

DATING PHYLOGENETICALLY BASAL EUDICOTS USING *rbcL* SEQUENCES AND MULTIPLE FOSSIL REFERENCE POINTS¹

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A molecular dating of the phylogenetically basal eudicots (Ranunculales, Proteales, Sabiales, Buxales and Trochodendrales sensu Angiosperm Phylogeny Group II) has been performed using several fossils as minimum age constraints. All *rbcL* sequences available in GenBank were sampled for the taxa in focus. Dating was performed using penalized likelihood, and results were compared with nonparametric rate smoothing. Fourteen eudicot fossils, all with a Cretaceous record, were included in this study for age constraints. Nine of these are assigned to basal eudicots and the remaining five taxa represent core eudicots. Our study shows that the choice of methods and fossil constraints has a great impact on the age estimates, and that removing one single fossil change the results in the magnitude of tens of million years. The use of several fossil constraints increase the probability of approaching the true ages. Our results suggest a rapid diversification during the late Early Cretaceous, with all the lineages of basal eudicots emerging during the latest part of the Early Cretaceous. The age of Ranunculales was estimated to 120 my, Proteales to 119 my, Sabiales to 118 my, Buxales to 117 my, and Trochodendrales to 116 my.

Key words: Cretaceous; dating; eudicots; fossils.

Today the big picture of angiosperm phylogeny is rather well understood, and based on analyses using multiple genes and extensive sampling (e.g., Soltis et al., 2000) a new classification system for flowering plants is now emerging, the Angiosperm Phylogeny Group II system (APG II, 2003). Better resolved phylogenies in combination with new methods for estimating divergence times (e.g., Sanderson, 1997; Huelsenbeck et al., 2000; Britton et al., 2002; Sanderson, 2002; Thorne and Kishino, 2002) and an increasing number of taxonomically identifiable fossils (e.g., Friis et al., 2005) have resulted in an ever growing interest in molecular dating of phylogenies (e.g., Aris-Brosou and Yang, 2002; Conti et al., 2002; Yang and Yoder, 2003; Schneider et al., 2004). So far the datings have given widely different results, although more recent studies tend to converge on similar ages (Sanderson et al., 2004; Bell and Donoghue, 2005). Especially datings of the lower nodes within the angiosperms, have given much older ages than those obtained from the fossil record (e.g., Wikström et al., 2001). For reviews of dating methods and studies see Magallón (2004) and Sanderson et al. (2004).

In this study we focus on the basal grade eudicots comprising the monophyletic groups Ranunculales, Proteales, Sabiales, and Trochodendrales sensu APG II (2003) (see Fig. 1). No previous dating focusing on these groups have been performed, although a few earlier studies have addressed questions on phylogeny and ages of these groups (e.g., Hoot et al., 1999; Magallón et al., 1999). Proteaceae has also been used by Martin and Dowd (1988) in an attempt to calibrate a molecular clock for the angiosperms.

The aim of this study is to present a molecular dating of the basal eudicots using multiple fossil reference-points based

on already published phylogenetic studies of the group. We do not attempt to contribute to phylogenetic reconstruction of eudicots, although our analysis contains the largest sampling for the group so far. All *rbcL* sequences of basal eudicot taxa available in GenBank as well as some newly sequenced species are included in the analysis (see Appendix). Divergence times were estimated using penalized likelihood (Sanderson, 2002) and nonparametric rate smoothing (Sanderson, 1997), with several fossils as minimum age constraints.

MATERIALS AND METHODS

Data set—The problem of undersampling in phylogenetic dating has so far received little attention. However, in a recent study Linder et al. (2005), show that undersampling results in “oversmoothed” results, i.e., overestimating the lower nodes and underestimating the higher nodes. This is real for all available dating methods when the number of taxa is under 150. Adding more taxa therefore seems to be more important for good age estimates, than adding longer sequences or additional genes. The problems are due to branch length being a function of both substitution rate and time. It has recently been shown by simulations (Britton, 2005) that these factors cannot be separated consistently by available dating methods. We therefore extended the taxon sampling as much as possible, using the most commonly sequenced gene, *rbcL*.

All *rbcL* sequences of phylogenetically basal eudicots (Ranunculales, Proteales, Sabiales, Buxales and Trochodendrales sensu APG II, 2003) recorded in GenBank were downloaded. A few multiple sequences and sequences with poly-N stretches were discarded. For underrepresented families additional sequences were added using herbarium material from the Botanical Museum in Uppsala (UPS). As placeholders for core eudicots we added sequences from orders and families considered basal in the core eudicots (APG II, 2003). Sequences from Ceratophyllaceae and Magnoliales were used as outgroups. The resulting data set consists of 114 sequences from basal eudicots, 92 sequences from core eudicot taxa and seven sequences from outgroup taxa. All taxa used in the study are listed in the Appendix. Alignment of sequences was done in Se-AI (Rambaut, 1996). The resulting matrix has 1401 characters, of which 797 are variable.

Tree—Using MacClade (Maddison and Maddison, 2003) we manually constructed a phylogenetic backbone constraint based on previous studies of eu-

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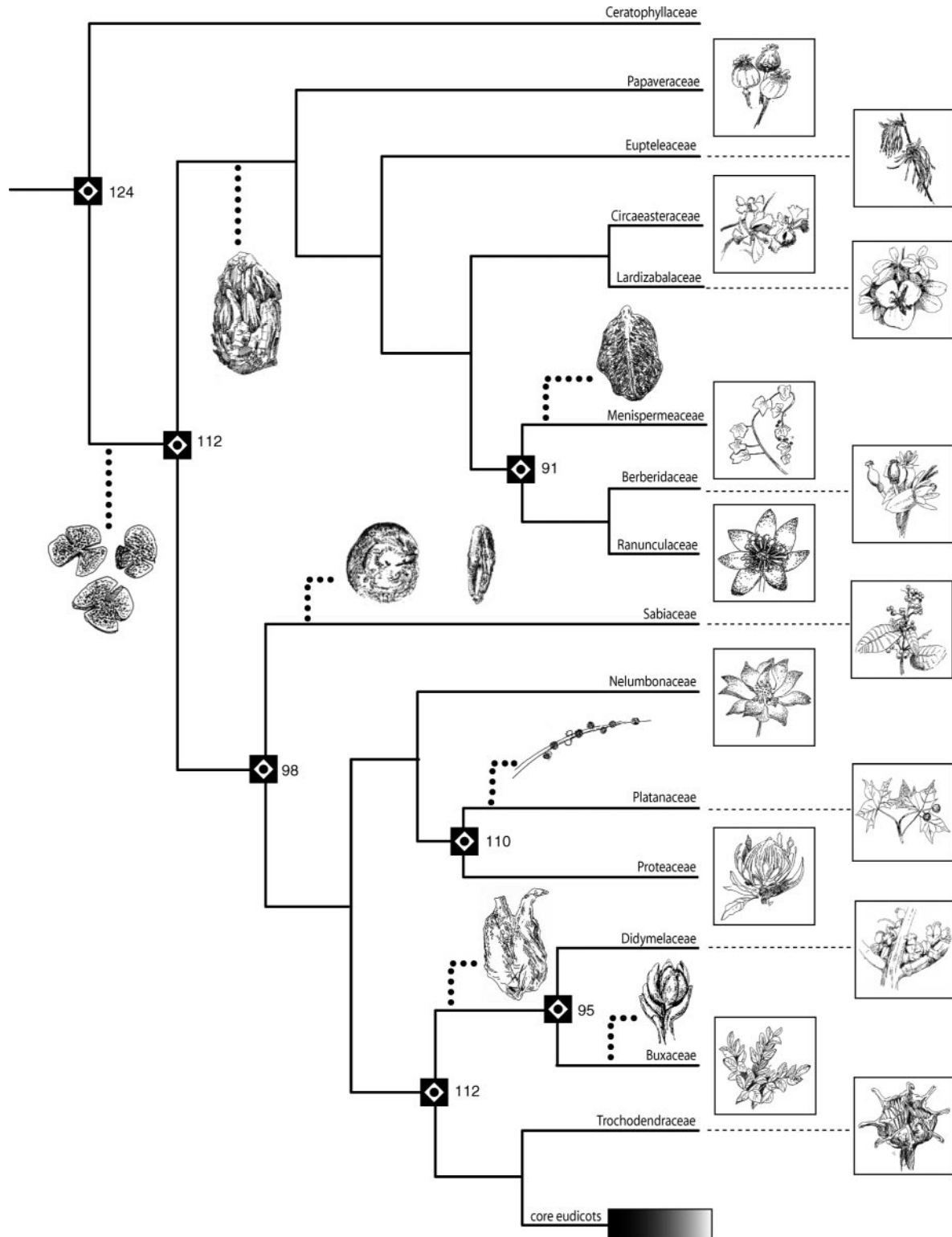


Fig. 1. The families of the basal eudicot grade and some of the fossils we used as constraints. Minimum age constraints for selected nodes are given in millions of years. The illustrated fossils are from top down *Teixeiraea lusitanica*, *Prototinomiscium vangerowii*, *Tricolpites*, *Insitiocarpus moravicus*, *Platanocarpus*, "Buxales," and *Spanomera marylandensis* (see Table 1). Drawings by Jerry Anderson.

dicot phylogeny. Orders were adopted from APG II and monophyly of families and genera was presupposed. References for all included clades are listed in the Appendix. Studies with extensive taxon sampling and combined data sets were preferred (Cuenoud et al., 2002; Fishbein et al., 2001; Hoot and Douglas, 1998; Hoot et al., 1997; Hoot et al., 1999; Soltis et al., 2003; Sosa et al., 2003; von Balthazar and Endress, 2002; Xiang et al., 2002). A clade was considered well supported and stable if found in more than one study and had more than 85% bootstrap/jackknife support. Clades with less than 85% support were collapsed to polytomies. The final backbone constraint contained 173 taxa, and can be obtained from the first author. Forty taxa could not be included in the constraint because they were not previously included in any phylogenetical studies.

A completely resolved tree with all taxa was obtained by a heuristic PAUP* 4.0 (Swofford, 2002) search. The 40 taxa not included in the backbone constraint were added to the matrix. Only trees consistent with the backbone constraint were retained at each step. The analysis resulted in two fully resolved most parsimonious trees differing only within Proteaceae and not affecting family interrelationships. The first one was saved, and the branch lengths were recalculated as length per nucleotide site using the maximum likelihood option in PAUP* applying the GTR+ Γ model with the default setting of 0.5 for the gamma shape parameter. Ideally, the latter is estimated from the data, but it increases computation time beyond that currently possible. The variation in branch lengths depending on model specification in optimization is, however, small, and does not have any impact on the age estimates in this study (Bremer et al., 2004). The tree with its branch lengths was used as the input tree for the dating programs.

Non-clocklike behavior—To check if the data set departed from a molecular clock we used the computer program PATH (Britton et al., 2002). PATH contains a built-in function that quickly estimates whether the observed substitution rate follows a molecular clock. The data set departed significantly from a molecular clock at 121 of the 212 nodes. Nodes that did not depart significantly from a molecular clock were only found within families. PATH also contains a dating method under the assumption that a clock exists. Since the clock was rejected we did not use PATH to estimate divergence times in this study.

Dating—Dating was done using penalized likelihood (PL) (Sanderson, 2002) as implemented in the r8s program (version 1.6) (Sanderson, 2003). For comparison, we also used nonparametric rate smoothing (NPRS) (Sanderson, 1997). NPRS allows rates to vary over the tree but minimizes a quadratic function of the rate changes in adjacent branches. This roughness penalty is in PL combined with a model-based likelihood approach. The penalty in PL is regulated by a smoothing parameter obtained by a cross-validation procedure. In our data set the optimal smoothing value was found to be ~100. Input files for NPRS and PL can be obtained from the first author upon request.

Age constraints from the early fossil record of eudicots—As fossil constraints, we have used all first occurrences in the Cretaceous of basal eudicots adopting a cautious approach using mainly reproductive organs such as flowers, fruits, and seeds. In a few cases, pollen grains and leaf impressions have also been used where sufficient distinctive characters are available for a systematic assignment at family or ordinal level. For absolute ages, the Cretaceous time scale of Gradstein et al. (1995) have been adapted. Table 1 summarizes the fossils we used as constraints.

The r8s program requires at least one reference point to be fixed. Tricolpate pollen, characterized by three slit-like apertures in a radial symmetrical arrangement around the equator, is a synapomorphy for eudicots not found in any other group of plants. The earliest fossils that can reliably be attributed to the eudicots are small tricolpate pollen grains from the Barremian of southern England (Hughes and McDougall, 1990) and from Equatorial Africa (Doyle, 1992). The abundance of fossil pollen in most sedimentary rock, together with the distinctiveness of tricolpate grains make it unlikely that tricolpate pollen should have had a long, undetected pre-Barremian history (Crane et al., 1989). We therefore use the first occurrence of tricolpate pollen

TABLE 1. Fossils used as minimum age constraints. Absolute ages are based on Gradstein et al. (1995).

Assigned to . . .	Fossil	Structure	Locality	Stratigraphic zone	Age	References
Eudicots	<i>Tricolpites</i>	tricolpate pollen grains	southern England	Barremian	124	(Hughes and McDougall, 1990)
Family Menispermaceae	<i>Protinomisium vangerowii</i>	fruit	Klíkov-Schichtenfolge, Czech Republic	Turonian	91	(Knobloch and Mai, 1986)
Order Ranunculales	<i>Teixeiraea lusitanica</i>	staminate flower	Vale de Agua locality, Portugal	late Aptian–early Albian	112	(von Balthazar et al., 2005)
Family Nelumbonaceae	<i>Nelumbites extenuinervis</i>	foliage, receptacle, fruit	Quantico locality, Virginia, USA	mid Albian	106	(Vakhrameev, 1952)
Family Platanaceae	<i>Platanocarpus brookensis</i>	inflorescence, infructescence, foliage	Brooke locality, Virginia, USA	early Albian	110	(Crane et al., 1993)
Family Proteaceae	<i>Beaupreopsis</i> and <i>Macadamialia</i> <i>Helicia</i> -type pollen	pollen	Australia, Antarctica	Santonian	85	(Dettmann and Jarzen, 1998)
Family Sabiaceae	<i>Instiitocarpus moravicus</i>	fruit	Peruc-schichten, Czech Republic	lower Cenomanian	98	(Knobloch and Mai, 1986)
Family Buxaceae	<i>Spanomera marylandensis</i>	flowers	Potomac, USA	late Albian	94	(Drinnan et al., 1991)
Order Buxales	"Buxales" unpublished	fruit, flowers	Vale de Agua locality, Portugal	late Aptian–early Albian	112	(Fris, unpublished manuscript)
Family Gunneraceae	<i>Retriicolpites microreticulatus</i>	pollen	Peru	Turonian	91	(Jarzen and Dettmann, 1989)
Family Droseraceae	<i>Paltaealdrovanda splendens</i>	fruit	Klíkov-Schichtenfolge, Czech Republic	mid-late Turonian	91	(Knobloch and Mai, 1986)
Order Saxifragales	<i>Divisestylus</i>	flowers, fruits	Old Crossman locality, New Jersey, USA	Turonian	91	(Hermesen et al., 2003)
Family Cornaceae	<i>Hironoia fusiformis</i>	fruit	Futaba Group, Kamitikaba, northeastern Japan	Coniacian	87	(Takahashi et al., 2002)
Family Ternstroemiaceae	<i>Eurya crassitesa</i>	flowers, fruits, seeds	Central Europe	Santonian	85	(Knobloch and Mai, 1986)

to fix the origin of eudicots to the Barremian, corresponding approximately to 124 Mya.

All other fossils were used as minimum age constraints. Any fossil with a reliable synapomorphy for a particular crown group in the tree is implicitly placed along the lineage from the stem node to the crown node of that group, meaning that the stem node is at least as old as the fossil, or has a minimum age equivalent to that of the fossil (Fig. 1).

Ranunculales—The Cretaceous record of well-documented Ranunculales fossils is sparse. This may partly be explained by the herbaceous or shrubby habit of most members of the order. Leaves of herbaceous plants often lack an abscission zone and typically weather while still attached to the plant. Problems in identifying synapomorphies for the group may also partly explain why so few reliable ranunculalean fossils have been described also from younger strata. Currently only a single floral structure and a few fruits from the Cretaceous have been assigned to the Ranunculales with certainty. A number of leaf fossils assigned to various families of the group are based solely on gross morphology, and none of these identifications have been confirmed by anatomical or micromorphological details although they may well be representatives of the family.

Teixiraea lusitanica is the oldest fossil that can be linked with some confidence to the Ranunculales, and it is used here as a minimum age constraint for the stem group Ranunculales (Fig. 1, Table 1). It is a small staminate flower described from the Early Cretaceous flora of Vale de Agua, Portugal (von Balthazar et al., 2005). The flower is staminate and actinomorphic with bracts and perianth organs apparently borne in a spiral arrangement without clear differentiation between the different organ categories. The androecium consists of many stamens of two sizes. Pollen grains are small, tricolpate and perforate with columellate infratectal layer. The fossil displays many characters found scattered in the Ranunculales. *Teixiraea* probably represents a separate, now extinct phylogenetic lineage within crown group Ranunculales or along the stem lineage (von Balthazar et al., 2005). The age of the flora is not fully established. A pre-Albian age (Barremian or Aptian) was suggested earlier for the Vale de Agua flora (e.g., Friis et al., 2001), but a late Aptian age or even slightly younger age (early Albian) has been suggested recently (for references see von Balthazar et al., 2005).

The Menispermaceae are represented in the Cretaceous of Europe by endocarps assigned to the fossil genus *Prototinosmium* (see Fig. 1, Table 1). The oldest record is from the Turonian of Central Europe (Knobloch and Mai, 1984, 1986). The endocarps were compared to those of extant Menispermaceae, tribe Tinosporeae, based on the distinct keel that runs along both the ventral and dorsal margin of the endocarp, and on a pair of characteristic cavities on the ventral side of the endocarp near the placenta.

Other Cretaceous taxa with features suggestive of Ranunculales include several impression fossils, most of which are described from the Potomac Group sediments of eastern North America (e.g., Doyle and Hickey, 1976) and from central and eastern Asia (e.g., Vakhrameev, 1952; Vakhrameev and Krassilov, 1979; Krassilov et al., 1983). Among these fossils are leaves from the Aptian of Maryland assigned to *Vitiphyllum multifidum* (Fontaine, 1889; Hickey and Doyle, 1977) and an axis with leaves and infructescences from the Albian of Kazakhstan described as *Caspiocarpus paniculiger* (Vakhrameev and Krassilov, 1979). None of these have sufficient characters preserved for a secure systematic placement. This is also true for leaves described from North America as *Winchellia triphylla* and assigned to the Berberidaceae (Lesquereux, 1893). *Ranunculacarpus quinquecarpellatus* is a reproductive organ from the Albian of the Kolyma Basin, Siberia, tentatively assigned to the Ranunculaceae (Samylin, 1960). Information currently available on this fossil, however, is insufficient for a definite systematic assessment.

An intriguing fossil plant of possible affinity to Ranunculaceae from the Early Cretaceous of Spain includes slender stems with strongly dissected leaves indicating an aquatic habit. It was first described as *Montsechites ferreri* (Teixeira, 1954) but later transferred to the extant taxon *Ranunculus aquatilis* (Blanc-Louvel, 1984). In gross morphology it is indeed very similar to modern Ranunculaceae, but the fossil has small spiny reproductive units in the axils of the leaves that are distinctly different from fruits of Ranunculaceae

and *Montsechites* cannot be included in this family based on the evidence currently available.

Sabiales—The Sabiaceae are represented in the Late Cretaceous floras of Europe by four species assigned to the two extant genera *Sabia* and *Meliosma*, and five species assigned to the fossil genus *Insitiocarpus* (Knobloch and Mai, 1986). The fossil species are based on fruit remains with distinct synapomorphies of the Sabiaceae including uni- or bilocular endocarps characterized by a deep funicular cavity with or without funicular plug, and semi-anatropous seeds with a thin seed wall (Knobloch and Mai, 1986). The oldest of the records is *Insitiocarpus moravicus* (Fig. 1, Table 1) described from the Cenomanian Peruc-Schichten in the Czech Republic (Knobloch and Mai, 1986). Fossil endocarps assigned to *Sabia* are slightly younger occurring from the Turonian and onwards and fossil *Meliosma* is known from the Maastrichtian and onwards (Knobloch and Mai, 1986).

Petrified twigs and stems of *Sabiocaulis sakurii* described from the Late Cretaceous of Hokkaido, Japan, have anatomical features closely similar to those of extant *Sabia* and may also represent an early occurrence in Asia of the family (Stopes and Fujii, 1910). The age of the fossils is late Turonian–Santonian (Nishida, 1991) and the wood is thus contemporaneous with the *Sabia* endocarps of Europe. According to Nishida (1991), however, the systematic position of *Sabiocaulis* is not fully resolved.

Proteales—All three families of the Proteales are present in the Cretaceous floras. The earliest reliable record of the Nelumbonaceae is leaves of *Nelumbites extenuinervis* (Table 1) and associated stems and reproductive remains described from the middle to early late Albian of Virginia, USA (Upchurch et al., 1994). The fossils are placed in the Nelumbonaceae based on the petalate, almost orbicular leaf shape with the point of petiole attachment inserted towards the center of the lamina, an agreement in venation pattern and a tendency for dimorphism in laminar shape. Associated tepals show shape and venation pattern comparable to that of extant *Nelumbo*, and impressions with circular protuberances show strong similarity to the floral receptacle of *Nelumbo*. Similar leaves were also reported from the early to middle Albian flora of western Kazakhstan as *Nelumbites tenuinervis* (Vakhrameev, 1952). These occur isolated, and the preservation is not as complete as for the North American specimens. *Nelumbites minimus*, also from the early to middle Albian of western Kazakhstan (Vakhrameev, 1952) and *Nelumbites* aff. *minimus* from the late Albian of Eastern Siberia (Samylin, 1968) have tiny leaves, about 1–2 cm in diameter. The Siberian material is better preserved, and have leaves attached to slender stems in an alternate arrangement distinctly different from the arrangement in extant *Nelumbo*. The affinity to Nelumbonaceae is not clear for these fossils.

Leaf and reproductive remains of Platanaceae are abundant throughout the middle and Late Cretaceous. The earliest occurrences are pistillate and staminate floral structures and associated leaves described from the early to middle Albian flora of the Brooke locality, Virginia, USA (Crane et al., 1993). The pistillate flowers, *Platanocarpus brookensis* (Table 1), are similar to flowers of extant *Platanus* in being densely clustered in spherical inflorescences. Synapomorphies of the group include the undifferentiated perianth, apocarpous gynoecium, and carpels with a single pendant and orthotropous to semi-orthotropous ovule. *Platanocarpus brookensis* is similar to other Cretaceous platanoid pistillate flowers in having five carpels in a radial symmetrical position, while pistillate flowers of extant *Platanus* have an irregular gynoecium usually with more than five carpels. The staminate structures, *Aquia brookensis*, associated with *Platanocarpus brookensis* consist of dispersed stamens only. Leaves associated with the reproductive structures are assigned to the fossil genus *Sapindopsis*. They are different from leaves of extant *Platanus* in their pinnate organization, but similar to *Platanus* in epidermal features (Crane et al., 1993). Several other species of Platanaceae are reported from the Early Cretaceous of eastern North America documenting that this group was well-established early in the history of angiosperms. *Platananthus potomacensis* and *Platanocarpus marylandensis* are staminate respectively pistillate flowers from the late Albian of Maryland (Friis et al., 1988), and *Hamatia elkneckensis* and *Platanocarpus elkneckensis* are corresponding platan-

oid fossils from the latest Albian of northeastern Maryland, USA (Pedersen et al., 1994).

The Proteaceae have an extensive fossil record in the southern hemisphere from the Late Cretaceous onward. The Cretaceous fossils are mainly dispersed pollen, while the Cainozoic record also includes abundant leaf fossils and reproductive organs. Pollen grains of Proteaceae are typically oblate, tricolporate, triporate, or biporate. The tricolporate and triporate forms may in general pollen morphology and structure be difficult to distinguish from pollen of several other eudicot families. However, thorough palynological studies of extant Proteaceae at the ultrastructural level have provided additional characters that can be used to assign dispersed pollen to the family, sometimes at the level of subfamily or tribes (for references, see Dettmann and Jarzen, 1998). The earliest dispersed pollen that can be included in crown group Proteaceae is *Beaupreopsis*-type pollen (subfamily Proteoideae) and *Macadamia/Helicia*-type pollen (subfamily Grevilleoideae) (Table 1), both appearing in the Santonian of Australia (Dettmann and Jarzen, 1998). The diversity of forms in the Late Cretaceous indicates an earlier origin, and several putative Proteaceae pollen are also known from the Turonian and onward in Australia (Dettmann and Jarzen, 1998), from the Santonian and onwards in Antarctica (Askin and Baldoni, 1998) and from the Cenomanian of Africa (Ward and Doyle, 1994). According to Dettmann and Jarzen (1998), the systematic affinity of the early Late Cretaceous records is, however, not fully established.

Buxales—The fossil genus *Spanomera* includes two species, *S. mauldinensis* and *S. marylandensis*, described from mid-Cretaceous strata of the Potomac Group sequence, USA (Drinnan et al., 1991). The species are based on well-preserved remnants of inflorescences and flowers as well as isolated fruits and stamens. Close relationship to extant Buxaceae is indicated by the opposite and decussate organization of the inflorescence and floral organs, short pedicelled staminate flowers, dorsifixed introrse to latrorse anthers with prominent pointed apical extension of connective, presence of a pistillode in staminate flowers, bicarpellate pistillate flowers, and carpels with a long decurrent stigma. Pollen grains of *Spanomera* are coarsely striate-rugulate and are distinct from extant Buxaceae in having simple tricolpate pollen, a feature that may be plesiomorphic for Buxales (Drinnan et al., 1991). The oldest of the *Spanomera* fossils is *S. marylandensis* (Table 1) described from the late Albian of Maryland (Drinnan et al., 1991). Other Early Cretaceous reproductive organs that may represent Buxales include undescribed staminate flowers and fruits from the Aptian-Albian Vale de Agua locality, Portugal (for discussion of age see von Balthazar et al., 2005). Two types of fruits, one bicarpellate and one tricarpetate are present, both with decurrent stigmatic areas and general morphology, suggesting a relationship with Buxales. The pollen grains are similar to those of *Spanomera* in aperture configuration and in the coarsely striate-rugulate exine structure. These latter fossils hence specify a minimum age for the stem group of Buxales (Fig. 1, Table 1).

Trochodendrales—There are no secure findings of Cretaceous Trochodendrales. *Trochodendroides* are fossil leaves resembling those of extant *Trochodendron*, but the family identity is uncertain (Doyle and Hickey, 1976; Crane, 1989). Fossil fruits and flowers, assigned to the extinct genus *Nordenkioldia* from the Paleocene, are the earliest fossils with distinct Trochodendrales synapomorphies (Crane et al., 1991).

Core eudicots—Five fossils were chosen as constraints within the core eudicots, three basal core eudicots, and two asterids.

Among basal core eudicots Gunneraceae, Droseraceae, and Saxifragales were chosen. Dispersed pollen assigned to the fossil taxon *Retitricolpites microreticulatus* is very similar to pollen of extant *Gunnera* in size, aperture configuration, dense reticulum, and sharp profiles of muri, and is generally accepted as a fossil member of the genus *Gunnera* (Brenner, 1968; Wanntorp et al., 2004). *Retitricolpites microreticulatus* is widespread in rocks of Late Cretaceous age from the southern hemisphere where it has been recorded from all continents. Records from the late Cenomanian to Santonian of Romania and India (Jarzen and Dettmann, 1989) are uncertain (Wanntorp et al., 2004) and the earliest reliable record of the genus is from the Turonian of Peru (Jarzen and Dettmann, 1989; Wanntorp et al., 2004). We have accepted this

identification and use it here as a minimum age constraint for the stem node of Gunneraceae (Table 1).

Palaealdrovanda splendens, described from the late Turonian-Santonian Kvikov beds of the Czech Republic, is based on small seeds closely resembling those of extant *Aldrovanda* (Droseraceae) (Knobloch and Mai, 1986). Synapomorphic characters that link the fossils seeds to extant *Aldrovanda* include hard testa with an outer epidermis of palisade cells and with a smooth, strongly reflecting surface, short micropylar neck, and extruding, pointed chalazal area (Knobloch and Mai, 1986, 1991). The fossil is used as a minimum age constraint for Droseraceae (Table 1).

Divisestylus brevistamineus, *D. longistamineus*, and *Divisestylus* sp. are three saxifragean taxa of fossil flowers and fruits described from the Late Cretaceous Old Crossman locality in New Jersey, USA (Hermesen et al., 2003). In morphology and floral organization, the fossils are closely similar to extant species of Itaceae and Saxifragaceae (Saxifragales), but cannot be placed in any family with certainty (Hermesen et al., 2003). The age of the fossils from Old Crossman Clay pit is generally cited as Turonian and we have here used *Divisestylus* (Table 1) to set the minimum age for Saxifragales at the Turonian.

The oldest fossil that can be related to Cornaceae is *Hironoia fusiformis*, an extinct genus and species collected from Futaba Group sediments at Kamitikaba, northeastern Japan (Takahashi et al., 2002). The fossil is based on small three-dimensionally preserved fruits (drupes) with remnants of a perianth. Synapomorphies of the endocarp wall and dehiscence valves observed for the fossil occur in Cornaceae, only in the two genera *Nyssa* and *Mastixia*. The fossil taxon cannot be placed with certainty in one of the two genera, and *Hironoia* is either an early member of the *Nyssa/Mastixia* clade or it may belong to the stem group leading to this clade. The age of the Futaba group sediments is established as early Coniacian to early Santonian, and fossil plants are thought to belong to the lower part of the sequence, which would give a minimum age for the *Nyssa/Mastixia* branch of early Coniacian (Table 1).

Fossils with synapomorphies indicating affinity to members of Pentaphylacaceae and Ternstroemiaceae (the two families are synonymized in APG II, 2003, but may be optionally treated as distinct) are common in the Late Cretaceous and represented by a variety of floral structures as well as fruits and seeds. The oldest fossils of this group are seeds with campylotropous organization and characteristic deep and strongly pitted cells of the outer seed wall. By these synapomorphies, they are clearly assignable to the modern genus *Eurya* and included in an extinct species, *E. crassitesta* (Knobloch and Mai, 1986) (Table 1). *Eurya* is known from several core samples and exposures in central Europe ranging in age from the Santonian onward (Knobloch and Mai, 1986).

RESULTS AND DISCUSSION

Stem and crown group ages for all orders and families within the phylogenetically basal eudicots and for major groups of core eudicots obtained by PL and NPRS are given in Table 2. Generally, the PL ages are younger than the ages obtained by NPRS. The differences are greater toward the terminals and smaller toward the root of the tree, see Table 2. In the following discussion, we refer to the results of the PL analysis when nothing else is stated. The PL results are also illustrated in Fig. 2.

The results of this study suggest that the time intervals between the divergences of the basal eudicot lineages are short. This is also what the fossil record indicates. Stem group ages for families are often in the Early Cretaceous and corresponding crown group ages mostly in the Late Cretaceous, indicating that evolution of many families took place in the mid Cretaceous.

The crown group age of eudicots, and consequently the stem group age of Ranunculales were estimated to 120 my. The stem groups of all the families of Ranunculales were present in the Santonian, 90 Mya. Compared to other stem and crown

TABLE 2. Estimated absolute ages for the crown and stem groups of the basal eudicots and some major clades within the core eudicots.

Group	Family	Stem group age inferred by PL	Crown group age inferred by PL	Stem group age inferred by NPRS	Crown group age inferred by NPRS
Eudicots		124	120	124	122
Ranunculales		120	114	122	121
	Papaveraceae	114	106	121	119
	Eupteleaceae	111	23	120	101
	Circaeasteraceae	107	72	116	84
	Lardizabalaceae	107	66	116	95
	Menispermaceae	105	70	116	80
	Ranunculaceae	90	73	104	87
	Berberidaceae	90	72	104	88
Proteales		119	115	121	121
	Proteaceae	110	85	119	96
	Platanaceae	110		119	
	Nelumbonaceae	115	3	121	108
Sabiales					
	Sabiaceae	118	91	122	119
Buxales		117	99	121	
	Buxaceae	99	63	118	111
Trochodendrales		116	45	120	107
Core eudicots		116	115	120	116
Gunnerales		115	90	112	55
Caryophyllales		114	99	116	102
Santalales		113	101	115	108
Saxifragales		108	102	108	102
Rosids		112	108	112	108
Geraniales		89	86	83	80
Crossosomatales		89	40	83	47
Asterids		112	109	112	109
Cornales		109	97	109	101
Ericales		109	103	109	99

group ages in this study the ages of the ranunculalean clades seem young. Because the ranunculalean clade constitutes about one third of the taxa of the study, but has only one fossil attached within the clade it is possible that the ages within Ranunculales are underestimated. Experimenting with removing other fossils in other clades gives younger ages. However, the estimated ages do not exclude the possibility that some Albian and Aptian leaf imprints, suggestive of Ranunculales, might actually be members of the order.

The stem group age of Sabiaceae was estimated to 118 my and the crown group to 91 my. The latter age is somewhat younger than the fossil *Insitiocarpus moravicus* that was used as minimum age constraint for the stem group Sabiaceae. The stem group of Proteales was estimated to 119 my. Nelumbonaceae was estimated to have originated in the Aptian, 115 Mya, about 10 my before its first occurrence in the fossil record. Proteaceae and Platanaceae diverged in the early Albian, 110 Mya. This age is very close to the first occurrence of reproductive parts and leaves of Platanaceae. The stem group age of Buxales was estimated to 117 my, and the split between Buxaceae and Didymelaceae estimated to 99 my. The stem group age of the small order Trochodendrales was estimated to 116 my.

The pattern of rapid divergence of major lineages continued within the core eudicots, with the divergence of core eudicots already in the Aptian, and the divergence of rosids and asterids a few million years later in the Albian.

Comparisons with other studies—A much-cited study by Wikström et al. (2001) using NPRS estimated the age of the eudicot clade to Late Jurassic. The study used a single internal calibration point for the angiosperms. However, because of

great differences in evolutionary rate between different branches of the angiosperm tree as demonstrated by several recent studies (e.g., Soltis et al., 2002), the use of a single fossil calibration point led to overestimation of the age of the deeper branches, as exemplified by the split between *Ceratophyllum* and eudicots in the Late Jurassic about 155 Mya. The ages of most of the families within the basal eudicot orders are of the same magnitude in the present study and the study of Wikström et al. The ages of the orders and lower branches within the Ranunculales however, are about 20 my older in the Wikström et al. study.

Magallón et al. (1999) used a more conservative approach when they discussed the eudicot origin. They did not perform actual dating, but instead plotted fossils on the consensus phylogenetic tree. The resulting stem group ages differ somewhat from those of the present study. This might be due to the use of some controversial fossils such as the Aptian *Trochodendroides* as age constraints. Differences in methodology makes comparisons of crown groups difficult.

Sanderson and Doyle (2001) estimated the age of the crown group Proteales to 48 my, but pointed out that both Nelumbonaceae and Platanaceae are known from the Albian, and subsequently that the estimated age was too young. The branches leading to *Nelumbo* and *Platanus* are relatively short, and the molecular clock method that Sanderson and Doyle used tends to push nodes below short branches toward younger ages. They concluded that the short branches may be the result of a slow down in substitution rate.

Methodological problems—Clearly an incorrect phylogeny could have great impact on age estimates, at least in lower nodes of the tree. In our data set, the family Sabiaceae is

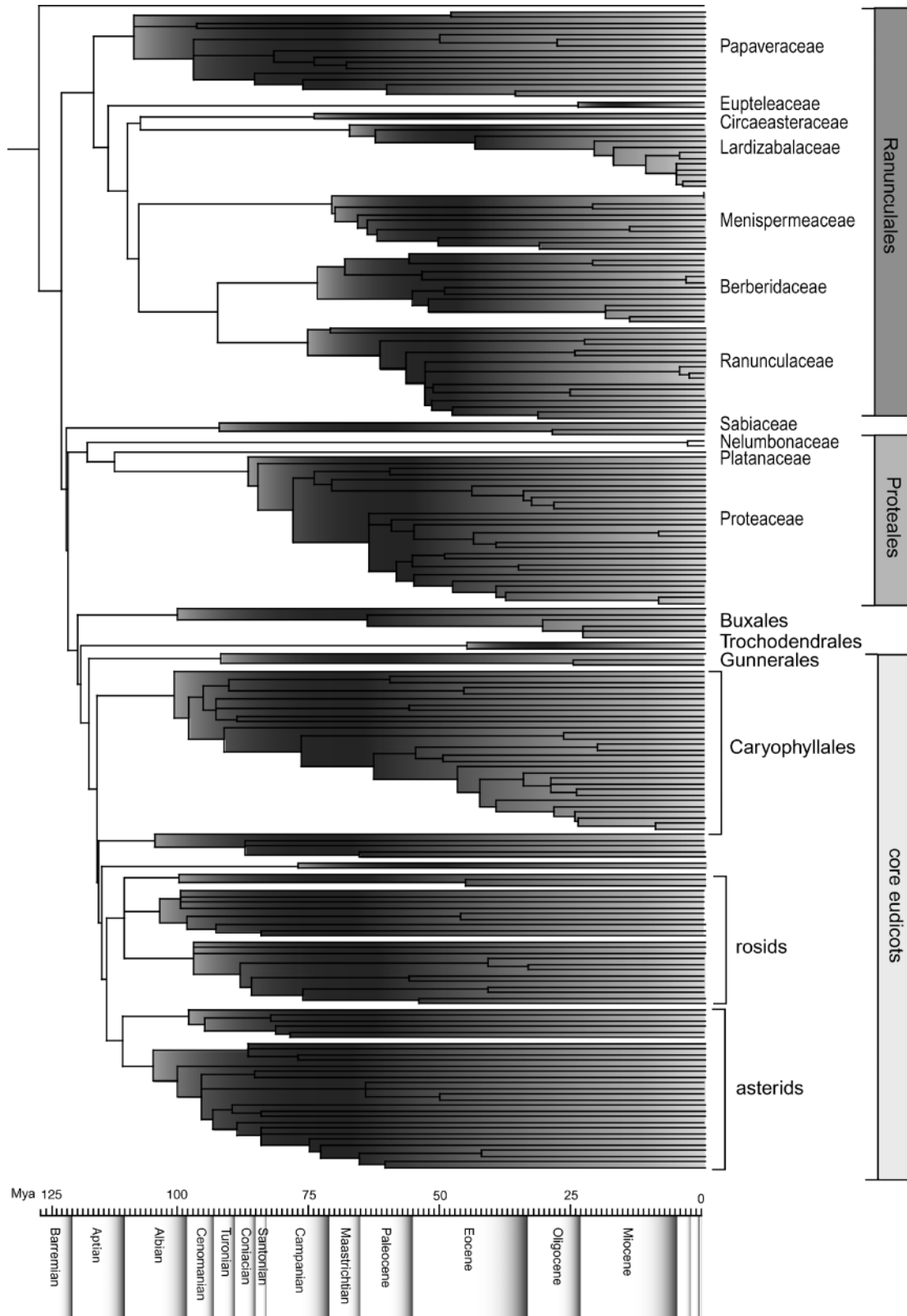


Fig. 2. Chronogram obtained from the penalized likelihood analysis. The stem group age of Ranunculales was estimated to 120 my, Proteales to 119 my, Sabiales to 118 my, Buxales to 117 my, and Trochodendrales to 116 my.

problematic because its placement has not been firmly established. In the most parsimonious trees of Soltis et al. (2000), Sabiaceae branch off after Proteaceae, but this relationship receives less than 50% support. In the study of Hilu et al. (2003) Sabiaceae branch off before Proteaceae, with just as weak support as in the Soltis et al. study, but with a posterior probability of 1.0. Both studies indicated that Sabiaceae might form a clade with Proteaceae. Because of the uncertain position of Sabiaceae we ran analyses with all these alternative topologies. They yielded no or only small differences in age estimates. It seems as if the results are robust to minor changes in topology, as exemplified by the alternative positions of Sabiaceae. This is consistent with the findings of Bremer et al. (2004), who explored how different error sources contribute to the variation in ages.

Calibration necessitates at least one node with a fixed age. Furthermore, the r8s program may result in excessively or even impossibly old age estimates if none of the lower nodes is fixed. In this study, the basalmost fossil reference point, and hence the best node to fix, is the stem group of eudicots. It is also suitable because the reference fossil is relatively well dated, i.e., the earliest occurrence of tricolpate pollen in the Barremian, about 124 Mya. The earliest appearance of tricolpate pollen, of course, may underestimate the stem group age of the eudicots. Tricolpate pollen, however, may have evolved several million years after the split of eudicots from their sister group. Alternatively tricolpate pollen may even have evolved before their first appearance in the fossil record, despite their absence from older palynofloras. The fact that different types of tricolpate pollen occurred almost simultaneously around the Barremian-Aptian transition, suggest an origin of eudicots prior to the first occurrence of tricolpate pollen, and some authors have argued that a 124 my age for eudicots is much too young (e.g., Wikström et al., 2001). Therefore we tested different analytical approaches to the calibration problem. We performed parallel analyses with the different fossils as separate, fixed-age constraints. This procedure was conducted for all the fossils included, using NPRS and PL—in total more than 20 analyses. These analyses all gave different ages for the stem eudicot node. The mean stem age for eudicots obtained by applying different fixed age nodes, was 144 my for NPRS, and 138 my for PL. The mean of all the different calibration points from the two methods is 141 my, that is about 17 my older than the earliest occurrence of tricolpate pollen. Hence, using fossils other than tricolpate pollen provides a stem group age for eudicots about $141 - 124 = 17$ my older. The range is very uncertain, however, and the variation depending on the reference fossil used as a fixed age constraint is large. Another way of calculating the “ghost range” for tricolpate pollen would be by the methods developed by stratigraphic palynologists (Holland, 2003). Confidence intervals on stratigraphic ranges can thus be calculated for fossils that are abundant and widely distributed. No such calculation has been done for tricolpate pollen, but other studies with this method suggest that the ghost range would be much smaller than 17 my, possibly only a few million years.

Considering the difficulties and uncertainties in obtaining a stem group age for eudicots with other reference fossils than the tricolpate pollen, we finally decided to use the original fixed age of 124 my for the stem group of eudicots, corresponding to the 124-my-old tricolpate pollen grains. Also, the pollen grain is a structure that is abundant and easily preserved; if there were any older fossil tricolpate pollen we

should be able to find them, since stratigraphic layers more than 124 my are available and actually contain monocolpate angiosperms and microspores from other seed plants. Even if we consider the possibility that specific pollinators inhibited the pollen from being widely dispersed and preserved, the problem of estimating the ghost range remains. We submit that the calibration approach that we have used is transparent, and could easily be falsified if older tricolpate pollen or perhaps even whole flower structures with in situ pollen were to be found before middle Barremian.

Some studies report similar results from PL and NPRS (e.g., Bremer et al., 2004). In the present study the age estimates differ (Table 2) between these methods. This is expected since we got a relatively high smoothing value from the cross validation process (100). This is probably due to some of the clades behaving more clock-like than other parts of the tree. These clades are mostly found within families, where up to 40% of the nodes do not deviate significantly from a molecular clock. This gives the ages in the PL analyses less freedom to vary than in the NPRS analyses.

Cretaceous firework or artifact from constraints or method?—All our methods rely on the assumption that there is an autocorrelation of evolution rates in adjacent lineages and that some kind of smoothing is reasonable, but could it be that rates change abruptly rather than gradually? This has been suggested by Sanderson and Doyle (2001). In palaeozoology there has been more debate on the rate of evolution. It has been proposed that events like the Cretaceous-Tertiary boundary mass extinction and the Cambrian Explosion could be artifacts of the rock record (e.g., Benton and Ayala, 2003, and references therein). Other scientists suggest that these events are real and that we should trust the fossil record (Benton et al., 2000).

Can dating be done without a clock?—To estimate divergence times consistently, we need precise fossil dates on all nodes where rate changes occur. Of course, this is not realistic, but in any case we should avoid to calibrate with only one or a few fossils, as is often done. If we had many fossils placed more or less even over the tree the choice of method would be less important. We need more fossils and these fossils must be well dated and as widely dispersed across the phylogenetic tree as possible. Excluding any of the fossils in this study, clearly gave younger ages for the clade to which the fossil was attached.

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APPENDIX. All genera sampled, GenBank accession numbers and references for placement in backbone constraint.

Group	Trochodendrales
Family. <i>Species</i> ; GenBank accession number; Reference for constraint.	Trochodendraceae. <i>Trochodendron aralioides</i> ; L01958.2; Hoot et al., 1999.
Ceratophyllales	Tetracentraceae. <i>Tetracentron sinense</i> ; L12668.1; Hoot et al., 1999.
Ceratophyllaceae; <i>Ceratophyllum demersum</i> ; D89473.1; —.	Proteales
Magnoliales	Nelumbonaceae. <i>Nelumbo nucifera</i> ; M77033.1; Hoot et al., 1999. <i>N. lutea</i> ; M77032.1; Hoot et al., 1999.
Annonaceae. <i>Polyalthia suberosa</i> ; AF193971; Soltis et al., 2000.	Platanaceae. <i>Platanus occidentalis</i> ; AF081073; Hoot et al., 1999.
Degeneriaceae. <i>Degenaria vitiensis</i> ; L12643.1; Soltis et al., 2000.	Proteaceae. <i>Adenanthos obovatus</i> ; U79165.1; Hoot and Douglas, 1998.
Eupomatiaceae. <i>Eupomatia bennettii</i> ; L12644.1; Soltis et al., 2000.	<i>Bellendena montana</i> ; U79177.1; Hoot and Douglas, 1998. <i>Brabejum stellatifolium</i> ; U79166.1; Hoot and Douglas, 1998. <i>Carnarvonia araliifolia</i> ; U79164.1; Hoot and Douglas, 1998. <i>Conospermum taxifolium</i> ; DQ099440; Hoot and Douglas, 1998. <i>Dilobeia thouarsii</i> ; DQ099448; —. <i>Dryandra drummondii</i> ; U79167.1; —. <i>Embothrium coccineum</i> ; U79168.1; Hoot and Douglas, 1998. <i>Gevuina avellana</i> ; U79169.1; Hoot and Douglas, 1998. <i>Grevillea robusta</i> ; AF197589; Hoot and Douglas, 1998. <i>Hakea myrtoides</i> ; U79170.1; —. <i>Isopogon latifolius</i> ; U79179.1; Hoot and Douglas, 1998. <i>Knightia excelsa</i> ; DQ099438; Hoot and Douglas, 1998. <i>Lambertia inermis</i> ; L11190.2; Hoot and Douglas, 1998. <i>Leucadendron laureolum</i> ; U79180.1; Hoot and Douglas, 1998. <i>Lomatia silaifolia</i> ; U79171.1; Hoot and Douglas, 1998. <i>Macadamia ternifolia</i> ; U79172.1; Hoot and Douglas, 1998. <i>Oreocallis mucronata</i> ; DQ099446; —. <i>Orothamnus zeyheri</i> ; U37132.1; —. <i>Persoonia lanceolata</i> ; U79178.1; —. <i>Petrophile biloba</i> ; U79181.1; Hoot and Douglas, 1998. <i>Placospermum coriaceum</i> ; AF093729; Hoot and Douglas, 1998. <i>Protea repens</i> ; U79182.1; Hoot and Douglas, 1998. <i>Roupala macrophylla</i> ;
Himantandraceae. <i>Galbulimima belgraveana</i> ; L12646.1; Soltis et al., 2000.	
Magnoliaceae. <i>Magnolia tripetala</i> ; AF206791.1; Soltis et al., 2000.	
Myristicaceae. <i>Myristica fragrans</i> ; AF206798.1; —.	
Basal eudicots	
Buxales	
Buxaceae. <i>Buxus sempervirens</i> ; AF093717; von Balthazar and Endress, 2002. <i>Pachysandra procumbens</i> ; AF203486; von Balthazar and Endress, 2002. <i>Sarcococca confusa</i> ; AF197588; von Balthazar and Endress, 2002. <i>Styloceras laurifolium</i> ; AF093733; von Balthazar and Endress, 2002.	
Didymelaceae. <i>Didymeles perrieri</i> ; AF061994.1; Hoot et al., 1999.	
Sabiales	
Sabiaceae. <i>Meliosma simplicifolia</i> ; AF197587; Hoot et al., 1999. <i>M. veitchiorum</i> ; AF206793.1; Hoot et al., 1999. <i>Sabia</i> sp.; L12662.2; Hoot et al., 1999.	

AF093728; Hoot and Douglas, 1998. *R. merophylla*; U79173.1; Hoot and Douglas, 1998. *Stenocarpus sinuatus*; U79174.1; Hoot and Douglas, 1998. *Telopea speciosissima*; U79175.1; Hoot and Douglas, 1998. *Xylozum pyriforme*; U79176.1; Hoot and Douglas, 1998.

Ranunculales

Berberidaceae. *Achlys triphylla*; L75868.2; —. *Berberis thunbergii*; AF139878; —. *Bongardia chrysogonum*; L75870.2; —. *Caulophyllum thalictroides*; AF190442; Hoot et al., 1999. *Caulophyllum robustum*; AF190441; —. *Diphylleia cymosa*; L75866.2; —. *Dysosma versipellis*; AF079454; —. *Epimedium koreanum*; L75869.2; —. *Holboellia parviflora*; AF398182; —. *H. grandiflora*; AF398181; —. *Jeffersonia diphylla*; L75867.2; —. *Mahonia bealei*; L75871.2; —. *Nandina domestica*; L75843.1; Hoot et al., 1999. *Podophyllum peltatum*; AF203488; Hoot et al., 1999. *Podophyllum emodi*; AF203487; —. *Ranzania japonica*; L75853.2; —. *Sinopodophyllum hexandrum*; AF079455; —.

Circaeasteraceae. *Circaeaster agrestis*; AF093720; Hoot et al., 1999.

Kingdoniaceae. *Kingdonia uniflora*; AF093719; Hoot et al., 1999.

Eupteleaceae. *Euptelea pleiosperma*; AY048174; —. *E. polyandra*; L12645.1; Hoot et al., 1999.

Lardizabalaceae. *Akebia trifoliata*; AF335305; Hoot et al., 1999. *Archakia apetala*; AF335306.1; —. *Boquila trifoliata*; D85690.1; Hoot et al., 1999. *Decaisnea fargesii*; L37916.2; Hoot et al., 1999. *Holboellia grandiflora*; AF398181.1; Hoot et al., 1999. *Lardizabala biternata*; L37919.2; Hoot et al., 1999. *Sargentodoxa cuneata*; AF093731; Hoot et al., 1999. *Sinofranchetia chinensis*; L37921.2; Hoot et al., 1999. *Stauntonia cavaleriana*; AY048173.1; Hoot et al., 1999. *S. hexaphylla*; L37922.2; Hoot et al., 1999. *S. duclouxii*; AF398183; Hoot et al., 1999.

Menispermaceae. *Abuta grandifolia*; DQ099443; Hoot et al., 1999. *Antizoma angustifolia*; DQ099437; *Chasmantera dependens*; DQ099445; —. *Cissampelos pareira*; AF197590; Hoot et al., 1999. *Cocculus trilobus*; D85696.1; Hoot et al., 1999. *Menispermum canadense*; AF190437; Hoot et al., 1999. *M. dauricum*; AF190436; —. *Pericampylus glaucus*; DQ099442; —. *Sciadotenia ramiflora*; DQ099439; Hoot et al., 1999. *Tinospora caffra*; L37923.2; Hoot et al., 1999.

Papaveraceae. *Argemone mexicana*; U86621.1; Hoot et al., 1997. *Corydalis nobilis*; AF093722; Hoot et al., 1997. *Dicentra eximia*; L37917.2; —. *Dicranostigma franchetiana*; U86624.1; Hoot et al., 1997. *Dendromecon rigidum*; U86623.1; Hoot et al., 1997. *Eschscholzia californica*; U86625.1; Hoot et al., 1997. *Glaucium flavum*; U86626.1; Hoot et al., 1997. *Hunnemannia fumariifolia*; U86627.1; Hoot et al., 1997. *Hypecoum imberbe*; U86628.1; —. *Macleaya cordata*; U86629.1; Hoot et al., 1997. *Papaver orientale*; L08764.1; Hoot et al., 1997. *Platystemon californicus*; U86630.1; Hoot et al., 1997. *Romneya coulteri*; U86632.1; Hoot et al., 1997. *Sanguinaria canadensis*; L01951.2; Hoot et al., 1997. *Stylophorum diphyllum*; U86633.1; Hoot et al., 1997.

Pteridophyllaceae. *Pteridophyllum racemosum*; U86631.1; Hoot et al., 1997.

Ranunculaceae. *Aconitum artemisiaefolium*; DQ099435; Hoot et al., 1997. *Actaea laciniata*; DQ099449; Hoot et al., 1997. *Aquilegia brevistyla*; DQ099444; Hoot et al., 1997. *Asteropyrum cavaleriei*; AF079453; *Bessia calthifolia*; AF079452; —. *Caltha appendiculata*; AF307908; Hoot et al., 1997. *Caltha palustris*; L02431.2; Hoot et al., 1997. *Clematis* sp.; AF193972; Hoot et al., 1997. *Coptis trifolia*; AF093730; Hoot et al., 1997. *Glaucidium palmatum*; L75848.2; Hoot et al., 1997. *Helleborus niger*; DQ099436; Hoot et al., 1997. *Hydrastis canadensis*; L75849.2; Hoot et al., 1997. *Myosurus minimus*; DQ099441; Hoot et al., 1997. *Psychrophila novae-zealandiae*; AF307907; —. *Ranunculus trichophyllus*; L08766.1; Hoot et al., 1997. *Thalictrum cultratum*; DQ099447; Hoot et al., 1997. *Xanthorhiza simplicissima*; L12669.1; Hoot et al., 1997.

Core eudicots

Aextoxicaceae. *Aextoxicon punctatum*; X83986.1; Soltis et al., 2000.

Berberidopsidaceae. *Berberidopsis corallina*; AJ235773.1; Soltis et al., 2000.

Dilleniaceae. *Dillenia indica*; L01903.2; Soltis et al., 2000. *Curatella americana*; AJ419729.1; —.

Gunnerales

Gunneraceae. *Gunnera hamiltonii*; AF093724; Soltis et al., 2003. *G. lobata*; AF307919.1; Soltis et al., 2003.

Myrothamnaceae. *Myrothamnus flabellifolius*; AF060707.1; Soltis et al., 2003.

Caryophyllales

Achatocarpaceae. *Phaulothamnus spinescens*; M97887.1; Cuénoud et al., 2002.

Aizoaceae. *Delosperma echinatum*; AJ235778.1; Cuénoud et al., 2002.

Amaranthaceae. *Amaranthus tricolor*; AB050127.1; Cuénoud et al., 2002.

Ancistrocladaceae. *Ancistrocladus korupensis*; AF206733.1; Cuénoud et al., 2002.

Asteropeiaceae. *Asteropeia micrasta*; AF206737.1; Cuénoud et al., 2002.

Basellaceae. *Basella alba*; M62564.1; Cuénoud et al., 2002.

Cactaceae. *Pereskia aculeata*; AF206805.1; Cuénoud et al., 2002.

Caryophyllaceae. *Dianthus caryophyllus*; M77699.1; Cuénoud et al., 2002.

Didiereaceae. *Alluaudia procera*; M62563.1; —.

Dioncophyllaceae. *Triphyophyllum peltatum*; Z97637.1; Cuénoud et al., 2002.

Droseraceae. *Drosera adelae*; AY096107.1; Cuénoud et al., 2002.

Drosophyllaceae. *Drosophyllum lusitanicum*; L01907.2; —.

Frankeniaceae. *Frankenia pulverulenta*; Z97638.1; Cuénoud et al., 2002.

Gisekiaceae. *Gisekia pharnacioides*; M97890.1; Cuénoud et al., 2002.

Halophytaceae. *Halophytum ameghinoi*; AJ403024.1; —.

Molluginaceae. *Mollugo verticillata*; M62566.1; Cuénoud et al., 2002.

Nepenthaceae. *Nepenthes alata*; L01936.2; Cuénoud et al., 2002.

Nyctaginaceae. *Bougainvillea glabra*; M88340.1; Cuénoud et al., 2002.

Physenaceae. *Physena* sp.; Y13116; Cuénoud et al., 2002.

Phytolaccaceae. *Phytolacca americana*; M62567.1; Cuénoud et al., 2002.

Plumbaginaceae. *Plumbago auriculata*; M77701.1; Cuénoud et al., 2002.

Polygonaceae. *Polygonum aviculare*; AF297127.1; Cuénoud et al., 2002.

Portulacaceae. *Portulaca grandiflora*; M62568.1; Cuénoud et al., 2002.

Rhabdodendraceae. *Rhabdodendron amazonicum*; Z97649; Cuénoud et al., 2002.

Sarcobataceae. *Sarcobatus vermiculatus*; AF132088; Cuénoud et al., 2002.

Simmondsiaceae. *Simmondsia chinensis*; AF093732; Cuénoud et al., 2002.

Stegnospermataceae. *Stegnosperma halimifolium*; M62571; Cuénoud et al., 2002.

Tamaricaceae. *Tamarix pentandra*; Z97650.1; Cuénoud et al., 2002.

Santalales

Loranthaceae. *Gaiadendron punctatum*; L26072.1; —.

Misodendraceae. *Misodendron brachystachyum*; L26074.1; —.

Olaaceae. *Heisteria parvifolia*; AJ131771.1; —.

Opiliaceae. *Opilia amentacea*; L26076.1; Cuénoud et al., 2002.

Santalaceae. *Santalum album*; L26077.1; Cuénoud et al., 2002.

Saxifragales

Altingiaceae. *Altingia* sp.; AF061996.1; Fishbein et al., 2001.

Cercidiphyllaceae. *Cercidiphyllum japonicum*; L11673.1; Fishbein et al., 2001.

Crassulaceae. *Penthorum sedoides*; L11197; Fishbein et al., 2001.

Daphniphyllaceae. *Daphniphyllum* sp.; L01901.2; Fishbein et al., 2001.

Grossulariaceae. *Ribes aureum*; L11204.2; Fishbein et al., 2001.

Haloragaceae. *Myriophyllum exalbescens*; L11195; Fishbein et al., 2001.

Hamamelidaceae. *Corylopsis pauciflora*; AF060710.1; Fishbein et al., 2001.

Iteaceae. *Itea virginica*; L11188.2; Fishbein et al., 2001.

Paoniaceae. *Paeonia suffruticosa*; AF274595; Fishbein et al., 2001.

Saxifragaceae. *Saxifraga stellaris*; AF374732; Fishbein et al., 2001.

Rosids

Aphloiaceae. *Aphloia theaeformis*; AF206735.1; Soltis et al., 2000.

Ixerbaceae. *Ixerba brexioides*; AF084475; Soltis et al., 2000.

Picramniaceae. *Picramnia polyantha*; AF127025; —.

Vitaceae. *Vitis rotundifolia*; AJ419718.1; Soltis et al., 2000.

Crossosomatales

Crossosomataceae. *Crossosoma bigelovii*; AY101844.1; Sosa and Chase, 2003.

Stachyuraceae. *Stachyurus praecox*; AJ235794.1; Sosa and Chase, 2003.

Staphyleaceae. *Staphylea trifolia*; AJ238406.1; Sosa and Chase, 2003.

Geraniales

Francoaceae. *Francoa sonchifolia*; L11184.2; Sosa and Chase, 2003.

Geraniaceae. *Pelargonium exstipulatum*; L14704.2; Sosa and Chase, 2003.

Hypseocharitaceae. *Hypseocharis* sp.; L14699.2; —.

Ledocarpaceae. *Wendtia gracilis*; L14708; —.

Melanthaceae. *Bersama lucens*; AJ235774.1; Soltis et al., 2000.

Vivianiaceae. *Viviania marifolia*; L14707.2; Soltis et al., 2000.

Asterids

Cornales

- Cornaceae. *Cornus walteri*; L11220.2; Xiang et al., 2002.
 Curtisiaceae. *Curtisia dentata*; L11222.2; Xiang et al., 2002.
 Grubbiaceae. *Grubbia tomentosa*; Z83141.1; Xiang et al., 2002.
 Hydrangeaceae. *Hydrangea macrophylla*; L11187.2; Xiang et al., 2002.
 Hydrostachyaceae. *Hydrostachys multifida*; U17879.1; Xiang et al., 2002.
 Loasaceae. *Loasa loxensis*; U17876.1; Xiang et al., 2002.

Ericales

- Actinidiaceae. *Actinidia chinensis*; L01882; Soltis et al., 2000.
 Balsaminaceae. *Impatiens discolor*; AB043533.1; Soltis et al., 2000.
 Clethraceae. *Clethra arborea*; AF421088; Soltis et al., 2000.
 Cyrillaceae. *Cyrilla racemiflora*; L01900; Soltis et al., 2000.
 Diapensiaceae. *Berneuxia thibetica*; AY049795; Soltis et al., 2000.
 Ebenaceae. *Diospyros virginiana*; L12613; Soltis et al., 2000.
 Ericaceae. *Vaccinium macrocarpon*; L12625; Soltis et al., 2000.

- Fouquieriaceae. *Fouquieria columnaris*; Z80210; Soltis et al., 2000.
 Lecythidaceae. *Barringtonia asiatica*; AJ247618; Soltis et al., 2000.
 Maesaceae. *Maesa myrsinoides*; Z80203; Soltis et al., 2000.
 Marcgraviaceae. *Marcgravia nepenthoides*; AF303129.1; Soltis et al., 2000.
 Myrsinaceae. *Ardisia crenata*; L12599; Soltis et al., 2000.
 Pentaphragmaceae. *Pentaphragma eurycoides*; AJ428891; Soltis et al., 2000.
 Polemoniaceae. *Phlox longifolia*; AF206809; Soltis et al., 2000.
 Primulaceae. *Androsace erecta*; AF395004; Soltis et al., 2000.
 Roridulaceae. *Roridula gorgonias*; L01950.2; Soltis et al., 2000.
 Sapotaceae. *Chrysophyllum oliviforme*; L12607.2; Soltis et al., 2000.
 Sarraceniacae. *Darlingtonia californica*; L42211.2; Soltis et al., 2000.
 Styracaceae. *Styrax americanus*; L12623; Soltis et al., 2000.
 Symplocaceae. *Symplocos paniculata*; L12624; Soltis et al., 2000.
 Tetrameristaceae. *Tetramerista* sp.; Z80199.1; Soltis et al., 2000.
 Theaceae. *Camellia japonica*; L12602; Soltis et al., 2000.
 Theophrastaceae. *Clavija eggersiana*; L12608; Soltis et al., 2000.

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