FJ626457	<sup>1</sup> Kim and Jansen (1996)
<i>Epimedium koreanum</i> Nakai	<sup>1</sup> L75869	<sup>2</sup> AB069837		FJ626458	USA: Chen Z-D 2002019 (PE)
<i>Gymnospermium microrrhynchum</i> Takht.	*EF173671		<sup>1</sup> AF325908		Shanxi: Wang Wei SX034 (PE)
<i>Jeffersonia diphylla</i> (L.) Pers.	<sup>1</sup> L75867	<sup>2</sup> AB069836	FJ626555	FJ626459	Sichuan: Chen et al. 960583 (PE)
<i>Mahonia bealei</i> (Fort.) Carr.	<sup>1</sup> L12657		FJ626556	FJ626460	<sup>1</sup> Liu et al. (2002)
<i>Nandina domestica</i> Thunb.	<sup>1</sup> L75843	<sup>2</sup> AB069830	<sup>1</sup> AB069833		<sup>1</sup> Kim and Jansen (1996), <sup>2</sup> Adachi et al., unpubl.;
<i>Plagiorhegma dubia</i> Maxim.	*EF173673		<sup>2</sup> AB069836	<sup>3</sup> U52604	Jilin: Zhou Y (PE)
<i>Podophyllum peltatum</i> L.	<sup>1</sup> AF093716	<sup>2</sup> AB069843	<sup>3</sup> AF325904	<sup>4</sup> DQ008614	Jilin: Chen Z-D 2047 (PE)
<i>Ranzania japonica</i> Ito	<sup>1</sup> L75853	<sup>2</sup> AB069829			<sup>1</sup> Adachi et al., unpubl.
<i>Sinopodophyllum hexandrum</i> (Royle) Ying	<sup>1</sup> AF079455		<sup>2</sup> AF325905		<sup>1</sup> Hoot et al. (1999), <sup>2</sup> Adachi et al., unpubl.;
<i>Vancouveria hexandra</i> C. Morren & Decne.	*EF173674	*DQ478616		FJ626463	<sup>3</sup> Liu et al. (2004), <sup>4</sup> Qiu et al. (2005)
		<sup>1</sup> AB069839			<sup>1</sup> Kim and Jansen (1996), <sup>2</sup> Adachi et al., unpubl.;
				<sup>2</sup> U52602	<sup>1</sup> Feng and Wang, unpubl.; <sup>2</sup> Liu et al. (2002)
					Qinghai (PE)
					Germany (cult.): Zhang M-L
					<sup>1</sup> Adachi et al., unpubl.; <sup>2</sup> Ro et al. (1997)
Menispermaceae					
<i>Albertisia laurifolia</i> Yamamoto	FJ626590	*EF143849	EF143880		Hainan: Hong Y-P 99371 (PE)
<i>Anamirta cocculus</i> (L.) Wight & Arn.	FJ626591	*EF143856	EF143887		Thailand: Wang H-C 103 (HIB)

<i>Arcangelisia gusanlung</i> Lo	FJ626592	*EF143852	EF143883	FJ626464	Hainan: Hong Y-P 99406 (PE)
<i>Aspidocarya uvifera</i> Hook. f. & Thoms.	FJ626593	*EF143853	EF143884	FJ626465	Yunnan: Hong Y-P 99190 (PE)
<i>Cissampelos paraira</i> Linn.	<sup>1</sup> AF197590*			<sup>2</sup> DQ008616	<sup>1</sup> Qiu et al. (1999), <sup>2</sup> Qiu et al. (2005)
		*EF143858	EF143889		Yunnan: Wang H-C BN-001 (HIB)
<i>Cocculus trilobus</i> (Thunb.) DC.	<sup>1</sup> L12642*				<sup>1</sup> Qiu et al. (1993)
		*DQ478611	EF143892	FJ626466	Guizhou: Hong Y-P H310 (PE)
<i>Cyclea hypoglauca</i> (Schauer) Diels	FJ626594	*EF143862	EF143894	FJ626467	Guangdong: Chen et al. 9812108 (PE)
<i>Diploclisia glaucescens</i> (Bl.) Diels	FJ626595	*EF143867	EF143899	FJ626468	South China Bot Gard (cult.): Hong Y-P 99403
<i>Hypserpa nitida</i> Miers	FJ626596	*EF143868	EF143900		Hainan: Hong Y-P 99378 (PE)
<i>Menispermum dauricum</i> DC.	<sup>1</sup> AF190436		<sup>2</sup> AF335293		<sup>1</sup> Xiang et al. (2000), <sup>2</sup> Wang et al. (2002)
		*DQ478613		FJ626469	Beijing (cult.): Hong Y-P 99095 (PE)
<i>Parabaena sagittata</i> Miers	FJ626597	*EF143854	EF143885	FJ626470	Guizhou: Hong Y-P H346 (PE)
<i>Pericampylus glaucus</i> (Lam.) Merr.	FJ626598	*EF143869	EF143901	FJ626471	Guangdong: Chen et al. 9812095 (PE)
<i>Pycnarrhena lucida</i> (Teijsm. & Binn.) Miq.	FJ626599	*EF143851	EF143882		Hainan: Hong Y-P HN167 (PE)
<i>Sinomenium acutum</i> (Thunb.) Redd. & Wils.	FJ626600	*EF143870	EF143902	FJ626472	Henan: Hong Y-P H006 (PE)
<i>Stephania longa</i> Lour.	FJ626601	*EF143875	EF143907	FJ626473	Guangxi: Hong Y-P H101 (PE)
<i>Tinomiscium petiolare</i> Hook. f. & Thoms.	*EF173675	*DQ478612	EF143888	FJ626474	Yunnan: Hong Y-P H142 (PE)
<i>Tinospora sinensis</i> (Lour.) Merr.	FJ626602	*EF143855	EF143886	FJ626475	Thailand: Wang H-C 109 (HIB)
Lardizabalaceae					
<i>Akebia quinata</i> (Houtt.) Decne.	<sup>1</sup> L12627	<sup>2</sup> AF542587	<sup>3</sup> AM397152	<sup>4</sup> AF389253	<sup>1</sup> Qiu et al. (1993), <sup>2</sup> Hilu et al. (2003), <sup>3</sup> Worberg et al. (2007), <sup>4</sup> Kim et al. (2004a)
<i>Archakebia apetala</i> (Xia et al.) Wu, Chen & Qin	<sup>1</sup> AF335306		<sup>1</sup> AF335288		<sup>1</sup> Wang et al. (2002)
		FJ626510			Gansu: Hong Y-P 99109 (PE)
<i>Boquila trifoliata</i> (DC.) Decaisne	FJ626603	FJ626511			Chile: Qin H-N 643 (PE)
			<sup>1</sup> AF335291		<sup>1</sup> Wang et al. (2002)
<i>Decaisnea insignis</i> (Griff.) Hook. f. et Thoms.	FJ626604	FJ626512			Hubei: Chen Z-D 961051 (PE)
			<sup>1</sup> AF335303	<sup>2</sup> AF389254	<sup>1</sup> Wang et al. (2002), <sup>2</sup> Kim et al. (2004a)
<i>Holboellia grandiflora</i> Reaub.	<sup>1</sup> AF398181		<sup>1</sup> AF335290		<sup>1</sup> Wang et al. (2002)
		FJ626513		FJ626476	Guizhou: Wang Wei 011 (PE)
<i>Lardizabala biternata</i> (Molina) Looser	<sup>1</sup> L37919	<sup>2</sup> AY437809	<sup>3</sup> AF335282	<sup>2</sup> DQ008618	<sup>1</sup> Hoot et al. (1995), <sup>2</sup> Qiu et al. (2005), <sup>3</sup> Wang et al. (2002)
<i>Parvatia brunoniana</i> spp. <i>elliptica</i> Griffith	<sup>1</sup> AF335307		<sup>1</sup> AF335283		<sup>1</sup> Wang et al. (2002)
		FJ626514			Guangxi: Wang Wei GH0425-3 (PE)
<i>Sargentodoxa cuneata</i> (Oliv.) Rehd. & Wils.	FJ626605	FJ626515			Yunnan: Hong Y-P 99238 (PE)
			<sup>1</sup> AF335301	<sup>2</sup> DQ008620	<sup>1</sup> Wang et al. (2002), <sup>2</sup> Qiu et al. (2005)
<i>Sinofranchetia chinensis</i> (Franch.) Hemsl.	*EF173676	FJ626516			Shanxi: Wang Wei SX040 (PE)
			<sup>1</sup> AF335284	<sup>2</sup> AF389255	<sup>1</sup> Wang et al. (2002), <sup>2</sup> Kim et al. (2004a)
<i>Stauntonia hexaphylla</i> (Thunb.) Decne.	FJ626606	FJ626517			Guangdong: Chen Z-D H29905 (PE)
			<sup>1</sup> AF335286		<sup>1</sup> Wang et al. (2002)

**Table 1.** (continued)

Taxon	GenBank accession numbers				Vouchers/References
	<i>rbcL</i>	<i>matK</i>	<i>trnL-F</i>	26S	
Circaceasteraceae					
<i>Circaeaster agrestis</i> Maxim.	FJ626607	FJ626518			Shanxi: Ren Y 001 (PE)
			<sup>1</sup> AF335298	<sup>2</sup> AF389246	<sup>1</sup> Wang et al. (2002), Kim et al. (2004a)
<i>Kingdonia uniflora</i> Balf. f. & W. W. Sm.	FJ626608	FJ626519			Shanxi: Ren Y 002 (PE)
			<sup>1</sup> DQ185598	<sup>2</sup> AF389245	<sup>1</sup> Zha and Sun, unpubl.; <sup>2</sup> Kim et al. (2004a)
Papaveraceae					
<i>Corydalis</i> sp.	FJ626609	FJ626520	FJ626561	FJ626477	Gansu: Hong Y-P 99105 (PE)
<i>Dicentra eximia</i> (Ker) Torrey	<sup>1</sup> L37917	<sup>2</sup> DQ182345	<sup>3</sup> AY145361	<sup>4</sup> AF389262	<sup>1</sup> Hoot et al. (1995), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Kim et al. (2004a)
<i>Eomecon chionantha</i> Hance	FJ626610	FJ626521	FJ626562	FJ626478	Guizhou: Wang Wei 059 (PE)
<i>Hylomecon japonica</i> (Thunb.) Prantl. & Kundig.	FJ626611	FJ626522	FJ626563	FJ626479	Jilin: Chen 2046 (PE)
<i>Hypecoum imberbe</i> Sm.	<sup>1</sup> U86628			<sup>2</sup> AF389263	<sup>1</sup> Hoot et al. (1997), <sup>2</sup> Kim et al. (2004a)
<i>Macleaya microcarpa</i> (Maxim.) Fedde	FJ626612	FJ626523	FJ626564		Beijing (cult.): Hong Y-P H542 (PE)
<i>Meconopsis quintuplinervia</i> Regel.	FJ626613	FJ626524	FJ626565	FJ626480	Shanxi: Wang Wei SX009 (PE)
<i>Papaver rhoeas</i> L.	FJ626614	FJ626525	FJ626566	FJ626481	Kunming (cult.): Wang Wei YN100 (PE)
<i>Pteridophyllum racemosum</i> Sieb. & Zucc.	<sup>1</sup> U86631			<sup>2</sup> AF389264	<sup>1</sup> Hoot et al. (1997), <sup>2</sup> Kim et al. (2004a)
		FJ626526	FJ626567		provided by Hoot: Chase 531 (K)
<i>Sanguinaria canadensis</i> L.	<sup>1</sup> L01951*	<sup>2</sup> DQ401350		<sup>3</sup> DQ008621	<sup>1</sup> Albert et al. (1992), <sup>2</sup> Qiu et al. (2006), <sup>3</sup> Qiu et al. (2005)
Eupteleaceae					
<i>Euptelea pleiospermum</i> Hook. f. & Thoms.	<sup>1</sup> AY048174		<sup>2</sup> AM39715		<sup>1</sup> Wang et al. (2002), <sup>2</sup> Worberg et al. (2007)
		FJ626527		FJ626482	Yunnan: Feng M. 967011 (PE)
<i>Euptelea polyandra</i> Sieb. & Zucc.	<sup>1</sup> L12645*			<sup>2</sup> AF389249	Qiu et al. (1993), <sup>2</sup> Kim et al. (2004a)
		FJ626528	FJ626568		Japan: Ren B-Q (PE)
Other basal eudicots					
<i>Buxus sempervirens</i> L.	<sup>1</sup> AF093717	<sup>2</sup> AF543728	<sup>3</sup> AY145357	<sup>4</sup> AF389243	<sup>1</sup> Hoot et al. (1999), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Kim et al. (2004a)
<i>Grevillea robusta</i> Cunn. & R. Br.	<sup>1</sup> AF197589			<sup>2</sup> DQ008612	<sup>1</sup> Qiu et al. (1999), <sup>2</sup> Qiu et al. (2005)
		FJ626529	FJ626569		Hainan: Hong Y-P 99354 (PE)
<i>Meliosma veitchiorum</i> Hemsl. View.	<sup>1</sup> AF206793			<sup>2</sup> AF389271	<sup>1</sup> Soltis et al. (1999), <sup>2</sup> Kim et al. (2004a)
		FJ626530	FJ626570		Henan: Hong Y-P 99061 (PE)
<i>Nelumbo lutea</i> (Willd.) Pers.	<sup>1</sup> DQ182337	<sup>1</sup> AF543740	<sup>1</sup> AY145359	<sup>2</sup> AF389259	<sup>1</sup> Müller et al. (2006), <sup>2</sup> Kim et al. (2004a)



<i>Nelumbo nucifera</i> Gaertn.	FJ626615	FJ626531	FJ626571	FJ626483	Beijing (cult.): Wang W 08010 (PE)
<i>Platanus occidentalis</i> L.	<sup>1</sup> L01943	<sup>2</sup> AF543747	<sup>3</sup> AY145358	<sup>4</sup> AF274662	<sup>1</sup> Albert et al. (1992), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Fishbein et al. (2001)
<i>Sabia swinhoei</i> Hemsl.	FJ626616	FJ626532	FJ626572		no voucher
				<sup>1</sup> AF389272	<sup>1</sup> Kim et al. (2004a)
<i>Tetracentron sinense</i> Oliv.	<sup>1</sup> L12668	<sup>2</sup> AM396504	<sup>2</sup> AM397165	<sup>3</sup> AF274670	<sup>1</sup> Qiu et al. (1993), <sup>2</sup> Worberg et al. (2007), <sup>3</sup> Fishbein et al. (2001)
<i>Trochodendron aralioides</i> Sieb. & Zucc.	<sup>1</sup> L01958	<sup>2</sup> AF543751	<sup>3</sup> AY145360	<sup>4</sup> AF274671	<sup>1</sup> Albert et al. (1992), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Fishbein et al. (2001)
Core eudicots					
<i>Altingia excelsa</i> Noron.	<sup>1</sup> DQ352374	<sup>2</sup> AF013037	<sup>1</sup> DQ352226	<sup>3</sup> AF479208	<sup>1</sup> Ickert-Bond and Wen (2006), <sup>2</sup> Li et al. (1997), <sup>3</sup> Soltis et al. (2003)
<i>Paeonia suffruticosa</i> Andr.	<sup>1</sup> AJ402982	<sup>2</sup> AF033593		<sup>3</sup> AF274659	<sup>1</sup> Savolainen et al. (2000a, b), <sup>2</sup> Sang et al. (1997), <sup>3</sup> Fishbein et al. (2001)
			FJ626573		Beijing (cult.): Wang W 08012 (PE)
<i>Hamamelis virginiana</i> L.	<sup>1</sup> DQ352368	<sup>2</sup> AF013046	<sup>1</sup> DQ352196	<sup>3</sup> AF036495	<sup>1</sup> Ickert-Bond and Wen (2006), <sup>2</sup> Li et al. (1997), <sup>3</sup> Kuzoff et al. (1998)
<i>Coriaria myrtifolia</i> L.	<sup>1</sup> L01897	<sup>2</sup> AB016459	<sup>3</sup> AY091824	<sup>4</sup> AY968406	<sup>1</sup> Albert et al. (1992), <sup>2</sup> Yokoyama et al. (2000), <sup>3</sup> Yang, unpubl.; <sup>4</sup> Zhang et al. (2006)
<i>Cornus mas</i> L.	<sup>1</sup> L11216	<sup>2</sup> AJ429275	<sup>2</sup> AJ430866	<sup>3</sup> AF297535	<sup>1</sup> Xiang et al. (1993), <sup>2</sup> Bremer et al. (2002), <sup>3</sup> Fan and Xiang (2001)
<i>Aextoxicon punctatum</i> Ruiz & Pav.	<sup>1</sup> X83986	<sup>2</sup> AF543720	<sup>3</sup> AY145362	<sup>4</sup> AF389239	<sup>1</sup> Savolainen et al. (1997), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Kim et al. (2004a)
<i>Vitis aestivalis</i> Michx.	<sup>1</sup> L01960	<sup>2</sup> AF274635	<sup>3</sup> AB235073	<sup>4</sup> AF479207	<sup>1</sup> Albert et al. (1992), <sup>2</sup> Fishbein et al. (2001), <sup>3</sup> Soejima and Wen (2006), <sup>4</sup> Soltis et al. (2003)
<i>Tetracera asiatica</i> (Lour.) Hoogl.	<sup>1</sup> AJ235796	<sup>2</sup> AY042665		<sup>3</sup> AF479097	<sup>1</sup> Savolainen et al. (2000a, b), <sup>2</sup> Cuenoud et al. (2002), <sup>3</sup> Soltis et al. (2003)
			FJ626574		Guangdong: Xu K-X 012 (PE)
Basal angiosperms					
<i>Acorus gramineus</i> L.	<sup>1</sup> D28866	<sup>2</sup> DQ182341	<sup>3</sup> AY145336	<sup>4</sup> AF036490	<sup>1</sup> Kawano, unpubl.; <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Kuzoff et al. (2003)
<i>Amborella trichopoda</i> Baill.	<sup>1</sup> L12628	<sup>2</sup> AF543721	<sup>3</sup> AY145324	<sup>4</sup> AF479238	<sup>1</sup> Qiu et al. (1993), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Soltis et al. (2003)
<i>Annona muricata</i> L.	<sup>1</sup> L12629	<sup>2</sup> AF543722	<sup>3</sup> AY145352	<sup>4</sup> DQ008634	<sup>1</sup> Qiu et al. (1993), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Qiu et al. (2005)
<i>Austrobaileya scandens</i> C. T. White	<sup>1</sup> L12632	<sup>2</sup> DQ182344	<sup>3</sup> AY145326	<sup>4</sup> AY095452	<sup>1</sup> Qiu et al. (1993), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Zanis et al. (2003)
<i>Brasenia schreberi</i> J. Gmelin	<sup>1</sup> M77031	<sup>2</sup> AF092973	<sup>3</sup> AY145329	<sup>4</sup> DQ008661	<sup>1</sup> Les et al. (1991), <sup>2</sup> Les et al. (1999), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Qiu et al. (2005)
<i>Calycanthus floridus</i> L. ( <i>occidentalis</i> Hook. & Arn. for 26S)	<sup>1</sup> L14291	<sup>2</sup> AF543730	<sup>3</sup> AY145349	<sup>4</sup> AY095454	<sup>1</sup> Olmstead et al. (1993), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Zanis et al. (2003)

**Table 1.** (continued)

Taxon	GenBank accession numbers				Vouchers/References
	<i>rbcL</i>	<i>matK</i>	<i>trnL-F</i>	26S	
<i>Canella winterana</i> (L.) Gaertn.	<sup>1</sup> AJ131928	<sup>2</sup> AF543731	<sup>3</sup> AY004152	<sup>4</sup> AY095455	<sup>1</sup> Qiu et al. (1993), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Karol et al. (2000), <sup>4</sup> Zanis et al. (2003)
<i>Ceratophyllum demersum</i> L.	<sup>1</sup> D89473	<sup>2</sup> AF543732	<sup>3</sup> AY145335	<sup>4</sup> AY095456	<sup>1</sup> Ueda et al. (1997), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Zanis et al. (2003)
<i>Hedyosmum arborescens</i> Sw.	<sup>1</sup> L12649	<sup>2</sup> DQ401339	<sup>3</sup> AY236750	<sup>4</sup> AF479226	<sup>1</sup> Qiu et al. (1993), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Zhang and Renner (2003), <sup>4</sup> Soltis et al. (2003)
<i>Illicium floridanum</i> Ellis	<sup>1</sup> DQ182334	<sup>1</sup> AF543738	<sup>2</sup> AY145325	<sup>3</sup> DQ008659	<sup>1</sup> Müller et al. (2006), <sup>2</sup> Borsch et al. (2003), <sup>3</sup> Qiu et al. (2005)
<i>Magnolia denudata</i> Desr.	<sup>1</sup> AY008913	<sup>2</sup> AF123465	<sup>3</sup> AY009052	<sup>4</sup> AF479244	<sup>1</sup> Kim et al., unpubl.; <sup>2</sup> Jin et al. (1999), <sup>3</sup> Su et al., unpubl.; <sup>4</sup> Soltis et al. (2003)
<i>Orontium aquaticum</i> L.	<sup>1</sup> AJ005632	<sup>2</sup> AF543744	<sup>3</sup> AY145338	<sup>4</sup> DQ008652	<sup>1</sup> Cho and Palmer (1999), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Qiu et al. (2005)
<i>Saruma henryi</i> Oliv.	<sup>1</sup> L12664	<sup>2</sup> AF543748	<sup>3</sup> AY145340	<sup>4</sup> DQ008644	<sup>1</sup> Qiu et al. (1993), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Borsch et al. (2003), <sup>4</sup> Qiu et al. (2005)
<i>Saururus cernuus</i> L.	<sup>1</sup> L14294	<sup>2</sup> AF543749	<sup>3</sup> AF332970	<sup>4</sup> AY095468	<sup>1</sup> Olmstead et al. (1993), <sup>2</sup> Müller et al. (2006), <sup>3</sup> Meng et al., unpubl.; <sup>4</sup> Zanis et al. (2003)
<i>Takhtajania perrieri</i> M. Baranova & J. Leroy	<sup>1</sup> AF206824	<sup>2</sup> DQ401371	<sup>3</sup> AY004146	<sup>4</sup> DQ008645	<sup>1</sup> Soltis et al. (1999), <sup>2</sup> Qiu et al. (2006), <sup>3</sup> Karol et al. (2000), <sup>4</sup> Qiu et al. (2005)

The accession numbers published in our previous studies (Wang et al., 2005, 2007a, b; Wang and Chen, 2007) are marked with an asterisk.

products were purified using a GFX<sup>TM</sup> PCR DNA and Gel Band Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) then directly sequenced. Sequencing reactions were conducted using the DYEnamic<sup>TM</sup> ET Dye Terminator Cycle Sequencing Kit (Amersham Pharmacia Biotech) and analyzed using MegaBACE<sup>TM</sup>1000 DNA Analysis Systems, following the manufacturer's protocols.

### Alignment and phylogenetic analysis

Three of the four DNA regions (all except *trnL-F*) were aligned using the default alignment parameters in CLUSTAL X (Thompson et al., 1997) with manual adjustment. Because of the great length variation in *trnL-F* regions, these were manually aligned in BioEdit (Hall, 1999) according to the rules outlined by Borsch et al. (2003). Following Borsch et al. (2003) and Worberg et al. (2007), we identified eight mutational hotspots in the *trnL-F* matrix, encompassing 1168 positions, which were excluded from the analyses. Two difficult-to-align regions in 26S rDNA, representing 45 sites, were also excluded from the analyses. Gaps were coded as missing data. All aligned matrices are available upon request from the corresponding author.

We used maximum parsimony (MP) and Bayesian inference (BI) to analyze each of the individual molecular data sets as well as the morphological data set. Because simultaneous analysis of combined data has been proposed as the best approach to phylogenetic inference (Kluge, 1989; Chippindale and Wiens, 1994; Nixon and Carpenter, 1996; Cognato and Vogler, 2001), we visually inspected the individual bootstrap consensus trees on a node to node basis to test the congruence among the individual DNA data sets by identifying contradictory nodes with >70% BS support as described in Mason-Gamer and Kellogg (1996). Since no strongly supported (>70% BS) nodes were found, data sets were combined as follows: first all molecular data were combined to obtain a combined molecular data set, then the combined molecular data set was combined with the morphological data set yielding a total evidence data set. The combined molecular data set included 137 taxa, in which the amount of missing data was: *rbcL* = 0.014% (2/137), *matK* = 0.007% (1/137), *trnL-F* = 0.036% (5/137), and 26S = 0.117% (16/137). The total evidence data set included 129 taxa, in which the amount of missing data was: *rbcL* = 0.008% (1/129), *matK* = 0.008% (1/129), *trnL-F* = 0.039% (5/129), and 26S = 0.124% (16/129).

Parsimony analyses were performed using PAUP\* version 4.0b10 (Swofford, 2003). Heuristic searches were conducted with 1000 replicates of random addition, one tree held at each step during stepwise addition, tree-bisection-reconnection (TBR) branch swapping,

MulTrees in effect, and steepest descent off. Bootstrapping was conducted with 1000 replicates, using a heuristic search strategy (five random addition replicates, saving five trees per replicate). The BI analyses were carried out using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). Each of the four regions (*rbcL*, *matK*, *trnL-F* and 26S rDNA) was assigned its own model of nucleotide substitution, as determined by the Akaike information criterion (AIC) in Modeltest version 3.06 (Posada and Crandall, 1998). The morphological data were run under the datatype = standard option, and only variable sites had the possibility of being sampled (coding = variable). For both the combined molecular data set and the total evidence data set, the prior assumption of rate heterogeneity across the data partitions was set at variable (ratepr = variable). For more information on the settings used, contact the first author or see the MrBayes manual on the Internet at <http://mrbayes.csit.fsu.edu/manual.php>. Searches were based on 1,000,000 generations with four chains of the Markov Chain Monte Carlo (MCMC). Runs were started from a random tree and allowed to proceed in parallel while sampling and recording the topology every 100 generations of the MCMC chain. Performance of individual runs was assessed and phylogenies compared between runs. Majority rule (>50%) consensus trees were constructed after removing the "burn-in period" samples (the first 15% of sampled trees).

In order to identify the diagnostic morphological characters at different taxonomic levels, we optimized selected characters onto the strict consensus tree from the MP analysis based on the total evidence data set using MacClade 4.06 (Maddison and Maddison, 2003).

## Results

### Molecular data

The aligned *trnL-F* matrix included 2777 positions, the aligned 26S rDNA sequences were 1345 nucleotides in length, and the aligned *rbcL* and *matK* data sets had 1395 and 1440 positions, respectively. Table 2 summarizes the number of variable and parsimony-informative sites and tree statistics for the molecular data sets.

The topologies based on individual DNA data sets were nearly identical except in some of the terminal branches (trees not shown, but available upon request from the corresponding author). The aligned matrix of the combined DNA data comprised 6957 characters, of which 2984 were variable and 2207 were potentially parsimony-informative. The MP analysis generated eight most parsimonious trees (MPTs) of 15,799 steps, with a CI of 0.33, RI of 0.66 and an RC of 0.22.

**Table 2.** Statistics from analyses of the molecular data sets. Tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC) were calculated based on parsimony-informative characters only.

Data set	No. taxa	Total length	No. variable character	No. informative character	No. of trees	Length of trees	CI	RI	RC	Model
<i>rbcL</i>	135	1395	633	458	105	3099	0.31	0.66	0.20	GTR+I+ $\Gamma$
<i>matK</i>	136	1440	1020	843	105	6111	0.32	0.68	0.22	TVM+I+ $\Gamma$
<i>trnL-F</i>	131	2777	718	557	11185	3426	0.40	0.72	0.29	TVM+I+ $\Gamma$
26S rDNA	121	1345	613	349	15	2993	0.30	0.58	0.18	GTR+I+ $\Gamma$
Combined molecular data	137	6957	2984	2207	8	15799	0.33	0.66	0.22	

Bayesian analysis yielded trees with topologies that are consistent with those retrieved with MP analysis except that *Hypecoum* is sister to the clade containing *Pteridophyllum*, *Dicentra* and *Corydalis* (PP 0.61), *Helleborus* is sister to the clade E (PP 0.92), *Asteropyrum* is sister to the clade D (PP 0.74), *Nigella* is sister to the clades B and C (PP < 0.50), and clades B-E, *Caltha*, *Asteropyrum*, *Helleborus*, and *Nigella* in Ranunculaceae form a poorly supported clade F (Fig. 1).

### Morphological data

The morphological data set generated 15 MPTs of 561 steps, with a CI of 0.22, RI of 0.76 and an RC of 0.16. Cladograms from BI and MP analyses were poorly resolved (Fig. 2). Both Menispermaceae and Papaveraceae are resolved and each is supported as a monophyletic group. Within Papaveraceae, *Pteridophyllum*, *Dicentra*, *Hypecoum*, and *Corydalis* formed a clade (BS 81%, PP 1.00). *Circaeaster* and *Kingdonia* formed a clade (BS 80%, PP 0.93).

### Total evidence data

The total evidence data set generated eight MPTs of 16,639 steps, with a CI of 0.35, RI of 0.65 and an RC of 0.23. Patterns of relationship are almost identical with those obtained from the combined molecular data except that support for Eupteleaceae as sister to other Ranunculales increased notably (BS 87%), *Pteridophyllum* and *Hypecoum* form a strongly supported (BS 96%) clade, and in Ranunculaceae, clade F is recovered, which unites clades B-E, *Caltha*, *Asteropyrum*, *Helleborus*, and *Nigella*, but is with poor support (Fig. 3).

The trees generated by BI were highly congruent with those of MP analysis except for the presence of several nodes with weak support (PP < 0.50) in Ranunculaceae: *Helleborus* is sister to the clade containing *Asteropyrum*, *Caltha* and Clade D, and *Nigella* is sister to the clades B-C (Fig. 3). All further discussion will be based on the total evidence trees (Fig. 3).

Ranunculales and its seven families are all strongly supported as monophyletic. Three major clades were identified within Ranunculales: Eupteleaceae, Papaveraceae and core Ranunculales. Papaveraceae and core Ranunculales form a clade (BS 87%, PP 0.98), sister to Eupteleaceae. Within core Ranunculales, Lardizabalaceae and Circaeasteraceae form a subclade with strong support (BS 87%, PP 1.00), which is sister to a subclade (BS 76%, PP 1.00) comprised of the other three families.

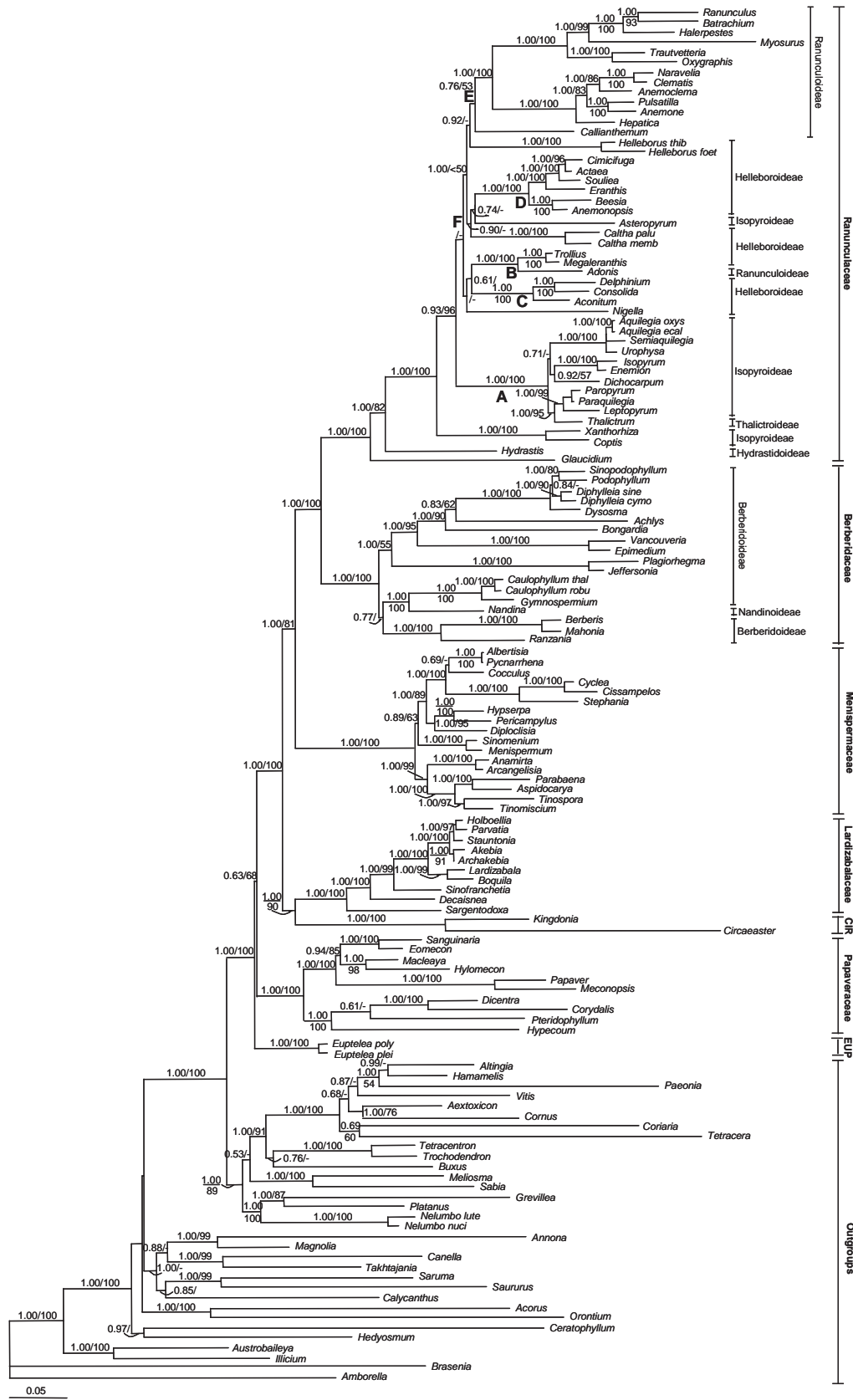
Papaveraceae is strongly supported (BS 100%, PP 1.00) as monophyletic and is divided into two subclades (each with 100% BS and 1.00 PP support), the first containing *Pteridophyllum*, *Hypecoum*, *Dicentra* and *Corydalis*; the second containing *Eomecon*, *Hylomecon*, *Macleaya*, *Meconopsis* and *Papaver*.

Within Lardizabalaceae, *Sargentodoxa* is sister to the remaining taxa of the family (BS 100%, PP 1.00), followed by *Decaisnea* (BS 100%, PP 1.00), then *Sinofranchetia* (BS 96%, PP 1.00).

Within Menispermaceae, two major clades are recognized. The first clade consists of tribe Pachygoneae Miers (represented by *Albertisia* and *Pycnarrhena*) and tribe Menispermeae DC., with the former embedded within the latter. The second clade is composed of tribes Tinosporeae Hook. f. & Thoms. and Fibraureae Diels, which are not supported as monophyletic. *Anamirta* of tribe Fibraureae and *Arcangelisia* of tribe Tinosporeae form a strongly supported (BS 100%, PP 1.00) subclade that is sister to a subclade (BS 95%, PP 1.00) comprised of *Tinomiscium* of tribe Fibraureae and *Tinospora* of tribe Tinosporeae, which in turn is sister to the *Aspidocarya-Parabaena* subclade (BS 100%, PP 1.00).

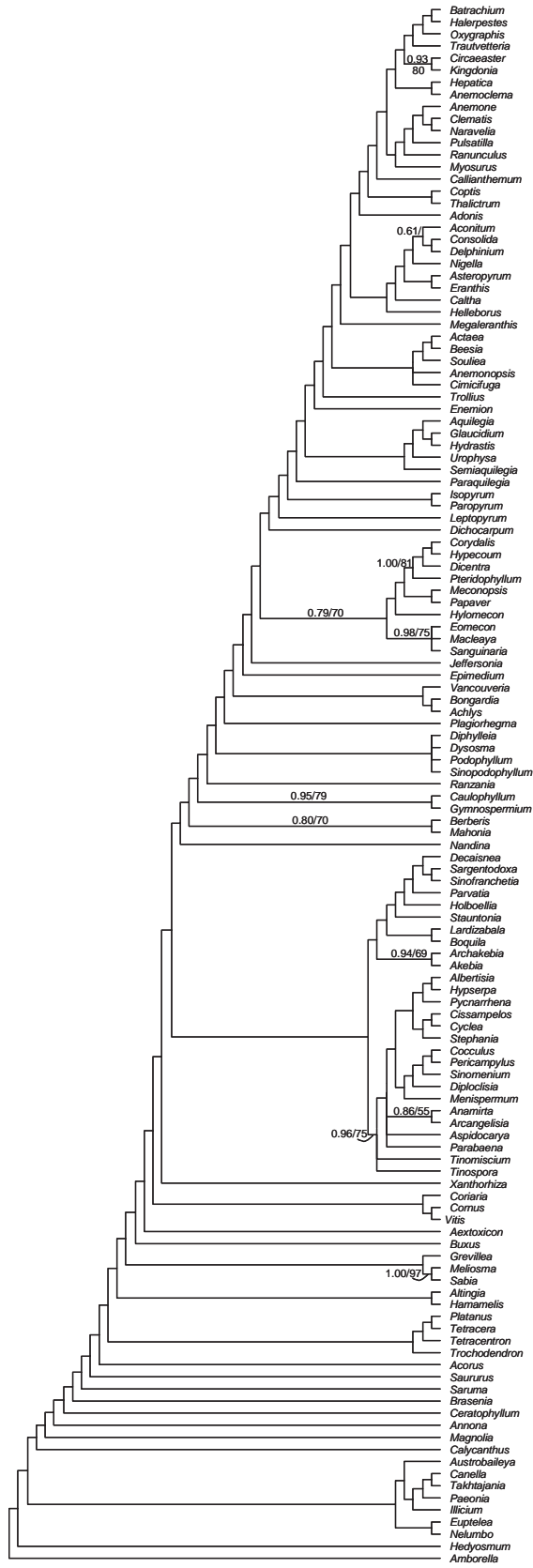
Within Berberidaceae, Nandinoideae Heintz is embedded within Berberidoideae Kostel and forms a clade with *Gymnospermium* and *Caulophyllum* (BS 99%, PP 1.00).

Ranunculaceae contains three major clades: *Glaucidium* (BS 100%, PP 1.00), *Hydrastis* (BS 76%, PP 1.00) and Ranunculaceae s. str. (sensu Takhtajan, 1997, excluding *Kingdonia*) (BS 100%, PP 1.00), with *Glaucidium* sister to the other two clades. Except for two monogeneric subfamilies (Thalictroideae and Hydrastidoideae), Isopyroideae, Helleboroideae and



**Fig. 1.** Phylogram obtained from Bayesian inference based on the combined molecular data set. Numbers above the branches are Bayesian posterior probabilities (>0.50) and bootstrap percentages (>50%), respectively. “–” indicates the nodes not found in MP trees. Familial classification is based on APG II (2003), subfamilial classifications based on Tamura (1993) for Ranunculaceae and Loconte (1993) for Berberidaceae. CIR = Circaeasteraceae, EUP = Eupteleaceae.





**Fig. 2.** Strict consensus tree of 15 MPTs of Ranunculales based on the morphological data set. Numbers above the branches are Bayesian posterior probabilities (>0.50) and bootstrap percentages (>50%), respectively.

Ranunculoideae of Tamura (1993) are not monophyletic. Within Ranunculaceae *s. str.*, the *Coptis-Xanthorhiza* clade (BS 100%, PP 1.00) is sister to the clade (BS 96%, PP 1.00) containing the remaining genera. Within the latter clade, five monophyletic groups are identified: clade A (BS 100%, PP 1.00) contains Thalictroideae and nine genera of Isopyroideae; clade B (BS 100%, PP 1.00) contains *Adonis* of Ranunculoideae and *Trollius* and *Megaleranthis* of Helleboroideae; clade C (BS 100%, PP 1.00) comprises tribe Delphinieae Warm.; clade D (BS 100%, PP 1.00) contains tribe Cimicifugeae Torrey & A. Gray together with *Beesia* and *Eranthis* of tribe Helleboreae DC.; clade E (BS 74%, PP 0.98) contains tribe Anemoneae DC. and tribe Ranunculeae DC. and *Callianthemum* of tribe Adonideae Kunth.

Mapping morphological characters onto the strict consensus tree from the MP analysis of the total evidence data set indicates that monophyly of Ranunculales and each of its seven constituent families, and inter-family and intra-family major clades are supported by morphology (Fig. 3). Specific morphological characters are discussed in detail below.

## Discussion

### Monophyly and synapomorphies of Ranunculales

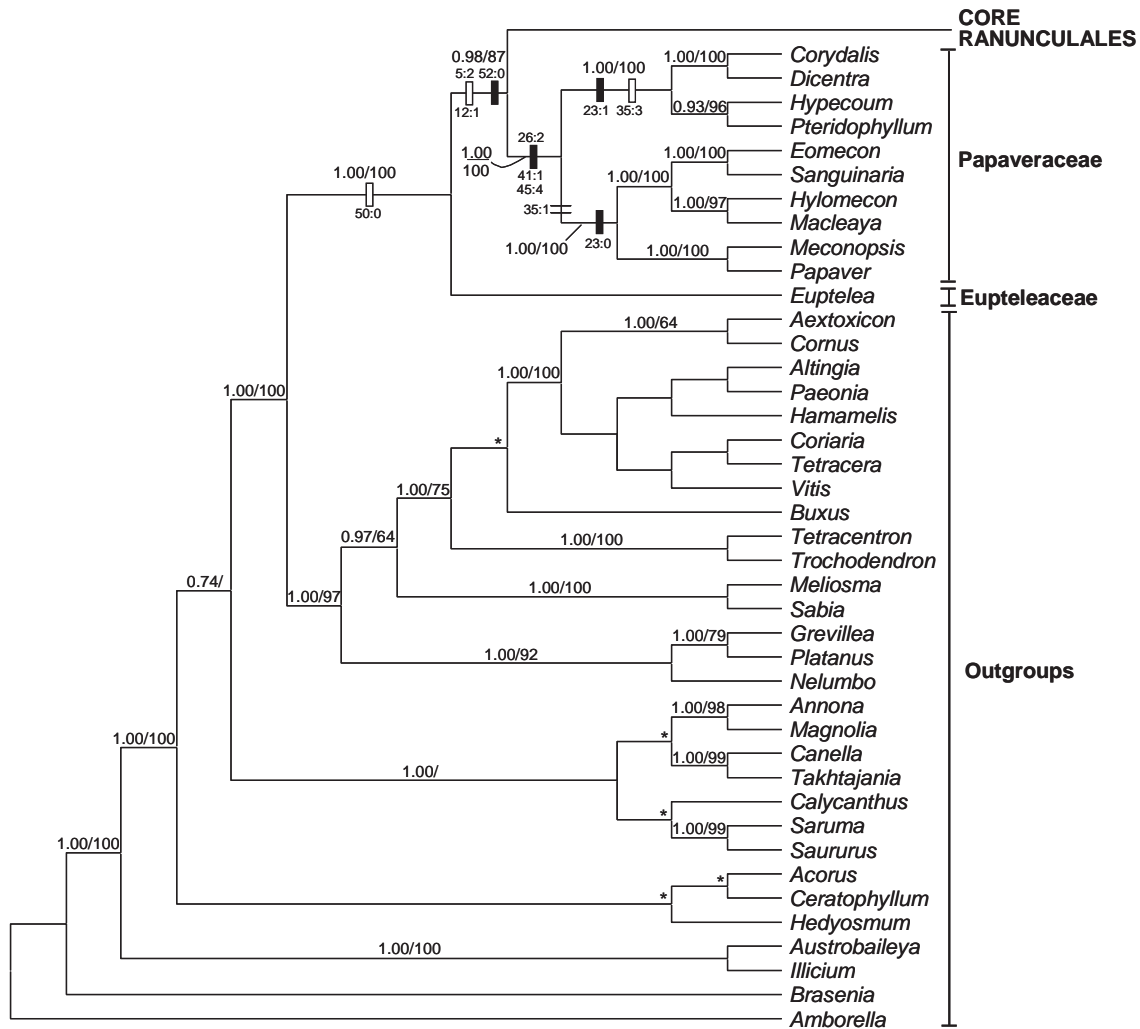
Ranunculales as well as each of its seven constituent families *sensu* APG II (2003) (Berberidaceae, Circaeasteraceae, Eupteleaceae, Lardizabalaceae, Menispermaceae, Papaveraceae, and Ranunculaceae) is strongly supported as monophyletic. Within the order, family inter-relationships are consistent with the results of previous molecular studies (e.g., Chase et al., 1993; Hoot and Crane, 1995; Hoot et al., 1999; Savolainen et al., 2000a, b; Soltis et al., 2000, 2003; Hilu et al., 2003; Kim et al., 2004a; Worberg et al., 2007), and combined molecular and morphological studies (Nandi et al., 1998; Doyle and Endress, 2000), but with our expanded taxon sampling, are resolved usually with greater support for clades found therein (Table 3).

Recent analyses (e.g., Chase et al., 1993; Hoot and Crane, 1995; Hoot et al., 1999; Savolainen et al., 2000a, b; Soltis et al., 2000, 2003; Hilu et al., 2003; Kim et al., 2004a; Worberg et al., 2007; this study) strongly support Eupteleaceae, a monogeneric family endemic to East Asia, as a member of Ranunculales. This conflicts with earlier morphological and anatomical studies (Hutchinson, 1973; Cronquist, 1988; Dahlgren, 1983; Endress, 1986, 1993; Kubitzki et al., 1993; Thorne, 2000; Wu et al., 2002), which considered that the genus belonged to the “lower” hamamelids and was related to Trochodendraceae (including *Trochodendron* and

*Tetracentron*) or *Cercidiphyllaceae*. *Eupteleaceae* differs from *Cercidiphyllaceae* in that stipules are absent (vs. present), seeds lack appendages (vs. with appendages), and in having strongly peltate carpels (vs. epeltate), and from *Trochodendraceae* in having longitudinal anther dehiscence (vs. valvate), carpels stipitate (vs. sessile), long anther connective tips (vs. short), ovary superior (vs. semi-inferior), and the micropyle formed from both integuments (vs. from inner integument only). Additionally, the chromosome number for *Trochodendron*, *Tetracentron* and *Cercidiphyllum* is  $n = 20, 23/24$  and 19, respectively (Ratter and Milne, 1976; Cronquist, 1981), whereas that of *Euptelea* is  $n = 14$  (Ratter and Milne, 1976; Pan et al., 1991), which is more similar to those found in *Lardizabalaceae* ( $n = 11, 14–16, 18$ ) (Wu and Kubitzki, 1993; Shi et al., 1994; Qin, 1997) and

*Menispermaceae* ( $n = 11–13$ ) (Kessler, 1993; Wang et al., 2004). Floral morphology (free carpels, basifixed anthers, whorled phyllotaxis) also supports a position within *Ranunculales* for *Eupteleaceae* (Ren et al., 2007).

A micropyle formed from both integuments is a synapomorphy of *Ranunculales* (*char.* 50:0), whereas one formed from the inner integument (found only in *Lardizabalaceae* and some *Ranunculaceae*) is independently derived (Fig. 3). Epicuticular wax tubules (*char.* 4:0) (Barthlott and Theisen, 1995) and large and homogeneous S sieve element plastids (*char.* 14:0) (Behnke, 1995) are ubiquitous in *Ranunculales*. However, S sieve element plastids are also present in other eudicots. At present, the epicuticular anatomy of *Eupteleaceae* is unknown. Thus, it is unclear whether



**Fig. 3.** Strict consensus tree of eight MPTs of *Ranunculales* based on the total evidence data set. Numbers above and below branches are Bayesian posterior probabilities (>0.50) and bootstrap percentages (>50%), respectively. “\*” indicates the nodes not found in Bayesian trees. Morphological character state changes within *Ranunculales* are indicated by boxes on the branches, with character numbers and states. The accelerated transformation (ACCTRAN) optimization is shown. Filled boxes represent non-homoplastic synapomorphies and empty boxes indicate homoplastic changes. Crosses indicate reversals and parallel lines indicate parallelisms.

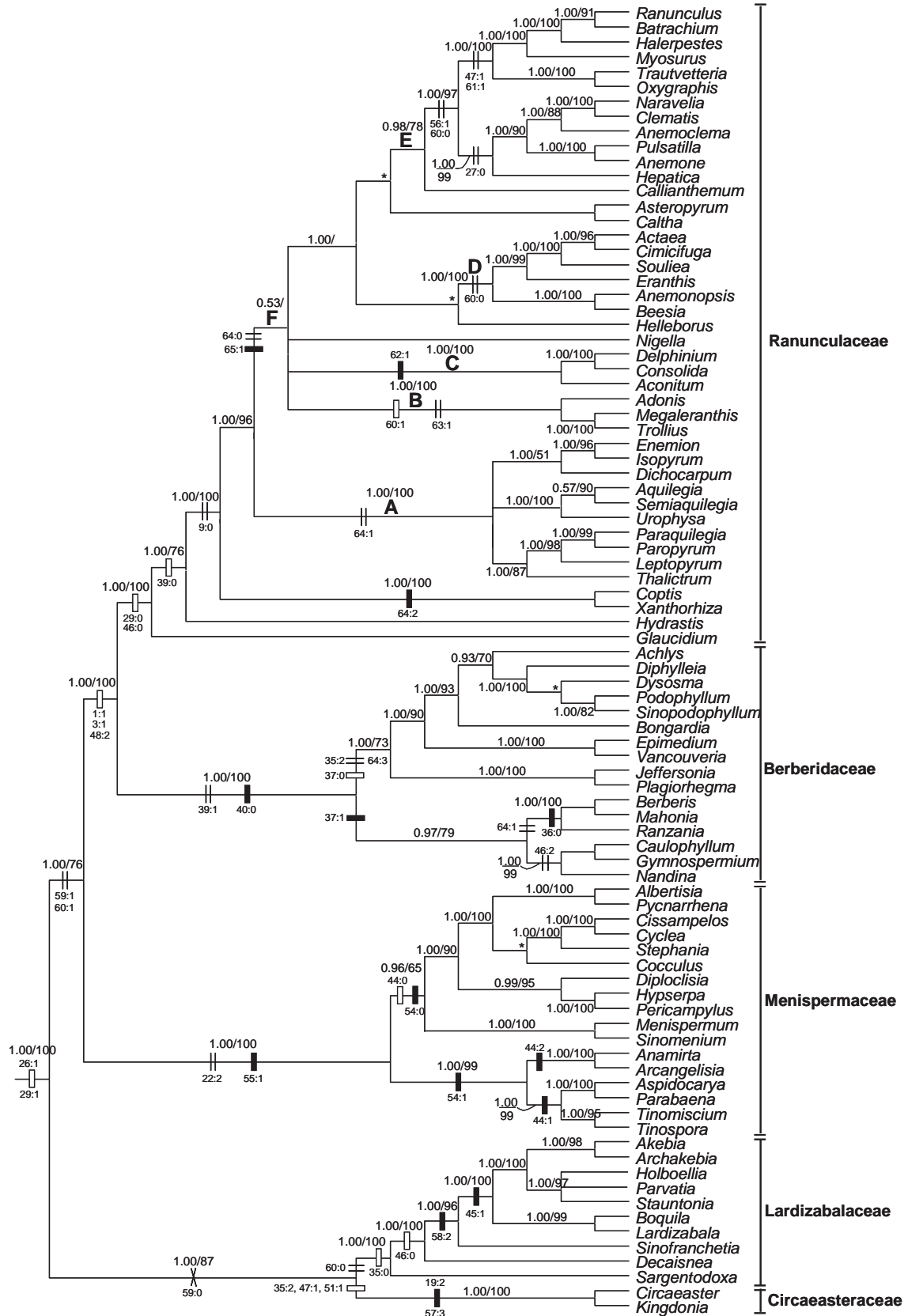


Fig. 3. (Continued)

**Table 3.** Comparative bootstrap/jackknife values for major clades in Ranunculales trees based on this study and several previous studies.

Clade	3-gene (Hoot et al., 1999)	3-gene (Soltis et al., 2000) (jack knife)	3-gene (Worberg et al., 2007)	4-gene (Soltis et al., 2003)	4-gene (Kim et al., 2004a)	4-gene (this study)	4-gene + morphology (this study)
Ranunculales	97	98	100	100	99	100	100
Berberidaceae	100	100	100	100	100	100	100
Circaeasteraceae	100	100	–	100	100	100	100
Lardizabalaceae	100	100	*	100	100	100	100
Menispermaceae	100	100	100	100	100	100	100
Papaveraceae	100	100	100	100	100	100	100
Ranunculaceae	99	90	*	100	87	100	100
<i>Glaucidium</i> sister to other Ranunculaceae	nr	nr	–	67	<50	82	76
Ranunculaceae + Berberidaceae	98	92	100	87	79	100	100
Ranunculaceae + Menispermaceae + Berberidaceae	88	70	nr	96	81	81	76
Circaeasteraceae + Lardizabalaceae	71	nr	–	71	53	90	87
Core Ranunculales	99	99	100	97	78	100	100
Eupteleaceae sister to other Ranunculales	nr	nr	80	78	70	68	87

“nr” (not recovered) indicates not recovered clade; “\*” indicates only one taxon sampled; “–” indicates taxa/clades that were not sampled.

the two characters represent synapomorphies of Ranunculales or not.

### Familial relationships within Ranunculales

Since the first molecular analysis that found that Eupteleaceae and Papaveraceae were early-diverging in Ranunculales (Chase et al., 1993), the exact relationship among Eupteleaceae, Papaveraceae and core Ranunculales has remained an open question. An investigation based on *rbcL*, *atpB* and 18S rDNA data (Hoot and Crane, 1995), found 87% BS support for a sister group relationship between Papaveraceae and the other Ranunculales. Another analysis with a more extensive taxon sampling and using the same 3 genes also supported this relationship, but with decreased BS support (Table 3; Hoot et al., 1999; Soltis et al., 2000). Conversely, with the addition of 26S rDNA data, Eupteleaceae was found to be sister to all other Ranunculales (Soltis et al., 2003; Kim et al., 2004a). Parsimony analysis of three rapidly evolving chloroplast genetic regions retrieved Eupteleaceae as the earliest-diverging lineage of Ranunculales with 80% BS support, whereas in ML analysis Eupteleaceae formed part of a tricotomy together with Papaveraceae and core Ranunculales (Worberg et al., 2007).

According to the total evidence data set with 99 taxa of the Ranunculales, Papaveraceae and core Ranunculales formed a clade (BS 87%, PP 0.98) sister to Eupteleaceae. The position of Eupteleaceae as sister to the rest of the order is also supported

by three morphological characters: ovules lacking tanniferous tissue (*char.* 52:0), simple perforation plates (*char.* 5:2) and wood with a storied structure (*char.* 12:1).

Monophyletic core Ranunculales has been strongly identified by several analyses (see Kim et al., 2004a; Worberg et al., 2007; this study) and there are a number of morphological characters supporting the clade, such as 3-merous perianths (*char.* 26:1) and stamens (*char.* 29:1), perianth parts and stamens opposite each other, and outer perianth members with three or more vascular traces (Stevens, 2001).

Circaeasteraceae as sister to Lardizabalaceae has been identified in several earlier analyses (Hoot and Crane, 1995; Hoot et al., 1999; Savolainen et al., 2000a; Soltis et al., 2003; Kim et al., 2004a), but the strong support for this relationship is new here. A morphological trait linking the two is the shared presence of cellular endosperm (*char.* 59:0)

Phylogenetic analyses have found that Menispermaceae, Berberidaceae and Ranunculaceae form a clade with Berberidaceae as sister to Ranunculaceae (e.g., Hoot et al., 1999; Savolainen et al., 2000a, b; Soltis et al., 2000, 2003; Hilu et al., 2003; Kim et al., 2004a; this study). In concurrence with this relationship is the shared presence in these families of nuclear endosperm (*char.* 59:1) and benzyloquinoline alkaloids (*char.* 60:1). Berberidaceae shares additional morphological similarities with Ranunculaceae, including herbaceous habit (*char.* 1:1; i.e., wood is derived), non-tuberous rhizome present (*char.* 3:1) and outer integument four or more cells thick (*char.* 48:2).

## Intrafamilial relationships

### Papaveraceae

The monophyly of Papaveraceae sensu APG II (2003) is strongly supported in our analyses, and this is bolstered by a number of traits such as 2-merous perianths (*char.* 26:2), syncarpous gynoecia (*char.* 41:1), and parietal placentae (*char.* 45:4). Papaveraceae is also considered to be a natural group in traditional classifications, but these have differing views as to the subdivision of the group (Hutchinson, 1973; Dahlgren, 1983; Cronquist, 1988; Kubitzki et al., 1993; Takhtajan, 1997; Thorne, 2007; Wu et al., 2002), due mainly to the positions of *Pteridophyllum* and *Hypocoum*. *Pteridophyllum* differs from other Papaveraceae in lacking both idioblasts and laticifers (Friedel, 1938) and also lacking calcium oxalate crystals in the inner epidermis of the outer integument (Brückner, 1985). Previously, *Hypocoum* was placed either in Fumariaceae (Lidén, 1993) or close to Fumariaceae *s. str.* (sensu Takhtajan, 1997). Based on molecular data, Hoot et al. (1997) suggested that *Pteridophyllum* is the earliest-diverging lineage of the group (BS 54%) and that *Hypocoum* and Fumariaceae *s. str.* form a clade (BS 80%), which is consistent with the results obtained from a morphological cladistic analyses using 39 characters (Kadereit et al., 1994). Loconte et al. (1995), however, concluded that *Pteridophyllum*, *Hypocoum* and Fumariaceae *s. str.* are embedded within Papaveraceae *s. str.* (sensu Takhtajan, 1997) based on a non-molecular cladistic analysis using 109 characters.

Our results strongly support a division of Papaveraceae sensu APG II (2003) into two major clades. The first contains *Pteridophyllum*, *Hypocoum* and Fumariaceae *s. str.* (represented by *Dicentra* and *Corydalis*), which share calyx not enclosing the floral bud (*char.* 23:1) and pollen with spinose exine sculpturing (*char.* 35:3); the second comprises Papaveraceae *s. str.* Our data suggest that there is a close relationship between *Hypocoum* and *Pteridophyllum*. It is important to keep in mind, however, that we were unable to obtain material of Eschscholzioid taxa of Papaveraceae *s. str.*, which could influence basal branching in the group. *Pteridophyllum* shares some morphological similarities with *Hypocoum*, including pinnately divided leaves, four stamens, and containing only protopine alkaloids (Wang, 1965), similarities already recognized by Fedde (1909, 1936). The Papaveraceae *s. str.* clade is strongly supported, but we are unable to discuss relationships therein further here due to the relatively limited taxon sampling in that part of the tree.

### Circaeasteraceae

This family is accepted by a majority of taxonomists, but the rank and position of the monotypic genus

*Kingdonia* have long been in dispute. It was placed in Ranunculaceae (Hutchinson, 1973; Thorne, 1992; Kubitzki et al., 1993; Wu et al., 2002) or Circaeasteraceae (Cronquist, 1988), or was given familial rank and positioned close to Circaeasteraceae *s. str.* (Dahlgren, 1980, 1983; Takhtajan, 1997). We found strong support for a close relationship between *Kingdonia* and *Circaeaster*, in concordance with previous molecular results (Hoot and Crane, 1995; Oxelman and Lidén, 1995; Hoot et al., 1999; Soltis et al., 2000, 2003; Kim et al., 2004a). *Circaeaster* and *Kingdonia* share a number of morphological similarities, including open dichotomous leaf venation (*char.* 19:2), pollen with striate exine sculpturing (*char.* 35: 2), unitegmic ovules (*char.* 47:1), tenuinucellar nucelli (*char.* 51:1), and the lack of an exotesta (*char.* 57:3).

### Lardizabalaceae

Traditionally, *Sargentodoxa* has been given familial rank and considered to be closely related to Lardizabalaceae *s. str.* (Hutchinson, 1973; Dahlgren, 1980, 1983; Cronquist, 1988; Takhtajan, 1997; Wu et al., 2002). A morphological analysis by Loconte and Estes (1989) suggested a close relationship between *Sargentodoxa* and *Boquila*, which led Thorne (1992) and Wu and Kubitzki (1993) to place *Sargentodoxa* in Lardizabalaceae. Molecular data place *Sargentodoxa* as sister to Lardizabalaceae *s. str.* (Hoot et al., 1995), based upon which Thorne (2000) assigned it subfamilial rank in Lardizabalaceae. More recent morphological cladistic (Wang and Li, 2002) and molecular (Wang et al., 2002) analyses concur with the results of Loconte and Estes (1989) and Hoot et al. (1995), respectively. Here, a monophyletic Lardizabalaceae sensu APG II (2003) is strongly supported, with *Sargentodoxa* as sister to all other genera. Non-molecular attributes congruent with this topology include pollen with psilate exine sculpturing (*char.* 35:0) and lack of benzyloisoquinoline alkaloids (*char.* 60:0). However, *Sargentodoxa* differs from the other genera of Lardizabalaceae in a number of morphological aspects, such as having lateral leaves without petiolules (personal observation) (vs. with petiolules), numerous and spirally arranged carpels (*char.* 38:0, 39: 0) (vs. three and whorled), a single ovule per carpel (*char.* 46:1) (vs. several to many), smooth seed testa (*char.* 58:0) (vs. mosaic or striate), chromosome number  $n = 11$  (Shi et al., 1994) (vs.  $n = 14–18$ ; Qin, 1997), and absence of triterpenoidal saponins (vs. presence) (Zheng and Yang, 2001). Thus, recognition of two subfamilies in Lardizabalaceae (Sargentodoideae and Lardizabaloideae) as proposed by Stevens (2001) is accepted here. Within Lardizabaloideae *Decaisnea* is unique in having pinnately compound leaves (vs. ternately or palmately), perforation plates scalariform (*char.* 5:0) (vs. simple), and seed testa with mosaic sculpturing (*char.* 58:1) (vs. striate). Although strongly



supported as sister to core Lardizabalaceae (sensu Hoot et al., 1995), *Sinofranchetia* has some unique morphological characters in the family, such as long cylindrical and completely sealed carpels lacking styles (Qin, 1989). It also shares some morphological similarities with *Decaisnea*, including marginal placentae (*char.* 45:0) and lateral petiolules less than 0.5 cm long. Therefore, tribal rank for both *Decaisnea* and *Sinofranchetia* as assigned by Qin (1997), is accepted here.

### Menispermaceae

This family is characterized by dioecy (*char.* 22:2), petioles often swollen at the base (Diels, 1910; Kessler, 1993), and drupaceous fruits (*char.* 55:1) with stylar scars (Diels, 1910). The family has customarily been divided into tribes based largely on characters of the fruit (endocarp morphology) and seed (presence or absence of endosperm and rumination and embryo straight or curved), which have been shown to be homoplastic (Ortiz et al., 2007; Wang et al., 2007b). Our results support recognition of two major clades in the family: the first comprises tribes Pachygoneae and Menispermeae, which share the presence of a basal stylar scar (*char.* 44:0) and non-foliaceous cotyledons (*char.* 54:0); the second comprises tribes Tinosporeae and Fibraureae, which share foliaceous cotyledons (*char.* 54:1). This is consistent with our previous results (Wang et al., 2007b) and with those of Ortiz et al. (2007), differing from the latter only in the position of *Tinomiscium*, which they found to be sister to all other Menispermaceae. We re-examined patterns of variation along the published *ndhF* sequence from *T. petiolare* by Ortiz et al. (2007) as part of large-scale phylogenetic survey of basal angiosperms and eudicots (unpublished), and found that it is probably a PCR-based artifact representing a fusion product between a species of *Cocculus* and a species of another eudicot (excluding Ranunculales) sequences (Ortiz also confirmed that her *ndhF* sequence was wrong; personal communication). In the present study (and also in Wang et al., 2007b), *Tinomiscium* and *Tinospora* form a strongly supported clade nested within tribe Tinosporeae, which is additionally supported by two morphological characters: stylar scar terminal (*char.* 44:1) and pollen grains with distinctly elongate endoapertures (Harley, 1985). Here we recognize two subfamilies within Menispermaceae: Menispermoideae and Tinosporoideae.

### Berberidaceae

The monophyly of Berberidaceae, which has been recovered by previous studies (Kim and Jansen, 1996, 1998; Kim et al., 2004b; Wang et al., 2007a), is also strongly supported here. All members of the family share the feature of having a single ascidiate carpel (*char.* 39:1, 40:0). The three major clades recognized in the family correspond to the chromosome base numbers,  $x = 6$ , 7

and 8/10, respectively (see Wang et al., 2007a). The  $x = 10$  group (*Nandina*) as sister to the  $x = 8$  group was recovered (also by Kim et al., 2004b; Wang et al., 2007a), which concurs with their shared possession of two to four ovules (*char.* 46:2) and similar paniculate inflorescences in which the lowermost branch originates from the axil of a leaf (Nickol, 1995). Members of the  $x = 6$  group were found to share two pollen characters: striate exine sculpturing (*char.* 35:2) and discontinuous endexine (*char.* 37:0). The  $x = 7$  group shares pollen grains with unstratified ectexine (*char.* 36:0), nectariferous glands at the base of the petals, and versatile stamens (Terabayashi, 1985). Therefore, we feel that subfamilial rank for these three clades is justified.

### Ranunculaceae

Ranunculaceae sensu APG II (2003) is strongly supported as monophyletic, which is further underscored by the presence of irregular stamen arrangement (*char.* 29:0) and ovules more than two per carpel (*char.* 46:0). The combined *rbcL*, *atpB* and 18S rDNA data (Hoot et al., 1999; Soltis et al., 2000) place *Glaucidium* as sister to *Hydrastis* with 60% bootstrap and 81% jackknife support, respectively. The same three genetic regions together with 26S rDNA, however, place *Glaucidium* as sister to the other Ranunculaceae (Soltis et al., 2003; Kim et al., 2004a), a relationship that is also supported by our analyses. Ranunculaceae *s.str.* share some morphological similarities with *Hydrastis* including carpels more than three (*char.* 39:0). *Glaucidium* exhibits several unique morphological characters, by which it differs from other Ranunculaceae, such as having four petals, two carpels slightly connate below (*char.* 41:1), seeds with broad wings at the margin, absence of benzyloquinoline alkaloids (*char.* 60:0), and chromosome number of  $n = 10$ , which prompted Stevens (2001) to suggest subfamilial rank for it. *Hydrastis* differs from Ranunculaceae *s.str.* in some characters such as non-V-shaped xylem (also found in *Glaucidium*; *char.* 8:1) and a chromosome number of  $x = 13$  (*char.* 64:5). *Hydrastis* was assigned subfamilial rank by Tamura (1993), which was accepted by Ro et al. (1997) and Stevens (2001).

Based on fruit type and ovule number, Tamura (1993) divided Ranunculaceae *s.str.* into four subfamilies: Helleboroideae, Isopyroideae, Ranunculoideae, and Thalictroideae. Except for his Thalictroideae, Tamura's (1993) subfamilies are not monophyletic (Figs. 1 and 3). Based on molecular phylogenetic analyses (e.g., Hoot, 1995; Jensen, 1995b; Johansson, 1995; Kosuge et al., 1995), Jensen et al. (1995) and Ro et al. (1997) regarded cytological characters, including chromosome base number and type (T-type and R-type), as important in recognizing subfamilies within Ranunculaceae *s.str.* We retrieved three major clades defined by their basic chromosome numbers:  $x = 9$ , corresponding to Coptidoideae;  $x = 7$ , corresponding to

Thalictrioideae (= Isopyroideae; Jensen et al., 1995) (clade A); and  $x = 8$ , corresponding to Ranunculoideae of Ro et al. (1997) (clade F), although with only poor support. The study by Hoot (1995) supported a monophyletic Ranunculoideae (BS 87%), but was based on a considerably lower taxon sampling. R-type chromosomes (*char.* 65:1) is a synapomorphic character for Ranunculoideae.

Within Thalictrioideae (clade A), *Thalictrum*, with achenes, is embedded within the group with follicles and forms a clade with *Leptopyrum*, *Paropyrum* and *Paraquilegia*, which is consistent with previous studies based on molecular data (Johansson and Jansen, 1993; Johansson, 1995; Wang and Chen, 2007).

Within Ranunculoideae (clade F), four clades (B–E) were recovered. Clade B comprises *Adonis*, *Megaleranthis* and *Trollius* (also found in Ro et al., 1999). This conflicts with earlier morphological studies (e.g., Tamura, 1967, 1993; Hutchinson, 1973; Wang, 1980; Takhtajan, 1997; Wu et al., 2002), which considered that *Adonis* was close to *Callianthemum*. *Adonis* differs from *Callianthemum* in that benzyloisoquinoline alkaloids and ferulic and sinapic acids are present (*chars.* 60:1 and 63:1, respectively) (vs. absent). These two characters are shared by *Adonis* and *Trollius*. Clade C corresponds to tribe Delphinieae sensu Tamura (1993), and is consistent with clades retrieved in previous studies (Hoot, 1995; Jensen, 1995b; Johansson, 1995). Its members share zygomorphic flowers and diterpene alkaloids (*char.* 62:1). Clade D corresponds to tribe Cimicifugeae of Compton and Culham (2002). A synapomorphy for this clade is lack of benzyloisoquinoline alkaloids (*char.* 60:0). Clade E largely corresponds to Ranunculoideae sensu Tamura (1993) (excluding *Adonis* and *Kingdonia*) and includes *Callianthemum* and tribes Anemoneae DC. and Ranunculeae DC. Synapomorphies for this clade are achene fruits (*char.* 56:1) and lack of benzyloisoquinoline alkaloids (*char.* 60:0). Historically, *Callianthemum* was considered to be closely related to *Adonis* (Tamura, 1967, 1993; Wang, 1980). However, chloroplast restriction site data place *Callianthemum* as sister to *Caltha* (BS 82–40%, depending on analytical approach used; Johansson, 1995), although the two genera differ in fruit type, ovule number and phytochemistry. At the same time, *Callianthemum* is distinguished from tribes Anemoneae and Ranunculeae in having bitegmic ovules (*char.* 47:0), lack of ranunculins (*char.* 61:0), and an ovule produced laterally from each margin of the carpel and supplied with a branch from each ventral bundle (Tamura, 1995). Therefore, tribal rank for it seems justified. Tribe Ranunculeae differs from tribe Anemoneae in having a single erect ovule at the base (vs. an ascending ovule produced from the middle of the adaxial ridge) and five petals (vs. lacking petals).

The relationships among clades B–E, *Nigella*, *Helleborus*, *Caltha* and *Asteropyrum* are still not resolved. Further study sampling more genes and taxa, especially

the *Nigella* group, could help resolve these relationships. Based on morphology and chromosome numbers, Langlet (1932) gave *Nigella* tribal rank. Jensen et al. (1995) assigned both *Caltha* and *Helleborus* tribal rank based on four independent molecular studies (Hoot, 1995; Jensen, 1995b; Johansson, 1995; Kosuge et al., 1995). Tribal rank for *Asteropyrum* was supported by our previous analyses (Wang et al., 2005).

### An updated classification of Ranunculales

The present study, with a broader representation of Ranunculales, has provided more robust support for many clades and new insights into the classification of the group. Previous classification systems have been based only on morphology except Ranunculaceae, for which Jensen et al. (1995) proposed a preliminary classification including less than 30 genera based on four independent molecular data sets (Hoot, 1995; Jensen, 1995b; Johansson, 1995; Kosuge et al., 1995). Our investigation has brought to light a high degree of homoplasy within Ranunculales, which helps explain why previous classifications based on morphology alone have been found to be taxonomically unsound for the most part. An updated subfamilial and tribal classification system for Ranunculales based on both morphological and molecular evidence is presented below (Fig. 4).

Order RANUNCULALES Juss. ex Bercht. & J. Presl. (1820)

Family Eupteleaceae K. Wilh. (1910)

Family Papaveraceae Juss. (1789)

Subfamily Fumarioideae Eaton (1836)

Subfamily Papaveroideae Eaton (1836)

Family Circaeasteraceae Hutch. (1926)

Family Lardizabalaceae R. Br. (1821)

Subfamily Sargentodoxoideae Thorne and Reveal (2007)

Subfamily Lardizabaloideae Kostel. (1833)

Tribe Decaisneae Reaub. (1906)

Tribe Sinofranchetiae H. N. Qin and

Y. C. Tang (1989)

Tribe Lardizabaleae DC. (1824)

Family Menispermaceae Juss. (1789)

Subfamily Menispermoideae W. Wang and

Z. D. Chen, Subfam. nov.:

Cotyledones appressae, subcarnosae; styli cicatricibus basibus praediti. Typus: *Menispermum* Linn., Sp. Pl. 1: 340. 1753.

Subfamily Tinosporoideae W. Wang and

Z. D. Chen, Subfam. nov.:

Cotyledones foliaceae; styli cicatricibus laterallibus vel terminalibus praediti. Typus: *Tinospora* Miers, Ann. Mag. Nat. Hist. ser. 2, 7: 35. 1851.

Family Berberidaceae Juss. (1789)

Subfamily Nandinoideae Heintze (1927)

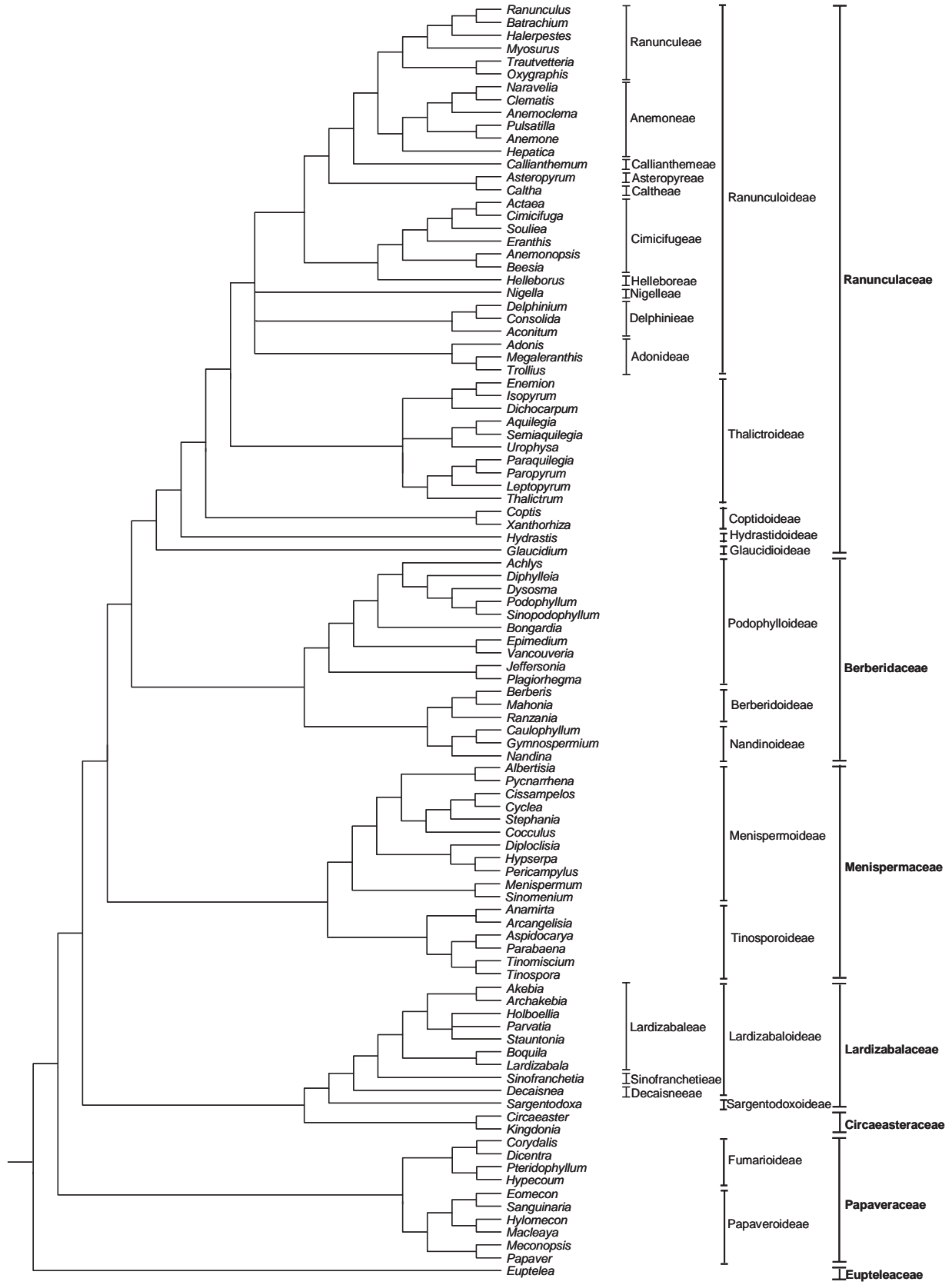


Fig. 4. Summary of a proposed updated subfamilial and tribal classification of Ranunculales based on the total evidence data set.

Subfamily Berberidoideae Kostel. (1836)  
 Subfamily Podophylloideae Eaton (1836)  
 Family Ranunculaceae Juss. (1789)  
 Subfamily Glaucidoideae (Tamura) Loconte (1995)  
 Subfamily Hydrastidoideae Engler (1903)  
 Subfamily Coptidoideae Tamura (1968)  
 Subfamily Thalictroideae Raf. (1868)  
 Subfamily Ranunculoideae Arn. (1832)  
 Tribe Adonideae Kunth (1838)  
 Tribe Delphinieae Schröd. (1909)  
 Tribe Nigelleae Schröd. (1909)  
 Tribe Helleboreae DC. (1817)  
 Tribe Cimicifugeae Torr. and A. Gray (1838)  
 Tribe Calthaeae Bercht. and J. Presl (1823)  
 Tribe Asteropyreae W. T. Wang and C. Y. Chang (1982)  
 Tribe Callianthemeae W. Wang and Z. D. Chen, Trib. nov.:  
 Plantae herbaceae perennes, haud ranunculiferae; Ovula 2, bitegminata; achenis globosis. Typus: *Callianthemum* C. A. Mey., Ledeb. Fl. Alt. 2: 336. 1830.  
 Tribe Anemoneae DC. (1817)  
 Tribe Ranunculeae DC. (1817)

## Acknowledgments

We sincerely thank Peter K. Endress, Wen-Tsai Wang and Yan-Cheng Tang for providing valuable comments and insightful suggestions, and Sara Hoot and Yin-Long Qiu for assistance in material collections. This research was supported by the National Basic Research Program of China (973 program 2007CB411601), the Knowledge Innovation Program of the Chinese Academy of Sciences no. KSCX2-YW-R-136, and National Natural Science Foundation of China Grant nos. 30800059 and 30530860.

## Appendix A. Morphological characters and character states used in this study

*Habit and anatomy:* Kubitzki et al. (1993), Barthlott and Theisen (1995), Behnke (1995), Carlquist (1995a), Takhtajan (1997), Nandi et al. (1998), Doyle and Endress (2000). Papaveraceae: Carlquist and Zona (1988); Lardizabalaceae: Carlquist (1984); Ranunculaceae: Carlquist (1995b), Tamura (1995); Berberidaceae: Carlquist (1995c); Menispermaceae: Carlquist (1996); Eupteleaceae: Li and Ren (2005).

1. Growth form: 0 = woody, 1 = herbaceous.
2. Herb: 0 = annual, 1 = perennial.
3. Rhizome: 0 = absent, 1 = present, non-tuberous, 2 = present, tuberous.

4. Epicuticular waxes: 0 = tubules, 1 = rodlets, 2 = platelets, 3 = absent. Epicuticular wax crystals in the Ranunculaceae *s. str.* (excluding *Hydrastis* and *Glaucidium*) are often absent, but platelets and tubules are sometimes present. Because the individual genera were not listed by Barthlott and Theisen (1995), we used *Ranunculus*, type genus of the family, to represent the Ranunculaceae *s. str.* and scored “023”.
5. Vessel perforations: 0 = scalariform, 1 = scalariform and simple in the same wood, 2 = simple. Ranunculaceae *s. str.*: Chen and Li (1990).
6. Vessel grouping: 0 = predominantly solitary, 1 = mostly pairs or multiples.
7. Tracheids: 0 = absent, 1 = present.
8. Xylem: 0 = V-shaped, 1 = non-V-shaped. *Hydrastis* and *Glaucidium*: Tobe and Keating (1985).
9. Paratracheal parenchyma: 0 = absent or scanty, 1 = well developed.
10. Homogeneous multiseriate rays: 0 = absent, 1 = present.
11. Rays: 0 = narrow (generally not more than four cell layers wide), 1 = wide.
12. Storied structure: 0 = absent, 1 = present.
13. Vessel side pitting: 0 = circular, only one or two rows, 1 = scalariform or transitional, 2 = opposite, 3 = alternate.
14. Sieve tube plastids: 0 = S-type, 1 = P-type.
15. Nodal anatomy: 0 = multilacunar, 1 = unilacunar, 2 = trilacunar, 3 = pentalacunar.  
*Leaf:* Kubitzki et al. (1993), Takhtajan (1997), Nandi et al. (1998), Doyle and Endress (2000), Wang and Li (2002).
16. Stipules: 0 = absent, 1 = present.
17. Leaf position: 0 = caulescent only, 1 = basal only, 2 = basal and caulescent.
18. Leaf arrangement: 0 = alternate, 1 = opposite, 2 = whorled, 3 = spiral, 4 = sub-decussate.
19. Major venation: 0 = pinnate, 1 = palmate, 2 = dichotomous, 3 = parallel.
20. Blade shape: 0 = obovate to elliptical to oblong, 1 = ovate, 2 = linear. These states were determined according to Doyle and Endress (2000).  
*Inflorescence and perianth:* Kubitzki et al. (1993), Takhtajan (1997), Doyle and Endress (2000), Wang and Li (2002). Ranunculaceae: Tamura (1995).
21. Inflorescence: 0 = solitary or occasionally with one to two additional later flowers, 1 = spike, raceme, or botryoid, 2 = richly branched (panicle or compound inflorescence or spikes, racemes, botryoids).
22. Sex: 0 = monoclinal, 1 = monoecious, 2 = dioecious.
23. Calyx: 0 = enclosing bud, 1 = not enclosing bud. Papaveraceae: Kadereit et al. (1994).
24. Perianth phyllotaxy: 0 = spiral, 1 = whorled, 2 = irregular.



25. Perianth whorl (series when phyllotaxy is spiral): 0 = more than two, 1 = two, 2 = one, 3 = absent.
26. Perianth arrangement (merosity): 0 = irregular, 1 = in threes, 2 = in twos or fours, 3 = in fives.
27. Nectar petals: 0 = absent, 1 = present.  
*Androecium and Pollen*: Loconte and Estes (1989), Kubitzki et al. (1993), Takhtajan (1997), Blackmore et al. (1995), Nandi et al. (1998), Wang and Li (2002). *Circaeasteraceae* and *Sargentodoxa*: Nowicke and Skvarla, 1982; *Ranunculaceae*: Hiepko (1995); *Hydrastis* and *Glaucidium*: Tobe and Keating (1985).
28. Androecium phyllotaxy: 0 = spiral, 1 = whorled, 2 = irregular.
29. Stamen arrangement: 0 = irregular, 1 = in threes, 2 = in twos, fours, or fives.
30. Stamen fusion: 0 = free, 1 = connate.
31. Connective apex: 0 = extended, 1 = truncated or smoothly rounded.
32. Orientation of dehiscence: 0 = introse, 1 = latrorse, 2 = extrorse.
33. Pollen aperture type: 0 = sulcate, 1 = colpate, 2 = porate, 3 = colporate, 4 = inaperturate, 5 = irregular.
34. Pollen aperture number: 0 = one, 1 = three, 2 = more than three, 3 = two.
35. Exine sculpturing: 0 = psilate or granulate, 1 = reticulate, 2 = striate, 3 = spinose.
36. Pollen ectexine: 0 = undifferentiated, 1 = differentiated into foot layer.
37. Pollen endexine: 0 = discontinuous, 1 = continuous.  
*Gynoecium*: Kubitzki et al. (1993), Takhtajan (1997), Endress and Igersheim (1999), Doyle and Endress (2000).
38. Gynoecium phyllotaxy: 0 = spiral, 1 = whorled, 2 = irregular.
39. Carpel number: 0 = more than 3, 1 = one, 2 = two, 3 = three.
40. Carpel form: 0 = ascidiate up to stigma, 1 = intermediate (both plicate and ascidiate zones present below the stigma) with ovule(s) on the ascidiate zone, 2 = completely plicate or intermediate with some or all ovule(s) on the plicate zone.
41. Carpel fusion: 0 = apocarpous (including pseudo-syncarpous), 1 = syncarpous (including at least basally).  
*Embryology*: Corner (1976), Takhtajan (1997), Igersheim and Endress (1997, 1998), Nandi et al. (1998), Endress and Igersheim (1999), Doyle and Endress (2000), Wang and Li (2002). *Acorus*: Igersheim et al. (2001); *Hydrastis* and *Glaucidium*: Tobe and Keating (1985); *Kingdonia*: Ren et al. (1998).
42. Pollen tube transmitting tissue: 0 = not prominently differentiated, 1 = one layer prominently differentiated, 2 = more than one layer differentiated.
43. Tanniferous tissue in the carpel wall: 0 = absent, 1 = present.
44. Styler scar: 0 = basal, 1 = terminal, 2 = lateral, 3 = ventral and terminal. *Menispermaceae*: Diels (1910), Kessler (1993).
45. Placentation type: 0 = marginal, 1 = laminar, 2 = basal, 4 = parietal.
46. Ovule number: 0 = more than two, 1 = one, 2 = mostly two (occasionally one or a few more than two). *Ranunculaceae*: Tamura (1995). These states were determined according to Doyle and Endress (2000).
47. Integument number: 0 = bitegmic, 1 = unitegmic.
48. Outer integument thickness: 0 = two cells, 1 = two and three to four, 2 = four and five, or more.
49. Inner integument thickness: 0 = two cells, 1 = two and three, or three, 2 = three and more.
50. Micropyle type: 0 = formed from both integuments, 1 = formed from inner integument only.
51. Nucellus: 0 = crassinucellar (including weakly so), 1 = tenuinucellar or pseudocrassinucellar.
52. Tanniferous tissue in the ovules: 0 = lacking, 1 = present.
53. Embryogeny: 0 = Onagrad, 1 = Caryophyllad, 2 = Asterad, 3 = Solanad, 4 = irregular.
54. Cotyledons: 0 = not foliaceous, 1 = foliaceous. *Menispermaceae*: Diels (1910), Kessler (1993). *Fruits and seeds*: Corner (1976), Takhtajan (1997), Doyle and Endress (2000), Wang and Li (2002). *Lardizabalaceae*: Qin (1997); *Ranunculaceae*: Tamura (1995); *Circaeaster*: Hu et al. (1990); *Kingdonia*: Hu and Tian (1985).
55. Fruit wall: 0 = fleshy, 1 = fleshy with hard endocarp (= drupe), 2 = dry.
56. Fruit dehiscence: 0 = dehiscent, 1 = indehiscent.
57. Exotesta: 0 = unspecialized, 1 = palisade or shorter sclerotic cells, 2 = tabular (wider than underlying cells), 3 = absent.
58. Testa sculpture: 0 = smooth, 1 = mosaic, 2 = striate. *Lardizabalaceae*: Xia and Peng (1989).
59. Endosperm development: 0 = cellular, 1 = nuclear, 2 = holobial.  
*Chemical compounds*: Loconte et al. (1995), Nandi et al. (1998).
60. Benzyloquinolines: 0 = absent (including rarely so), 1 = present. The monophyletic origin of benzyloquinoline alkaloids has been confirmed (Liscombe et al., 2005, and references therein). Basal angiosperms: Wang (1965); *Ranunculales*: Zhu and Xiao (1991), Jensen (1995a); other angiosperms: Takhtajan (1997).
61. Ranunculins: 0 = absent, 1 = present. *Ranunculaceae*: Ruijgrok (1966), Jensen (1995a). Ruijgrok (1966) used gas chromatography to investigate 24 genera of *Ranunculaceae*, and his results



indicated that Ranunculins are only present in tribes Anemoneae and Ranunculeae.

62. Diterpene alkaloids: 0 = absent, 1 = present. Ranunculaceae: Xiao (1980), Jensen (1995a). Xiao (1980) used gas chromatography to identify the compounds of 26 genera of Ranunculaceae, and his results indicated that Diterpene alkaloids were the characteristic of Delphinieae (including three genera) and are absent in other Ranunculaceae.
63. Ferulic + sinapic acid: 0 = absent (including rarely so), 1 = present. Ranunculaceae: Based on Jensen

(1995a) (including 26 genera), Ferulic + sinapic acid is present in *Trollius* and *Adonis* in large mounts, in *Thalictrum* in small amounts, but absent in other genera.

Cytology

64. Chromosome basic number (x): 0 = eight, 1 = seven, 2 = nine, 3 = six, 4 = five, 5 = more than nine. Berberidaceae: Loconte and Estes (1989); Ranunculaceae: Tamura (1993).
65. Chromosome type: 0 = T(halictrum)-type, 1 = R(anunculus)-type. *Asteropyrum*: Yang et al. (1993); other Ranunculaceae: Tamura (1993).

## Appendix B

Data matrix of 65 morphological characters. Inapplicable states are coded with a dash (-) and polymorphic taxa with letters: *a* = 0/1; *b* = 0/2; *c* = 0/3; *d* = 0/4; *e* = 0/1/2; *f* = 0/1/3/ *g* = 0/2/3; *h* = 1/2; *i* = 1/2/3; *j* = 1/3; *k* = 2/3; ? = missing data.

Taxon	Character number						
	0000000001	1111111112	2222222223	3333333334	4444444445	5555555556	66666
<i>Taxon</i>	1234567890	1234567890	1234567890	1234567890	1234567890	1234567890	12345
<i>Achlys</i>	111?2?????	???0??1-11	10?-310110	1?11210-10	-???210210	???21??10	?0?3?
<i>Aconitum</i>	1ah?h?????	???0?02011	00?0001000	1111c1??c?	0???00?211	a?0?20??11	01101
<i>Acorus</i>	110?0?0?0?	???1011032	10?1110110	10001?1?131	111??00101	111?000?00	?????
<i>Actaea</i>	111?1?????	???0000211	h0?0000000	1111c1??1?	0???000211	a?0?011?10	00001
<i>Adonis</i>	110?1?????	???0002011	00?0000000	1111?10002	0???010211	a??211?11	00101
<i>Aextoxicon</i>	0-?0?1?10	?01??00000	22?1130?ba	00j101?12?	1???20111	0???21????	?????
<i>Akebia</i>	0-0200?01	1130200001	1h?1010110	0211010132	010?100111	002?001200	?????
<i>Albertisia</i>	0-???????	?????00001	22?1010111	1?1111?10?	0???002????	???011?11	?????
<i>Altingia</i>	0-?0?1?10	?030210000	h1?-3-0120	01bb11?122	111??h002a	01??210?00	?????
<i>Amborella</i>	0-?0?1??0	0000200001	h1?0000000	00000?0000	001??10111	01??100?0?	?????
<i>Anamirta</i>	0-?200?00	113?200011	22?1010??1	1?3111?13?	0??202????	???111?11	?????
<i>Anemoclema</i>	111?1?????	?????02201	10?0030000	0???3??0?	0???01????	???21??10	?0?01
<i>Anemone</i>	11a?h?0??	???0b02h11	e0?0000000	1abb31?002	0???011—	1?c?211?10	10001
<i>Anemonopsis</i>	111???????	?????002011	10?0001000	1011c1??0?	0???000211	a??20??10	00001
<i>Annona</i>	0-?b10?a0	1011200c00	00?1010000	02000??h02	011??h0211	000?ba0?01	?????
<i>Aquilegia</i>	111?h?0??	???0b02011	10?1031000	111131?102	0???000211	a?d?201?11	00010
<i>Arcangelisia</i>	0-???????	?????00011	22?1010111	1?3111?13?	0??202????	???111?11	?????
<i>Archakebia</i>	0-???????	?????200001	1h?1010110	021101013?	0???100???	???00?200	?????
<i>Aspidocarya</i>	0-???????	?????00011	22?1010111	1?3111?13?	0??102????	???111?11	?????
<i>Asteropyrum</i>	110?0?????	?????02h11	00?0001000	11h2?1??0?	0???000???	1??20??11	?0001
<i>Austrobaileya</i>	0-?001??0	00i0200100	10?0000000	00001?0000	011??00211	01??000?0?	?????
<i>Batrachium</i>	1a0?2?????	?????02011	00?0001000	1??????0?	0???01????	???21??10	?0?01
<i>Beesia</i>	111?1?????	?????02011	h0?0030000	111131??1?	0???00????	a??20??10	?0?01
<i>Berberis</i>	0-0210001	1130200001	20?1011110	1?5-001-10	-10?200210	000?011?11	?0?1?
<i>Bongardia</i>	112?2?????	???0??1-01	h0?1011110	1?11210-10	-???200210	???20??10	?0?3?
<i>Boquila</i>	0-?2a1?01	113?200001	0h?1011111	021101013?	0???100???	???01?200	?????
<i>Brasenia</i>	111?0?1???	???0100e10	00?1110110	1b000??100	000??h0101	00??201?20	?????
<i>Buxus</i>	0-?001?00	00h1100000	11?1120120	002211?1k2	1?0??20211	000?ba1?00	?????
<i>Callianthemum</i>	111?1?????	???0?0h011	00?0001000	11??31?002	0???020211	a??21??10	00001

<i>Caltha</i>	11a?2??0??	??0202011	e0?0000000	11ih310?0?	0??00?211	a?0?201?11	00001
<i>Calycanthus</i>	0–1211?00	001110010a	00?0000000	02031??002	?01??20221	0a??200?01	?????
<i>Canella</i>	0–0001?a0	0021200000	h0?10101a1	12001??102	111??b0110	01??001??0	?????
<i>Caulophyllum</i>	11102?????	??0?000a1	20?1011110	1?11111-10	-10?2220210	000?00??11	?0?0?
<i>Ceratophyllum</i>	110?–??0?	?0?0?10010	01?-3-0000	0h4-0??-11	-01??11—	002?210?00	?????
<i>Cimicifuga</i>	111?1?????	??0002011	20?000a000	1111c1??02	010?000211	a00?20??10	00001
<i>Circaeaster</i>	10-01?????	??0100421	20?0000000	10112100i2	001?021—	1?1?213-0?	?????
<i>Cissampelos</i>	0–?201?01	113?200011	22?1010??1	1?3111?-1?	-??0021—	0?00112?11	?????
<i>Clematis</i>	0–?210011	1130200111	b0?0000000	aabb?1??02	0??011—	1?0?21??10	10001
<i>Cocculus</i>	0–?200?01	1130200011	22?1010110	1?3111?132	0110020101	0000112?11	?????
<i>Consolida</i>	10-?2?????	??0?02011	10?0001000	1111c1?11?	0??00?211	a??20??11	01101
<i>Coptis</i>	111?1?????	????202-11	b0?00c1000	112231?002	0??000211	a?0?20??11	00020
<i>Coriaria</i>	0–32?0?00	?120?10110	1a?1130120	00ji01?10?	a????10111	0??211?10	?????
<i>Cornus</i>	0–?0?1?00	?011210a10	20?11k0120	1?3101?10?	1????11—	1??11??a1	?????
<i>Corydalis</i>	1a10??????	??0102301	1011020120	1?1h31?12?	1??400110	0?1?201?11	?????
<i>Cyclea</i>	0–???????	????000011	22?1010??1	1?3111?-1?	-??002????	??011??11	?????
<i>Decaisnea</i>	0–?000?00	1110200001	ha?1011111	0211010132	010?000211	002?001100	?????
<i>Delphinium</i>	1a0?110011	1130?02011	10?0001000	1111c1?1c2	010?00?211	a00?20??11	01101
<i>Dicentra</i>	111???????	??0100301	1011020120	1?h31?122	110?400110	001?201?11	?????
<i>Dichocarpum</i>	111?h?????	?????01-11	10?0031000	111h31??2?	1??00?0???	??220??11	?0?30
<i>Diphylleia</i>	111?2?????	??0?000011	20?1010110	1?11?10-10	-??000210	?0?01??10	?0?3?
<i>Diploclisia</i>	0–???????	????000011	22?1010110	1?3111?13?	0??002????	??011??11	?????
<i>Dysosma</i>	112?2??0??	?????00a11	e0?1010110	1?11210-10	-??000210	?0?01??10	?0?3?
<i>Enemion</i>	11a?1?????	?????02011	10?0030000	1a1131??0?	0??00?0???	??220??11	00010
<i>Eomecon</i>	111???????	??0101011	2001020100	1?2211?12?	1??400???	??220??11	?????
<i>Epimedium</i>	111b210001	1130?02-11	h0?1021120	1?11210-10	-??000210	0??201?11	?0?3?
<i>Eranthis</i>	112?2?????	??0?02211	00?0001000	1a??10?0?	0??000211	a?0?20??11	00001
<i>Euptelea</i>	0–?001?00	10i0100000	00?-3-0100	011h11?001	001?0h0210	01j?212?00	?????
<i>Glaucidium</i>	1112110111	103?002011	00?1?20000	1?11c10?22	1??000221	1?0?20?10	?0?0?
<i>Grevillea</i>	0–?h11?a0	10202000a0	10?1120120	aa2i11?-12	-11??h0011	012?h1a?a0	?????
<i>Gymnospermium</i>	112?2?????	?????10011	10?1011110	1?11111-10	-??220210	0??20??11	?0?0?
<i>Halerpestes</i>	110?h?????	?????02011	a0?0001000	111131??0?	0??01????	??221??10	?0?01
<i>Hamamelis</i>	0–30?1?10	?030210000	h0?11k0120	01h111?122	111??h021a	011?210?00	?????
<i>Hedyosmum</i>	0–?a01?00	1000110100	0h?-310–0	00031??-10	-00??10111	00a?a00?00	?????
<i>Helleborus</i>	11a?110011	1130?0b011	b0?0001000	1a??110?02	1??00a211	a??201?11	10001
<i>Hepatica</i>	111?h?????	??0?02211	00?0000000	1a1b?1?0?	0??011—	1?j?21??10	10001
<i>Holboellia</i>	0–?200?01	1130200001	1h?101111a	0211010132	0??100111	0?2?01?200	?????
<i>Hydrastis</i>	1112110111	1130002011	00?1?10000	1a11210?02	010?020210	00?0a1?11	?0?50
<i>Hylomecon</i>	111???????	??0?0h011	1001020100	1?1111?12?	1??400010	??2201?11	?????
<i>Hypocoum</i>	10-0???????	??0101-01	1011020120	121331?122	110?400110	001?201?11	?????
<i>Hypserpa</i>	0–?201?01	113?200011	22??000??a	1?3111?002	01?002????	00?011??11	?????
<i>Illicium</i>	0–?a01?00	00h0100000	00?000000a	1b031??00a	011??a0211	012?ba1?00	?????
<i>Isopyrum</i>	111?1?????	????202011	20?0031000	111131??i?	0??000211	a?3?20??11	00010
<i>Jeffersonia</i>	111?211010	113??1-11	00?2001200	1?11210-10	-10?000210	00?201?11	?0?3?
<i>Kingdonia</i>	111?1?????	??0101-21	00?0030000	1h11210002	0??011—	1?1?213-0?	?????
<i>Lardizabala</i>	0–?201?00	113?210001	a2?1011111	021101013?	0??100111	0?2?01?200	?????
<i>Leptopyrum</i>	10-?2?????	?????02a11	20?0031000	111131??0?	010?001—	a0??20??11	00010
<i>Macleaya</i>	110???????	??0?02011	2001020100	1?2211?12?	1??410???	??2201?11	?????
<i>Magnolia</i>	0–1a11?00	001a0?0300	00?10a0000	0b000??002	00a??10210	0a0?2a0?01	?????
<i>Mahonia</i>	0–?210001	1130200001	20?1011110	1?5-001-10	-??000210	0?0?011?11	?0?1?
<i>Meconopsis</i>	110???????	??0?01001	e001020100	1?1111?10?	1??400???	??220??11	?????
<i>Megaleranthis</i>	111???????	?????02-11	00?0001000	11??2?????	0??00?0???	??220??1?	?0?01
<i>Meliosma</i>	0–3h?1?00	?010?00000	3e?10h0120	0?3111??2?	1????21—	0??11??2?	?????
<i>Menispermum</i>	0–0???????	??0200011	22?1010110	1?3111?132	01?002????	00?011??11	?????
<i>Myosurus</i>	10-???????	??0?02011	10?0001000	11??????02	0??011—	1?0?21?10	10001

<i>Nandina</i>	0-0211001	1130?00001	20?1010110	1?11011-10	-10?020210	000?01??11	?0?4?
<i>Naravelia</i>	0-???????	?????00111	b0?0000000	00??3??0?	0??01?????	???21??1?	?0?01
<i>Nehumbo</i>	11200?1???	?20?10?11	00?00b0000	0b1101?200	001??10221	012?210?11	?????
<i>Nigella</i>	10-?2?????	?20?02011	00?0001000	12?????0?	1??000211	a?3?201?11	00131
<i>Oxygraphis</i>	110?1?????	?????01-11	00?0001000	11??31?002	0??011—	???21??10	?0?01
<i>Paeonia</i>	a1h10?1?00	0030300001	00?0030001	12jh11?10?	0??00210	0??201?10	?????
<i>Papaver</i>	1a00211?11	1130?01001	0001020100	1?1111?10?	1??400110	0?3?201?11	?????
<i>Parabaena</i>	0-???????	?????00011	22?1010111	1?3111?13?	0??102????	??1111?11	?????
<i>Paraquilegia</i>	111?1?????	?????202a11	10?0031000	111131??0?	0??000???	???20??11	?0?10
<i>Paropyrum</i>	111???????	?????202a11	20?0031000	111131??0?	0??000???	???20??1?	?0?10
<i>Parvatia</i>	0-???????	?????200001	11?1011111	0211010132	0??100???	?2?01?200	?????
<i>Pericampylus</i>	0-???????	?????00011	22?101011a	1?3111?13?	0??002????	??0111?11	?????
<i>Plagiorhegma</i>	111?2?????	?20?01-11	00?1011110	1?11210-10	-??000210	?2?20??11	?0?3?
<i>Platanus</i>	0-0a01?01	1010010010	10?1hh0120	011111?102	011??h0121	01??2a0?10	?????
<i>Podophyllum</i>	11112?????	?????00111	00?1010110	1?11210-10	-??000210	0?0?011?10	?0?3?
<i>Pteridophyllum</i>	1112???????	?????01-01	1011020120	1?1i31?12?	1??420???	???20??11	?????
<i>Pulsatilla</i>	111?2?????	?????02211	00?0000000	1abb31?002	0??011—	1?0?21??10	10001
<i>Pycnarrhena</i>	0-?201?01	113?200001	22?1010111	1?3111?13?	0??302????	??0111?11	?????
<i>Ranunculus</i>	1aagh?????	?20?0b02011	b0?0001000	1bbbc????02	010?011—	100?211?10	10001
<i>Ranzania</i>	111?2?????	?????0-11	00?1011110	1??001-10	-??000210	0??01??1?	?0?1?
<i>Sabia</i>	0-3h1?00	?010?00001	ja?10h0120	?b3111?122	1?1??21—	01??11??2?	?????
<i>Sanguinaria</i>	111???????	?20?01011	0001020100	1?2211?12?	1??400210	0?3?201?11	?????
<i>Sargentodoxa</i>	0-?2?????	?20?020001	11?1011110	021101000?	0??010111	0??01?000	?????
<i>Saruma</i>	110111?00	1111100011	00?121?111	02d00??102	100??00011	003?210?01	?????
<i>Saururus</i>	110?01??0	1?10010011	10?-3-0110	11000??102	101??b0010	012?ba0?00	?????
<i>Semiaquilegia</i>	112?1?????	?20?02011	10?0031000	111131??0?	0??000???	???20??11	00010
<i>Sinofranchetia</i>	0-?200?00	1130200001	1h?1011110	0211010132	010?000111	002?01?200	?????
<i>Sinomenium</i>	0-???????	?20?020001	22?1010110	1?3111?13?	0??002????	??0111?11	?????
<i>Sinopodophyllum</i>	111?2?????	?20?00-11	00?1010110	1?11?10-10	-??000210	0?0?011?10	?0?3?
<i>Souliea</i>	111?1?????	?????02011	10?0001000	115?c1??i?	0??000???	a??20??10	?0?01
<i>Stauntonia</i>	0-?200?01	1130200001	1h?1011111	0211010132	0??100111	0?2?01?200	?????
<i>Stephania</i>	0-???????	?20?020001	22?1010??1	1?2111?-12	-??0020?-	0?00112?11	?????
<i>Takhtajania</i>	0-?0?1??0	102a200000	h0?10k0100	12001??eb2	011??0011a	01??001?00	?????
<i>Tetracentron</i>	0-?0?1??0	1010210001	10?1120120	111111?102	11??00021	010?212?10	?????
<i>Tetracera</i>	0-?a?1?10	?010e10000	e0?1130?01	0?jh11?10?	a????001h0	0?0?202?10	?????
<i>Thalictrum</i>	111?h10011	1130002e11	20?0000000	112231?002	010?010211	a?0?21??11	00020
<i>Tinomiscium</i>	0-???????	?????00011	22?1010110	1?1111?13?	0??102????	??1111?11	?????
<i>Tinospora</i>	0-?200?01	1130200011	22?1010110	1?1111?13?	0??1021—	0?01112?11	?????
<i>Trautvetteria</i>	111???????	?????002011	20?0001000	1111?1??0?	0??001—	1??21??10	10001
<i>Trochodendron</i>	0-?0?1??0	1010a10001	10?1120000	111111?002	111??00021	011?212?00	?????
<i>Trollius</i>	111?1?????	?20?02011	e0?0001000	111121??0?	0??000211	a?4?20??11	00101
<i>Urophyssa</i>	111???????	?????02011	10?0031000	111131??0?	0??000???	???20??1?	?0?10
<i>Vancouveria</i>	111?2?????	?20?0?1-11	h0?1011110	1?11210-10	-??000210	0??201?10	?0?3?
<i>Vitis</i>	0-?2?0?10	?11111001a	20?11k012a	003111?10?	1??h0211	0?2?111?10	?????
<i>Xanthorhiza</i>	0-?210000	113?00?01	b0?1031120	102?310?02	010?020211	a00?20??11	00020

## References

- APG, 1998. An ordinal classification for the families of flowering plants. *Ann. Missouri Bot. Gard.* 85, 531–553.
- APG II, 2003. An update of the Angiosperm Phylogeny group classification for the orders and families of flowering plants: APG II. *Bot. J. Linn. Soc.* 141, 399–436.
- Barthlott, W., Theisen, I., 1995. Epicuticular wax ultrastructure and classification of Ranunculiflorae. *Plant Syst. Evol. (Suppl.)* 9, 39–45.
- Behnke, H.D., 1995. Sieve-element plastids, phloem proteins, and the evolution of the Ranunculanae. *Plant Syst. Evol. (Suppl.)* 9, 25–37.
- Blackmore, S., Stafford, P., Persson, V., 1995. Palynology and systematics of Ranunculiflorae. *Plant Syst. Evol. (Suppl.)* 9, 71–82.

- Borsch, T., Hilu, K.W., Quandt, D., Wilde, V., Neinhuis, C., Barthlott, W., 2003. Noncoding plastid *trnT-trnF* sequences reveal a well resolved phylogeny of basal angiosperms. *J. Evol. Biol.* 16, 558–576.
- Brückner, C., 1985. Frucht und amenanatomie von *Pteridophyllum racemosum* Sieb. et. Zucc. Bull. und die position der monotypischen Gattung in den Papaverales. Feddes Reperit 96, 199–213.
- Carlquist, S., 1984. Wood and stem anatomy of Lardizabalaceae, with comments on the vining habit, ecology and systematics. *Bot. J. Linn. Soc.* 88, 253–277.
- Carlquist, S., 1995a. Wood anatomy of Ranunculiflorae: a summary. *Plant Syst. Evol. (Suppl.)* 9, 11–24.
- Carlquist, S., 1995b. Wood and bark anatomy of Ranunculaceae (including *Hydrastis*) and Glaucidiaceae. *Aliso* 14, 65–103.
- Carlquist, S., 1995c. Wood anatomy of Berberidaceae: ecology and phylogenetic considerations. *Aliso* 14, 85–103.
- Carlquist, S., 1996. Wood and stem anatomy of Menispermaceae. *Aliso* 14, 155–170.
- Carlquist, S., Zona, S., 1988. Wood anatomy of Papaveraceae, with comments on vessel restriction patterns. *IAWA Bull.* 9, 253–267.
- Chase, M.W., 2004. Monocot relationships: an overview. *Am. J. Bot.* 91, 1645–1655.
- Chase, M.W., Soltis, D.E., Olmstead, R.G., Morgan, D., Les, D.H., Mishler, B.D., Duvall, M.R., Price, R.A., Hillis, H.G., Qiu, G.Y.Y., Kron, K.A., Rettig, J.H., Conti, E., Palmer, J., Manhart, J.R., Sytsma, K.J., Michaels, H.J., Kress, W.J., Karol, K.G., Clark, W.D., Hedren, M., Gaut, B.S., Jansen, R.K., Kim, K.J., Wimpee, C.F., Smith, J.F., Furnier, G.R., Strauss, S.H., Xiang, Q.Y., Plunkett, G.M., Soltis, P.S., Swensen, S.M., Williams, S.E., Gadek, P.A., Quinn, C.J., Eguarte, L.E., Golenberg, E., Learn, G.H.J., Graham, S.W., Barret, S.C.H., Dayanandan, S., Albert, V.A., 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Ann. Missouri Bot. Gard.* 80, 528–580.
- Chen, Y.Z., Li, Z.L., 1990. Comparative studies of perforation plate structures of vessels in Ranunculaceae. *Acta Bot. Sinica* 32, 245–251.
- Chen, Z.D., Wang, X.Q., Sun, H.Y., Han, Y., Zhang, Z.X., Zou, Y.P., Lu, A.M., 1998. Systematic position of the Rhoipteleaceae: evidence from nucleotide sequences of the *rbcL* gene. *Acta Phytotax. Sinica* 36, 1–7.
- Chippindale, P.T., Wiens, J.J., 1994. Weighting, partitioning, and combining characters in phylogenetic analysis. *Syst. Biol.* 43, 278–287.
- Compton, J.A., Culham, A., 2002. Phylogeny and circumscription of tribe Actaeae (Ranunculaceae). *Syst. Bot.* 27, 502–511.
- Cognato, A.I., Vogler, A.P., 2001. Exploring data interaction and nucleotide alignments in a multiple gene analysis of *Ips* (Coleoptera: Scolytinae). *Syst. Biol.* 50, 758–780.
- Corner, E.J.H., 1976. *The Seeds of Dicotyledons*. Cambridge University Press, Cambridge.
- Cronquist, A., 1981. *An Integrated System of Classification of the Flowering Plants*. Columbia University Press, New York.
- Cronquist, A., 1988. *The Evolution and Classification of Flowering Plants*, second ed. New York Botanical Garden, New York.
- Dahlgren, G., 1980. A revised system of classification of the angiosperms. *Bot. J. Linn. Soc.* 80, 91–124.
- Dahlgren, R., 1983. General aspects of angiosperm evolution and macro-systematics. *Nord. J. Bot.* 3, 119–149.
- Diels, L., 1910. Menispermaceae. In: Engler, A. (Ed.), *Das Pflanzenreich IV*, 94. Engelmann, Leipzig.
- Doyle, J.A., Endress, P.K., 2000. Morphological phylogenetic analysis of basal angiosperms: comparison and combination with molecular data. *Int. J. Plant Sci.* 161, S121–S153.
- Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf material. *Phytochem. Bull.* 19, 11–15.
- Endress, P.K., 1986. Floral structure, systematics, and phylogeny in Trochodendrales. *Ann. Missouri Bot. Gard.* 73, 297–324.
- Endress, P.K., 1993. Eupteleaceae. In: Kubitzki, K., Rohwer, J.G., Bittrich, V. (Eds.), *The Families and Genera of Vascular Plants II*. Springer, Berlin, pp. 299–301.
- Endress, P.K., Igersheim, A., 1999. Gynoecium diversity and systematics of the basal eudicots. *Bot. J. Linn. Soc.* 130, 305–393.
- Fedde, F., 1909. Papaveraceae–Hypecoideae et Papaveraeae–Papaveroideae. In: Engler, A. (Ed.), *Das Pflanzenreich IV*, Vol. 104. Engelmann, Leipzig, pp. 1–430.
- Fedde, F., 1936. Papaveraceae. In: Engler, A., Harms, H. (Eds.), *Die natürlichen Pflanzenfamilien 17b*, second ed. Engelmann, Leipzig, pp. 5–145.
- Friedel, J., 1938. Note sur la structure anatomique de *Pteridophyllum racemosum* Sieb. et. Zucc. *Bull. Soc. Bot.* 85, 406–408.
- Hall, T.A. (Ed.), 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41, 95–98.
- Harley, M.M., 1985. Pollen morphology and taxonomy of the tribe Fibraureae (Menispermaceae). *Kew Bull.* 40, 553–565.
- Hiepko, P. (Ed.), 1995. Ranunculaceae. In: *Natürl. Pflanzenfam II*. 17a (4). Duncker and Humblot, Berlin.
- Hilu, K.W., Borsch, T., Müller, K., Soltis, D.E., Soltis, P.S., Savolainen, V., Chase, M.W., Powell, M.P., Alice, L.A., Evans, R., Sauquet, H., Neinhuis, C., Slotta, T.A.B., Rohwer, J.G., Campbell, C.S., Chatrou, L.W., 2003. Angiosperm phylogeny based on *matK* sequence information. *Am. J. Bot.* 90, 1758–1776.
- Hoot, S.B., 1995. Phylogeny of the Ranunculaceae based on *atpB*, *rbcL* and 18S nuclear ribosomal DNA sequence data. *Plant Syst. Evol. (Suppl.)* 9, 241–251.
- Hoot, S.B., Crane, P.R., 1995. Interfamilial relationships in the Ranunculidae based on molecular systematics. *Plant Syst. Evol. (Suppl.)* 9, 119–131.
- Hoot, S.B., Culham, A., Crane, P.R., 1995. Phylogenetic relationships of the Lardizabalaceae and Sargentodoxaceae: chloroplast and nuclear DNA sequence evidence. *Plant Syst. Evol. (Suppl.)* 9, 195–199.
- Hoot, S.B., Kadereit, J.W., Blattner, F.R., Jork, K.B., Schwarzbach, A.E., Crane, P.R., 1997. Data congruence and phylogeny of the Papaveraceae *s.l.* based on four data

- sets: *atpB* and *rbcL* sequences, *trnK* restriction sites, and morphological characters. *Syst. Bot.* 22, 575–590.
- Hoot, S.B., Magallón, S., Crane, P.R., 1999. Phylogeny of basal eudicots based on three molecular data sets: *atpB*, *rbcL*, and 18S nuclear ribosomal DNA sequences. *Ann. Missouri Bot. Gard.* 86, 1–32.
- Hu, Z.H., Tian, L.X., 1985. Studies on morphology of *Kingdonia uniflora* F. Balfour et W W Smith III. The morphology and anatomy of flowers, fruits and seeds. *Acta Phytotax. Sinica* 23, 170–178.
- Hu, Z.H., Yang, J., Jing, R.Q., Dong, Z.M., 1990. Morphology studies on *Circaea agrestis* II. Morphology and anatomy of flower, fruit and seed. *Cathaya* 2, 77–88.
- Hutchinson, J., 1973. The Families of Flowering Plants Arranged According to a New System Based on their Probable Phylogeny. Clarendon Press, Oxford.
- Igersheim, A., Buzgo, M., Endress, P.K., 2001. Gynoecium diversity and systematics in basal monocots. *Bot. J. Linn. Soc.* 136, 1–65.
- Igersheim, A., Endress, P.K., 1997. Gynoecium diversity and systematics of the Magnoliales and winteroids. *Bot. J. Linn. Soc.* 124, 213–271.
- Igersheim, A., Endress, P.K., 1998. Gynoecium diversity and systematics of the paleoherbs. *Bot. J. Linn. Soc.* 127, 289–370.
- Jensen, U., 1995a. Secondary compounds of the Ranunculiflorae. *Plant Syst. Evol. (Suppl.)* 9, 85–97.
- Jensen, U., 1995b. Serological legumin data and the phylogeny of the Ranunculaceae. *Plant Syst. Evol. (Suppl.)* 9, 217–227.
- Jensen, U., Hoot, S.B., Johansson, J.T., Kosuge, K., 1995. Systematics and phylogeny of the Ranunculaceae—a revised family concept on the basis of molecular data. *Plant Syst. Evol. (Suppl.)* 9, 273–280.
- Johansson, J.T., 1995. A revised chloroplast DNA phylogeny of the Ranunculaceae. *Plant Syst. Evol. (Suppl.)* 9, 253–261.
- Johansson, J.T., Jansen, R.K., 1993. Chloroplast DNA variation and phylogeny of the Ranunculaceae. *Plant Syst. Evol.* 187, 29–49.
- Kadereit, J.W., Blattner, F.R., Jork, K.B., Schwarzbach, A., 1994. Phylogenetic analysis of the Papaveraceae *s.l.* (incl. Fumariaceae, Hypecoaceae, and *Pteridophyllum*) based on morphological characters. *Bot. Jahrb. Syst.* 116, 361–390.
- Kessler, P.J.A., 1993. Menispermaceae. In: Kubitzki, K., Rohwer, J.G., Bittrich, V. (Eds.), *The Families and Genera of Vascular Plants II*. Springer, Berlin, pp. 402–418.
- Kim, S., Soltis, D.E., Soltis, P.S., Zanis, M.J., Suh, Y., 2004a. Phylogenetic relationships among early-diverging eudicots based on four genes: were the eudicots ancestrally woody? *Mol. Phylogenet. Evol.* 31, 16–30.
- Kim, Y.D., Jansen, R.K., 1996. Phylogenetic implications of *rbcL* and ITS sequence variation in the Berberidaceae. *Syst. Bot.* 21, 81–396.
- Kim, Y.D., Jansen, R.K., 1998. Chloroplast DNA restriction site variation and phylogeny of the Berberidaceae. *Am. J. Bot.* 85, 1766–1778.
- Kim, Y.D., Kim, S.H., Kim, C.H., Jansen, R.K., 2004b. Phylogeny of Berberidaceae based on sequences of the chloroplast gene *ndhF*. *Biochem. Syst. Ecol.* 32, 291–301.
- Kluge, A.G., 1989. A concern for evidence and a phylogenetic hypothesis of relationships among Epicrates (Boidae, Serpentes). *Syst. Zool.* 38, 7–25.
- Kosuge, K., Sawada, K., Denda, T., Adachi, J., Watanabe, K., 1995. Phylogenetic relationships of some genera in the Ranunculaceae based on alcohol dehydrogenase genes. *Plant Syst. Evol. (Suppl.)* 9, 263–271.
- Kubitzki, K., Rohwer, J.C., Bittrich, V. (Eds.), 1993. *The Families and Genera of Vascular Plants II*. Springer, Berlin.
- Langlet, O., 1932. Über Chromosomenverhältnisse und Systematik der Ranunculaceae. *Svensk Bot. Tidskr.* 26, 381–401.
- Li, H.F., Ren, Y., 2005. The variation of perforation plates of vessels in the secondary xylem of *Euptelea pleiosperma* (Eupteleaceae). *Acta Phytotax. Sinica* 43, 1–11.
- Li, R.Q., Chen, Z.D., Lu, A.M., Soltis, D.E., Soltis, P.S., Manos, P.S., 2004. Phylogenetic relationships in Fagales based on DNA sequences from three genomes. *Int. J. Plant Sci.* 165, 311–324.
- Li, X.X., Zhou, Z.K., 2007. The higher-level phylogeny of monocots based on *matK*, *rbcL* and 18S rDNA sequences. *Acta Phytotax. Sinica* 45, 113–133.
- Lidén, M., 1993. Fumariaceae. In: Kubitzki, K., Rohwer, J.G., Bittrich, V. (Eds.), *The Families and Genera of Vascular Plants II*. Springer, Berlin, pp. 310–318.
- Liscombe, D.K., MacLeod, B.P., Loukanina, N., Nandi, O., Facchini, P.J., 2005. Evidence for the monophyletic evolution of benzyloquinoline alkaloid biosynthesis in angiosperms. *Phytochemistry* 66, 1374–1393.
- Loconte, H., Campbell, L.M., Stevenson, D.W., 1995. Ordinal and familial relationships of Ranunculid genera. *Plant Syst. Evol. (Suppl.)* 9, 99–118.
- Loconte, H., Estes, J.R., 1989. Phylogenetic systematics of Berberidaceae and Ranunculales (Magnoliidae). *Syst. Bot.* 14, 565–579.
- Maddison, D.R., Maddison, W.P., 2003. *MacClade 4.06: Analysis of Phylogeny and Character Evolution*. Sinauer Associates, Sunderland, MA.
- Mason-Gamer, R., Kellogg, E., 1996. Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Syst. Biol.* 45, 524–545.
- Nandi, O., Chase, M.W., Endress, P.K., 1998. A combined cladistic analysis of angiosperms using *rbcL* and non-molecular data sets. *Ann. Missouri Bot. Gard.* 85, 137–212.
- Nickol, M.G., 1995. Phylogeny and inflorescence of Berberidaceae—a morphological survey. *Plant Syst. Evol. (Suppl.)* 9, 327–340.
- Nickrent, D.L., Blarer, A., Qiu, Y.L., Soltis, D.E., Soltis, P.S., Zanis, M., 2002. Molecular data place Hydnoraceae with Aristolochiaceae. *Am. J. Bot.* 89, 1809–1817.
- Nixon, K.C., Carpenter, J.M., 1996. On simultaneous analysis. *Cladistics* 12, 221–241.
- Nowicke, J.W., Skvarla, J.J., 1982. Pollen morphology and the relationships of *Circaea*, *Kingdonia* and *Sargentodoxa* to the Ranunculales. *Am. J. Bot.* 69, 990–998.
- Ortiz, R.D.C., Kellogg, E.A., van der Werff, H., 2007. Molecular phylogeny of the moonseed family (Menispermaceae): implications for morphological diversification. *Am. J. Bot.* 94, 1425–1438.



- Oxelman, B., Lidén, M., 1995. The position of *Circaeaster* – evidence from nuclear ribosomal DNA. *Plant Syst. Evol. (Suppl.)* 9, 189–193.
- Pan, K.Y., Lu, A.M., Wen, J., 1991. Chromosome number and development of gametophytes in *Euptelea pleiospermum* (Eupteleaceae). *Acta Phytotax. Sinica* 29, 439–444.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Qin, H.N., 1989. An investigation on carpels of Lardizabalaceae in relation to taxonomy and phylogeny. *Cathaya* 1, 61–82.
- Qin, H.N., 1997. A taxonomic revision of the Lardizabalaceae. *Cathaya* 8–9, 1–214.
- Qiu, Y.L., Dombrowska, O., Lee, J., Li, L., Whitlock, B.A., Bernasconi-Quadroni, F., Rest, J.S., Davis, C.C., Borsch, T., Hilu, K.W., Renner, S.S., Soltis, D.E., Soltis, P.S., Zanis, M.J., Cannone, J.J., Gutell, R.R., Powell, M., Savolainen, V., Chatrou, L.W., Chase, M.W., 2005. Phylogenetic analysis of basal angiosperms based on nine plastid, mitochondrial, and nuclear genes. *Int. J. Plant Sci.* 166, 815–842.
- Qiu, Y.L., Li, L., Hendry, T.A., Li, R., Taylor, D.W., Issa, M.J., Ronen, A.J., Vekaria, M.L., White, A.M., 2006. Reconstructing the basal angiosperm phylogeny: evaluating information content of the mitochondrial genes. *Taxon* 55, 837–856.
- Ratter, J.A., Milne, C., 1976. Chromosome counts in primitive angiosperms: II. *Notes R. Bot. Gard. Edinb.* 35, 143–145.
- Ren, Y., Li, H.F., Zhao, L., Endress, P.K., 2007. Floral morphogenesis in *Euptelea* (Eupteleaceae, Ranunculales). *Ann. Bot.* 100, 185–193.
- Ren, Y., Wang, M.L., Hu, Z.H., 1998. *Kingdonia*, embryology and its systematic significance. *Acta Phytotax. Sinica* 36, 423–427.
- Ro, K.E., Han, H.Y., Lee, S.T., 1999. Phylogenetic contributions of partial 26S rDNA sequences to the tribe Helleboreae (Ranunculaceae). *Korean J. Biol. Sci.* 3, 9–15.
- Ro, K.E., Keener, C.S., McPherson, B.A., 1997. Molecular phylogenetic study of the Ranunculaceae: utility of the nuclear 26S Ribosomal DNA in inferring intrafamilial relationships. *Mol. Phylogenet. Evol.* 8, 117–127.
- Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics (Oxford, England)* 19, 1572–1574.
- Ruijgrok, H.W.L., 1966. The distribution of ranunculin and cyanogenic compounds in the Ranunculaceae. In: Swain, T. (Ed.), *Comparative Phyto-chemistry*. Academic Press, London, UK, pp. 175–186.
- Savolainen, V., Chase, M.W., Hoot, S.B., Morton, C.M., Soltis, D.E., Bayer, C., Fay, M.F., De Bruijn, A., Sullivan, S., Qiu, Y.L., 2000b. Phylogenetics of flowering plants based upon a combined analysis of plastid *atpB* and *rbcL* gene sequences. *Syst. Biol.* 49, 306–362.
- Savolainen, V., Fay, M.F., Albach, D.C., Backlund, A., van der Bank, M., Cameron, K.M., Johnson, S.A., Lledó, M.D., Pintaud, J.C., Powell, M., Sheenan, M.C., Soltis, P.S., Soltis, D.E., Weston, P., Whitten, W.M., Wurdack, K.J., Chase, M.W., 2000a. Phylogeny of the eudicots: a nearly complete familial analysis based on *rbcL* gene sequences. *Kew Bull.* 55, 257–309.
- Shi, J.X., Ren, Y., Di, W.Z., 1994. The taxonomic studies on Sargentodoxaceae. *Acta Bot. Boreal.* 14, 99–103.
- Soltis, D.E., Sinters, A.E., Zanis, M.J., Kim, S., Thompson, J.D., Soltis, P.S., Ronse de Craene, L.P., Endress, P.K., Farris, J.S., 2003. Gunnerales are sister to other core eudicots: implications for the evolution of pentamery. *Am. J. Bot.* 90, 461–470.
- Soltis, D.E., Soltis, P.S., Chase, M.W., Mort, M.E., Albach, D.C., Zanis, M., Savolainen, V., Hahn, W.H., Hoot, S.B., Fay, M.F., Axtell, M., Swensen, S.M., Nixon, K.C., Farris, J.S., 2000. Angiosperm phylogeny inferred from a combined data set of 18S rDNA, *rbcL*, and *atpB* sequences. *Bot. J. Linn. Soc.* 133, 381–461.
- Soltis, D.E., Soltis, P.S., Nickrent, D.L., Johnson, L.A., Hahn, W.J., Hoot, S.B., Sweere, J.A., Kuzoff, R.K., Kron, K.A., Chase, M.W., Swensen, S.M., Zimmer, E.A., Chaw, S.M., Gillespie, L.J., Kress, W.J., Sytsma, K.J., 1997. Angiosperm phylogeny inferred from 18S ribosomal DNA sequences. *Ann. Missouri Bot. Gard.* 84, 1–49.
- Stevens, P.F., 2001 (onwards). Angiosperm Phylogeny Website. Version 7, May 2006 (and more or less continuously updated since). <<http://www.mobot.org/MOBOT/research/APweb/>>.
- Swofford, D.L., 2003. PAUP\*: phylogenetic analysis using parsimony (\*and other methods), version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Taberlet, P., Gielly, L., Pautou, G., Bouvet, J., 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* 17, 1105–1109.
- Takhtajan, A., 1997. *Diversity and Classification of Flowering Plants*. Columbia University Press, New York.
- Tamura, M., 1967. Morphology, ecology and phylogeny of the Ranunculaceae VII. *Sci. Rep. Osaka Univ.* 16, 21–43.
- Tamura, M., 1993. Ranunculaceae. In: Kubitzki, K., Rohwer, J.G., Bittrich, V. (Eds.), *The Families and Genera of Vascular Plants II*. Springer, Berlin, pp. 563–583.
- Tamura, M., 1995. Reproductive structures. In: Hiepko, P. (Ed.), *Natürl. Pflanzenfam II. 17a (4) Duncker and Humblot*, Berlin, pp. 41–70.
- Terabayashi, S., 1985. The comparative floral anatomy and systematics of the Berberidaceae. II. Systematic considerations. *Acta Phytotax. Geobot.* 36, 1–13.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* 24, 4876–4882.
- Thorne, R.F., 1992. Classification and geography of the flowering plants. *Bot. Rev.* 58, 225–348.
- Thorne, R.F., 2000. The classification and geography of the flowering plants: dicotyledons of the class Angiospermae. *Bot. Rev.* 66, 442–624.
- Thorne, R.F., 2007. An updated classification of the class Magnoliopsida (“Angiospermae”). *Bot. Rev.* 73, 67–182.
- Tobe, H., 2002. Hydrastidaceae. In: Kubitzki, K. (Ed.), *The Families and Genera of Vascular Plants V*. Springer, Berlin, pp. 405–409.
- Tobe, H., Keating, R.C., 1985. The morphology and anatomy of *Hydrastis* (Ranunculaceae): systematic reevaluation of the genus. *Bot. Mag. Tokyo* 98, 291–316.

- Wang, F., Li, D.Z., Yang, J.B., 2002. Molecular phylogeny of the Lardizabalaceae based on *trnL-F* sequences and combined chloroplast data. *Acta Bot. Sinica* 44, 971–977.
- Wang, F., Li, D.Z., 2002. Cladistic analysis of the Lardizabalaceae based on morphological data. *Acta Bot. Yunnan.* 24, 445–454.
- Wang, H.C., Meng, A.P., Li, J.Q., He, Z.C., 2004. Chromosome numbers for eight species in five genera of Menispermaceae. *J. Jap. Bot.* 79, 241–246.
- Wang, W., Chen, Z.D., 2007. Generic level phylogeny of Thalictroideae (Ranunculaceae) – implications for the taxonomic status of *Paropyrum* and petal evolution. *Taxon* 56, 811–821.
- Wang, W., Chen, Z.D., Liu, Y., Li, R.Q., Li, J.H., 2007a. Phylogenetic and biogeographic diversification of Berberidaceae in the northern hemisphere. *Syst. Bot.* 32, 731–742.
- Wang, W., Li, R.Q., Chen, Z.D., 2005. Systematic position of *Asteropyrum* (Ranunculaceae) inferred from chloroplast and nuclear sequences. *Plant Syst. Evol.* 255, 41–54.
- Wang, W., Wang, H.C., Chen, Z.D., 2007b. Phylogeny and morphological evolution of tribe Menispermeae (Menispermaceae) inferred from chloroplast and nuclear sequences. *Perspect. Plant Ecol. Evol. Syst.* 8, 141–154.
- Wang, W.T., 1980. Ranunculaceae. In: *Flora Reipublicae Popularis Sinicae*, Vol. 28. Science Press, Beijing, pp. 241–255.
- Wang, Z.X., 1965. Relationships of some families of dicotyledons and some genera of Papaveraceae viewed from the standpoint of their isoquinoline alkaloids. *Acta Bot. Sinica* 13, 284–295.
- Worberg, A., Quandt, D., Barnikse, A.M., Löhne, C., Hilu, K.W., Borsch, T., 2007. Towards understanding early eudicot diversification: insights from rapidly evolving and non-coding DNA. *Org. Divers. Evol.* 7, 55–77.
- Wu, Z.Y., Kubitzki, K., 1993. Lardizabalaceae. In: Kubitzki, K., Rohwer, J.G., Bittrich, V. (Eds.), *The Families and Genera of Vascular Plants II*. Springer, Berlin, pp. 361–365.
- Wu, Z.Y., Lu, A.M., Tang, Y.C., Chen, Z.D., Li, D.Z., 2002. Synopsis of a new “polyphyletic–polychronic–polytopic” system of the angiosperms. *Acta Phytotax. Sinica* 40, 289–322.
- Xia, Q., Peng, Z.X., 1989. A study on the seed of Lardizabalaceae and Sargentodoxaceae, (1) A SEM examination of testa. *Acta Phytotaxon. Sinica* 27, 273–276.
- Xiao, P.G., 1980. A preliminary study of the correlation between phylogeny, chemical constituents and pharmaceutical aspects in the taxa of Chinese Ranunculaceae. *Acta Phytotaxon. Sinica* 18, 142–153.
- Yang, Q.E., Gong, X., Gu, Z.J., Wu, Q.A., 1993. A karyomorphological study of five species in the Ranunculaceae from Yunnan, with a special consideration on systematic positions of *Asteropyrum* and *Calathodes*. *Acta Bot. Yunnan.* 15, 179–190.
- Zanis, M.J., Soltis, P.S., Qiu, Y.L., Zimmer, E., Soltis, D.E., 2003. Phylogenetic analyses and perianth evolution in basal angiosperms. *Ann. Missouri Bot. Gard.* 90, 129–150.
- Zheng, Q.A., Yang, C.R., 2001. Chemotaxonomic study on the family of Lardizabalaceae. *Chinese Bull. Bot.* 18, 332–339.
- Zhu, M., Xiao, P.G., 1991. Distribution of benzylisoquinolines in magnoliidae and other taxa. *Acta Phytotax. Sinica* 29, 142–155.