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Simultaneous parsimony jackknife analysis of 2538 *rbc*L DNA sequences reveals support for major clades of green plants, land plants, seed plants and flowering plants

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Abstract: The ever-larger data matrices resulting from continuing improvements in DNA sequencing techniques require faster and more efficient methods of phylogenetic analysis. Here we explore a promising new method, parsimony jackknifing, by analyzing a matrix comprising 2538 sequences of the chloroplast gene rbcL. The sequences included cover a broad taxonomic range, from cyanobacteria to flowering plants. Several parsimony jackknife analyses were performed, both with and without branch-swapping and multiple random addition sequences: 1) including all positions; 2) including only first and second codon positions; 3) including only third positions; and 4) using only transversions. The best resolution was obtained using all positions. Removal of third positions or transitions led to massive loss of resolution, although using only transversions somewhat improved basal resolution. While branch-swapping improved both resolution and the support found for several groups, most of the groups could be recovered by faster simple analyses. Designed to eliminate groups poorly supported by the data, parsimony jackknifing recognizes 1400 groups on the basis of all *rbcL* positions. These include major taxa such as green plants, land plants, flowering plants, monocots and eudicots. We include appendices of supported angiosperm families, as well as larger groups.

The DNA sequence of the chloroplast encoded *rbcL* gene is the most widely used for phylogenetic reconstruction of plants. The gene codes for the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase, commonly abbreviated RuBisCo, the enzyme responsible for carbon dioxide fixation in photosynthesis. After its initial use for phylogenetic reconstruction a decade ago (RITLAND & CLEGG 1987, ZURAWSKI & CLEGG 1987), it soon became the first choice in plant molecular systematics and today thousands of species have been sequenced. The best-known study is the analysis of 500 *rbcL* sequences compiled by a team of 42 systematists (CHASE & al. 1993). This and a number of simultaneously published studies highlighted the problems of analyzing large data sets (e.g. DUVALL & al. 1993, OLMSTEAD & al. 1993).

Algorithms for finding most parsimonious trees, as implemented in Hennig 86 (FARRIS 1988), MEGA (KUMAR & al. 1993), NONA (GOLOBOFF 1993) and PAUP (SWOFFORD 1993), were developed for working on small data sets. For the everlarger matrices that are now being produced, they are inefficient and unacceptably time-consuming. Since only superficial searches are possible with these programs, the resulting trees are often far from optimal, and the consensus is likely to be misleadingly well resolved, being based on only a few of the many possible solutions.

One of the problems is the frequent occurrence of multiple solutions, sometimes so numerous that they exceed what is possible to store and evaluate with available computer technology. This problem was encountered in the 500-sequence analysis by CHASE & al. (1993), which suffered from 'unquestionable absence of many trees at the same level of optimality'. Another problem in phylogenetic reconstruction is the assessment of support for individual clades. Two commonly used procedures for estimating branch support are bootstrapping (FELSENSTEIN 1985) and Bremer support analysis (BREMER 1988, 1994; KÄLLERSJÖ & al. 1992), but they are time-consuming and unrealistic for large data sets given currently available software.

Until recently, the only available solution to these problems has been to reduce the size of the data matrix, i.e. to discard information. A smaller sample of taxa can be selected to save time, or an attempt can be made to make structure more obvious by a priori removing putatively homoplastic characters such as third codon positions or transitions. Discarding information is risky, however. Analyses of data sets with a small number of taxa covering a wide taxonomic range, may give different results depending on exactly which taxa have been chosen (OLMSTEAD & al. 1993, FARRIS & al. 1996). Removing transitions or third positions means fewer informative positions and a potential loss of resolution.

Recently, a more promising approach has been suggested. The jackknifing procedure described by FARRIS & al. (1996) was developed to deal specifically with the problems of analyzing large data sets. The method combines jackknifing with fast, efficient parsimony algorithms, and a new way of storing trees. The multiple trees produced through jackknifing are combined to a single tree with well supported clades only, thereby solving the problem of multiple solutions and providing a fast method for assessment of branch support. This procedure is implemented in the parsimony jackknife program *Jac* (FARRIS 1996), with which it is possible to analyze matrices even larger than the 500-sequence matrix of CHASE & al. (1993).

Here we present a parsimony jackknife analysis of 2538 rbcL sequences, possibly the largest phylogenetic analysis ever undertaken. The purpose of this analysis is threefold, (1) to test the efficiency of parsimony jackknifing on an extremely large data set, (2) to explore the possibility of simultaneously analyzing phylogenetic relationships in widely different groups at widely different levels of the taxonomic hierarchy, and (3) to investigate the amount of support from rbcL data for different groups at different levels of the phylogeny of green plants, including the land plants, the seed plants, and the flowering plants in particular.

Data and methods

CHASE & al.'s (1993) original data set was used as a starting point. To this matrix were added sequences compiled from GenBank and from unpublished sequences at the Molecular Systematics Laboratories at Royal Botanic Gardens, Kew, the Swedish Museum of Natural History, Stockholm, the Department of Systematic Botany, Uppsala University, and the Botanical Institute, University of Copenhagen. The number of sequences was limited by computer memory, since there was an upper limit to the size of the data matrix that could be processed with the internal computer memory available (in this particular case, 32 Mb). The sequences were aligned using AssemblyLign, Oxford Molecular Group, Inc. Several sequences from cyanobacteria and green algae had to be omitted due to difficulties in alignment. Only sequences for which alignments were straightforward were included in the final data matrix, which comprises 2538 sequences, 1428 bp long. It should be noted that several sequences are not complete; many lack bases at the 5', or the 3' end. These absent bases have been replaced by the IUPAC code "N". The sequences have not been scrutinized for errors or stop codons. In some cases we have received the same sequence from different sources, e. g. sequences included in the original matrix of CHASE & al. (1993) were later extracted also from GenBank. Identical, superfluous sequences have been removed, but in a few cases there have been minor discrepancies between the two versions, and we have then retained both in the matrix.

Two versions of the Jac program (FARRIS 1996) were used. Both versions randomly delete a fraction of e^{-1} of the characters, perform a quick heuristic parsimony search, and reiterate the two steps a specified number of times (FARRIS & al. 1996). The original version uses a fast search, without branch-swapping. The modified, later version has an even faster tree-building algorithm, which allows a branch swapper to be used at each replication (FARRIS & al. 1998). Also, with this version it is possible to perform several random-addition sequences per replicate.

For the original *Jac* analysis, without branch-swapping, 1000 replications were used, except in the analyses of reduced data (see below). The output consists of a single tree comprising those clades present in at least 50% of the trees generated by the 1000 searches (see FARRIS & al. 1996 for additional explanations). The first complete analysis comprised the entire sequences with all positions (first, second, and third) and all changes (transversions and transitions) included and weighted equally. A series of analyses with reduced data sets were also performed. Since the first analysis revealed that jackknife frequencies did not change significantly after 500 replicates, these additional analyses were restricted to 500 rather than 1000 reiterations. The second analysis used only the first and second positions in the sequences and a third analysis only the third position. A fourth analysis utilized transversions only, transitions being ignored throughout the sequences (in all positions). All analyses were run on a 133 MHz Pentium.

Using the modified, new version of *Jac* with branch-swapping, the same analyses were performed. To ensure that the addition order of taxa did not influence the results, five random-addition sequences were performed for each replicate. Both Pentiums (133–200 MHz) and PowerPCs (66 MHz) were used for these analyses.

Results

Our initial analysis using parsimony jackknifing without branch-swapping required 99 hours on our 133 MHz Pentium. The *Jac* version used for the analysis with branch-swapping has a stop-restart function, making it possible to move the analysis between computers; our use of this feature precludes a precise time estimate. A relevant comparison can, however, be made on the basis of per replicate speed. The original *Jac* required 356 seconds per replicate. With the new version with branch-swapping the time is increased to approximately 1304.5 seconds.

The tree resulting from analysis of all positions, with branch-swapping, includes 1400 supported groups. The tree has a total of 67482 steps in informative characters, of these 15602, 12335, and 39545 are distributed among the 1st, 2nd and 3rd positions, respectively. 46098 of the steps comprise transversions, whereas only 21384 are transitions.

When comparing the results from the original and the new *Jac* it is obvious that including a combination of branch-swapping and random-addition sequences does make a positive difference (Table 1). A comparison shows that the first tree has 83 groups not present in the second tree, whereas the second tree has 124 unique groups. Most of the differences concern groups with low jackknife frequencies (<60%). The groups of the first tree are generally small, 51% of them consist of two or three taxa. Only 11% have ten or more taxa, with a largest group of 27. In the second, branch-swapped tree 30% of the new groups have two or three taxa, and 41% ten or more. For groups present in both trees the jackknife frequencies are often approximately the same, especially for smaller groups. For deep nodes, setting off more ancient groups, branch-swapping tends to increase jackknife frequencies (see Table 1). Analyses of the reduced data sets (see below) show a

	All data	1st & 2nd positions	3rd position	Transversions only
Number of positions				
Total number of positions	1428	952	476	1428
Informative positions	1235	764	471	1064
Resolution of tree				
Number of supported clades	\$			
without branch-swapping	1359	410	1284	655
with branch-swapping ¹	1400	431	1327	706
Support for major groups				
(jackknife frequencies with	out branch-s	wapping)		
Streptophytes	55%	64%	_	71%
Land plants	70%	_	58%	80%
Seed plants	75%	_	55%	69%
Flowering plants	87%	. —	87%	61%
(jackknife frequencies with	branch-swa	pping ¹)		
Streptophytes	62%	76%	_	80%
Land plants	96%	_	97%	90%
Seed plants	98%	85%	65%	99%
Flowering plants	100%		100%	99%

Table 1. Some results from the analyses using all, first and second positions, third position, or transversions only. "–" indicates support of <50%. ¹Combined with five random addition sequences per replicate

similar tendency, and in all of them branch-swapping led to more resolved trees. Since the trees resulting from branch-swapping analyses represent better hypotheses of phylogeny, we will limit our discussion to these below.

The reduced analyses comprising the first and second positions, the third position, or transversions only, yielded less resolution but no conflicting results, except for a few clades being weakly supported by low jackknife values (below 55%). As with the full data set, branch-swapping resulted in more resolved trees, with higher jackknife frequencies for deeper nodes. Because we feel there is no more phylogenetic information to be gained by these approaches, we restrict our discussion to the resulting lower resolution and support for some major groups (Table 1). Using only the first and second positions reduces resolution drastically (to less than 1/3), with 431 supported clades compared to 1400 using all positions. This approach also fails to recognize the major groups of land plants and flowering plants. Using only the third position reduces resolution marginally, from 1400 to 1327 supported clades. Streptophytes are among the lost clades. Using only transversions reduces resolution by half, to 706 supported clades. The major groups of streptophytes, land plants, seed plants and flowering plants are all recognized, but resolution at higher levels is poor.

Obviously, it is not possible to reproduce the entire tree of 2538 sequences here. Details of the tree may be obtained from the first author upon request. Figure 1 shows the basal branches of the tree, with the flowering plants (angiosperms) as a single branch. Resolution is not shown within genera and orders of green algae (*Volvocales* and *Charales*) and within several families of ferns, cycads, and conifers. The trees show clades with jackknife frequencies of at least 50% and provide also the number of sequences within each group. The whole tree is rooted between cyanobacteria and eukaryotes, a split supported at the 100% level. Streptophytes, i.e. the green algal lineage leading to land plants ('charophytes' in a wide sense) and the land plants, are supported by a jackknife value of 62.4%, land plants by 96.8%, seed plants by 98.9%, and flowering plants by the maximum value of 100%. Mosses are not supported as monophyletic, but the sampling of this group is extremely limited. Ferns and conifers are also not supported as clades, but the possibility that they represent monophyletic groups is not contradicted by the tree.

Several details of relationships within the flowering plants are given in Appendices 1–5. The flowering plants are represented by 2230 sequences. There are 401 families included, as listed in Appendices 1–4. Appendix 5 lists a number of monophyletic groups including two or more families. Several of these groups correspond to commonly recognized orders. A number of larger groups are also supported, for example, monocots, eudicots, rosids (*Rosidae*), and caryophyllids (*Caryophyllidae*, the families of the order *Caryophyllales* and several others). Asterids do not appear as a clade in the tree, but their possible monophyly is not contradicted by the tree.

Discussion

Analysis. Our study shows that parsimony jackknifing makes it perfectly feasible to work with large data sets. The second analysis of the complete data set, using branch-swapping with 1000 replicates, each replicate with five randomaddition replicates, required approximately two weeks of computer time. Improvements in algorithms, computer programming, and hardware developments will no doubt speed up the calculations even more. For example, in 1995, a parsimony jackknife re-analysis of CHASE & al.'s (1993) original data set was presented by VICTOR ALBERT at the San Diego AIBS meeting. That analysis took 3.5 hours to execute on a 133 MHz Pentium (1000 replicates, without branchswapping). Today, with the new *Jac*, the same analysis takes 15 minutes on a 200 MHz Pentium Pro.

The efficiency of the new *Jac* allowed us to incorporate branch-swapping and random-addition sequences in the analysis. When applied to our 2538-sequence matrix some groups from the original tree were lost, whereas several new groups were recognized. Many of these groups have low support (50-55%), and their gain or loss may be attributed to sampling error. A closer comparison shows that the lost groups are often found at the tips of the tree, including only a few taxa, whereas gained nodes are more evenly distributed over the tree, and several represent more ancient groups. The net result is that branch-swapping in combination with random-addition sequences has resulted in a better resolved tree (1400 groups, compared to 1359 without branch-swapping). Among the new groups are monocots and eudicots (see below), and, as can be seen in Table 1, jackknife frequencies for ancient groups are considerably higher. The same effect can be seen in the analyses using subsets of the matrix. For instance, when analyzing only 1st and 2nd positions without branch-swapping, the group "seed plants" is not recognized, but with branch-swapping it is set off by a frequency of 85%.

Whether or not branch-swapping and/or random-addition sequences will make a difference depends on the data. In many cases they affect the jackknife frequencies marginally, but sometimes branch-swapping is necessary to obtain accurate results. BENGT OXELMAN (pers. comm.) has pointed out that the *hennig* algorithm used in the old *Jac* tends to underestimate support for ancient groups set off by few characters. This certainly seems to be the case in our present study. In rare cases it may also overestimate support (PABLO GOLOBOFF, pers. comm.). A detailed discussion of jackknifing and branch-swapping is presented elsewhere (FARRIS & al. 1998). We recommend the use of more exact calculations, if possible, since they improve accuracy and provide more robust hypotheses of relationships. However, for extremely large data sets (> 5000 taxa), "simple" jackknife parsimony is still the only choice.

Our taxon sampling includes representatives from broadly divergent groups at widely different levels of the taxonomic hierarchy. When we started collecting data we feared that the diverse, unbalanced sampling would inevitably lead to increased homoplasy and loss of resolution. This has not been the case. Most of the sequences have been used in more restricted studies, of specific taxonomic groups, e.g. the *Asterales* (GUSTAFSSON & BREMER 1997) or the *Malpighiales* (FAY & al. 1997). When comparing well supported groups from those studies with our tree there is a general agreement both in numbers of groups and jackknife frequencies (Tables 2, 3). In fact, support often increases with addition of more information. Poor sampling may lead to spurious groupings, as has been discussed in earlier papers (CHASE & al. 1993, OLMSTEAD & al. 1993, FARRIS & al. 1996).

Jackknife%		Таха		Taxon
2538-tree	46-tree	2538-tree	46-tree	
100%	100%	2	2	Unnamed group
100%	99.9 %	2	2	Argophyllaceae
100%	99.9 %	3	3	Alseuosmiaceae
98.7%	96.9%	9	7	Campanulaceae
97.9%	99.0%	2	2	Unnamed group
97.7%	97.9%	2	2	Stylidiaceae
91.7%	82.4%	3	2	Unnamed group
89.6%	78.1%	10	2	Unnamed group
85.5%	67.9%	3	2	Unnamed group
84.0%	88.0%	8	6	Unnamed group
80.9%	70.8%	26	4	Asteraceae
76.0%	95.8%	23	2	Unnamed group
74.6%	81.9%	42	11	Unnamed group
64.3%	<50%	13	4	Goodeniaceae
60.5%	64.1%	2	2	Unnamed group
54.3%	56.0%	3	3	Calyceraceae
52.2%	<50%	68	36	Asterales
51.7%	<50%	5	4	Menyanthaceae
<50%	64.3%	29	7	Unnamed group

Table 2. Comparison of jackknife frequencies (no branch swapping applied) in compatible groups in the 2538-sequence analysis and in a 46-sequence analysis of the order *Asterales* (GUSTAFSSON & BREMER 1997). Numbers in bold represent exactly the same sequences in both analyses

RICE & al. (1997: 559) have objected to parsimony jackknifing on the astonishing grounds that it requires "abandoning" the parsimony criterion! They seem to have arrived at this idea by misinterpreting the fact that a parsimony jackknife tree need not itself be a most-parsimonious tree. For ambiguous data, the parsimony jackknife tree instead represents a most-parsimonious tree from which poorly supported groups have been removed. However, retaining only better-founded groups hardly means that parsimony has been abandoned. Nor did RICE & al. (1997) really believe so themselves, for they counted among "reasons for preferring parsimony" that several most-parsimonious trees "can be reduced to a consensus tree to give an indication of uncertainty in an analysis."

A consensus tree consists only of groups with positive Bremer support and need not itself be a most-parsimonious tree. RICE & al. (1997) who themselves employed a consensus, could then scarcely object to removing poorly-supported groups. To further undercut their own position, they went on to point out that, "Parsimony jackknifing yields a single, often unresolved tree containing clades that are expected to appear in the consensus of all most-parsimonious trees". In their view, apparently, removing poorly-supported groups is beneficial – unless parsimony jackknifing is used to accomplish this more easily! Their opposition to resampling, however, is even weaker than that. Parsimony jackknifing provides an

Jackknife%		Taxa		Taxon
2558-tree	31-tree	2538-tree	31-tree	
100%	100%	17	2	Malpighiaceae
100%	100%	8	2	Unnamed group
100%	99.7%	4	3	Linaceae
100%	99.6%	14	3	Violaceae
100%	99.6%	5	4	Unnamed group
100%	99.6%	4	4	Unnamed group
99.9%	99.9%	2	2	Unnamed group
99.9%	99.1%	2	2	Ochnoideae
99.7%	94.7%	6	8	Unnamed group
99.7%	90.5%	3	3	Quiinaceae
93.2%	94.8%	2	2	Unnamed group
82.7%	72.1%	2	2	Passifloraceae
81.3%	95.9%	2	2	Humiriaceae
74.4%	96.4%	4	2	Unnamed group
66.8%	61.3%	2	2	Unnamed group
60.9%	77.9%	11	2	Unnamed group
<50%	53.5%	>50	3	Unnamed group
<50%	50.6%	6	3	Unnamed group

Table 3. Comparison of jackknife figures (no branch swapping applied) in compatible groups in the 2538-sequence analysis and in a 31-sequence analysis of the order *Malpighiales* (FAY & al. 1997). Numbers in bold represent exactly the same sequences in both analyses

indication of the different degrees of support for groups that are retained, while the consensus tree by itself does not.

1st, 2nd, and 3rd positions. The total number of changes in informative characters, calculated from the parsimony jackknife tree is 67482. Of these 23.1, 18.3, and 58.6% occur in the 1st, 2nd, and 3rd position, respectively. It is frequently assumed that when changes occur more often in the third position, they are likely to contain more homoplasy. Following the same reasoning, if first and second positions are more conservative they should provide more phylogenetically reliable information. This assumption is not supported by our analysis. On the contrary, even though changes are more frequent in the third position, a large proportion of the groups found in analysis of the complete data set is lost when third positions are omitted (Table 1). Comparatively few groups are lost if the data are restricted to the third position only (from 1400 to 1327 clades). The phylogenetic information conveyed by the third position becomes even more obvious when the number of resolved clades is compared to the number of variable (informative) positions in the data. The first and second positions together contain 764 informative positions which support 431 clades, whereas 471 informative third positions support 1327 clades (Table 1). It seems that nothing is to be gained by excluding part of the data, especially not third positions.

Transversions and transitions. It is also often assumed that transitions should contain more homoplasy and that transversions should provide more reliable

phylogenetic information. The present study does not bear out this assumption. On our tree, transversions (46098 steps) occur more than twice as frequently as transitions (21384 steps). Further, an analysis with transversions only (1064 informative characters) results in much lower resolution (Table 1; from 1400 to 706 clades). There is a massive loss of resolution within the angiosperms. However, support for the very old, phylogenetically basal groups seems to increase if the data are restricted to transversions only, e.g. support for streptophytes increases from 62% to 80%. Hence, this may be a possible strategy for analyzing the oldest relationships, but not to be recommended in analyses restricted to land plants or subgroups of green algae.

Green plants. MANHART (1994) analyzed a set of 38 green plant rbcL sequences focusing on the green algal relatives of land plants and the pteridophytes (ferns and their allies). He concluded that analysis of these relationships with the *rbcL* gene seemed problematic, presumably due to limited phylogenetic information in the sequences but possibly also to sampling problems. Sampling of green algae is also rather limited in our data set and resolution among the green algae is low. There is good support for two well established orders, Volvocales and Charales. The latter order is one of the last algal branches in the green algal lineage leading to the land plants. This lineage is usually referred to as the 'charophytes' (MATTOX & STEWART 1984, MANHART 1994, MISHLER & al. 1994), and includes not only Charales but several other groups, e.g. Coleochaete, Klebsormidium, and the conjugates (Netrium, Roya, Cylindrocystis, Mesotaenium, Mougeotia, Zygnema, Spirotaenia, Sirogonium, and Spirogyra in Fig. 1). Charophytes and land plants together form the streptophytes (JEFFREY 1982, BREMER 1985). This group is recognized by the *rbc*L data, but supported with a comparatively low jackknife value of 62.4%. The sister group of the land plants according to our analysis is Coleochaete and Charales together, this latter pair being supported by a moderately high jackknife value of 79.1%.

Land plants. The old lineages of the land plants, i.e., the major bryophyte and pteridophyte groups, are not well represented in the data set. Resolution is also low, involving a polytomy of 15 land plant lineages (Fig. 1), and some are weakly supported (a jackknife frequency of less than 60%) and possibly spurious groups, such as the unexpected grouping of the two sequences representing liverworts (Bazzania) and sphenopsids (Equisetum). (In the tree based on transversions this grouping is not present. Instead, Bazzania appears as the sister to all other land plants.) Bryophyte and vascular land plant interrelationships were reviewed by MISHLER & al. (1994) but their review was based on other sources of data. A more comprehensive analysis of major bryophyte and pteridophyte interrelationships based on *rbcL* sequences remains to be done. Within the ferns, there are many more *rbcL* sequences available, and a comprehensive analysis was done by HASEBE & al. (1995). Their trees are more resolved than the jackknife tree, which does not support a fully resolved phylogeny of ferns based on *rbcL* sequences. There is, however, a general agreement with several larger, well supported groups, such as the water ferns (Marsileaceae, Azollaceae, Salviniaceae; 97.9%), the tree ferns together with three smaller families (Cyatheaceae, Dicksoniaceae, Loxomataceae, Metaxyaceae, Plagiogyriaceae; 95.3%), and the higher leptosporangiate ferns of ten families from Aspleniaceae to Polypodiaceae in Fig. 1 (99.6%; cf. HASEBE & al.



1995). Two of the families stand out as grossly paraphyletic, *Dryopteridaceae* and *Dennstaedtiaceae* (WOLF 1995). The former constitute a paraphyletic assemblage at the base of the higher leptosporangiate ferns, whereas the latter are a paraphyletic assemblage at the base of a more inclusive group, which includes also the water ferns and tree ferns and is supported by 89% of the replicates in the jackknife tree (Fig. 1).

Seed plant interrelationships have been the subject of much controversy (e.g. Doyle & al. 1994, NIXON & al. 1994). Our jackknife analysis identifies three well supported monophyletic groups of extant seed plants, angiosperms (or flowering plants; 100%), Gnetales (Ephedra, Gnetum, Welwitschia; 100%), and more unexpectedly, all other seed plants together or 'gymnosperms s.str.', i.e. Ginkgo, cycads, and conifers, supported at 93.4%. Conifers are not supported as monophyletic but a resolution with *Pinaceae* as sister group to other conifers is not contradicted by the tree. Taxus, Amentotaxus, and Cephalotaxus are well nested in the conifers, in a group together with Cupressaceae and Taxodiaceae (99.9%). The latter family is paraphyletic. In the jackknife tree the Gnetales are sister to all other seed plants, angiosperms and gymnosperms s. str. being sister groups, but this grouping is supported by only 58.1% of the replicates. It is not supported by morphological data, which indicate a sister group relationship between Gnetales and angiosperms. The arrangement in the 2538-sequence jackknife tree, as well as that of the liverwort and Equisetum mentioned above, illustrates that groupings with low jackknife values (e.g. below 60%) may not be reliable.

During the compilation of the present matrix several preliminary analyses were performed while new data were being added. The groups occurring in these smaller data sets are consistent with the tree presented here, with one exception: the interrelationships of angiosperms, conifers, cycads and *Gnetales*. Depending on the taxon sampling, *Gnetales*, conifers, or conifers+*Ginkgo*+cycads have all appeared as the closest sister groups to the angiosperms, although often there is a basal polytomy of all of these groups. In the tree based on transversions only *Gnetales*+conifers+cycads+*Ginkgo* form the sister group of the angiosperms. At this point, it seems unlikely that sequences from *rbcL* alone will tell us which extant seed plants are most closely related to flowering plants.

Flowering plants. The angiosperms come out as a strongly monophyletic group, supported at the maximum 100% level. The two major groups of flowering plants, monocots and eudicots, are also supported as monophyletic, but with the somewhat low values of 66.4 and 60.7, respectively. Eudicots exclude the phylogenetically primitive flowering plants, which form ten unresolved branches at the flowering plant node of Fig. 2. Among them are the two commonly recognized orders *Laurales* and *Magnoliales*. The former is moderately well supported (75.8%) but the latter is only weakly supported (54.0%) and excludes *Canellaceae*

Fig. 1. The deep branches of the jackknife tree from parsimony analysis of 2538 *rbcL* sequences. At each node, the figure is the frequency of jackknife replicates containing that branch (branches appearing in less than 50% of the 1000 replicates are not retained). After each taxon, the first figure is the number of sequences included, the second is the frequency of replicates in which the group appears as a clade. A dash indicates an unsupported taxon that does not appear as a clade (not found in 50% of the 1000 replicates)



and Winteraceae, which are sometimes included in Magnoliales. The alleged Magnolia fossil, also placed outside Magnoliales in Fig. 2, is a sequence reported to have been obtained from a Miocene species (GOLENBERG & al. 1990). An interesting clade consists of the orders Nymphaeales (water lilies; Nymphaeaceae, Barclayaceae, Cabombaceae) and Illiciales (Illiciaceae, Schizandraceae) together with the two isolated families Austrobaileyaceae and Amborellaceae (64.2%). This clade was first identified by OIU & al. (1993) in their rbcL study of magnoliid groups. All clades in Fig. 2 are present in the trees reported by QIU & al., who also discussed various interrelationships among the clades and the sequence of the branching at the base of the tree. For example, in several rbcL analyses (e.g. CHASE & al. 1993, QIU & al. 1993, ALBERT & al. 1994), the genus Ceratophyllum (Ceratophyllaceae) appears as the sister group of all other flowering plants. This and other arrangements of the basal branches are not supported by our jackknife analysis. The rbcL sequences thus do not appear to be informative with respect to the difficult problem of resolving the deepest branches of the angiosperms.

The monocots have been the subject of a number of large-scale cladistic analyses using rbcL sequences, by DUVALL & al. (1993) and CHASE & al. (1995a, b). As expected, the clades supported by our jackknife analysis appear also in the trees reported by these authors, whereas many branches in their trees are absent in our tree; such branches lack support from the jackknife procedure. Acorus (Acoraceae) appears as the sister group of all other monocots, the latter being supported by a jackknife value of 66.9%, a moderately high value, just above the support for the monocots as a whole (including Acorus), which are maintained at 66.4%. The next clade consists of the two orders Arales (Araceae and Lemnaceae, Lemna being nested inside Araceae) and Alismatales (in a wide sense represented by the 11 families listed under Alismatales in Appendix 5) and a single sequence of Tofieldiaceae (from the genus Pleea, usually classified in the tribe Tofieldieae of Melanthiaceae; CHASE & al. 1995a, cf. TAKHTAJAN 1994). The Arales/Alismatales/ Tofieldiaceae clade is not well supported (55.1%), but appears as the sister group of all remaining monocots (excluding Acorus). This major monocot clade, comprising all but Acorus (Acoraceae) and the Arales/Alismatales/Tofieldiaceae clade, is supported at 68.8%. The clade is not well resolved. It comprises a basal polytomy of 20 branches, three of them representing the large orders Asparagales, Liliales and Zingiberales (SMITH & al. 1993), the other 17 branches one or a few families each.

The two large orders Asparagales and Liliales were originally conceived as groups by HUBER (1969) and DAHLGREN & CLIFFORD (1982; also DAHLGREN & al.

Fig. 2. The major branches of flowering plants (angiosperms) in the jackknife tree from parsimony analysis of 2538 *rbcL* sequences. At each node, the figure is the frequency of jackknife replicates containing that branch (branches appearing in less than 50% of the 1000 replicates are not retained). After each taxon, the first figure is the number of sequences included, the second is the frequency of replicates in which the group appears as a clade. A dash indicates an unsupported taxon that does not appear as a clade (not found in 50% of the 1000 replicates). For further resolution of the terminal branches, see Appendix 5

1985), and later as a result of the rbcL analyses modified mainly by transfer of Orchidaceae and Iridaceae from Liliales to Asparagales (DUVALL & al. 1993, CHASE & al. 1995a, RUDALL & CUTLER 1995). Asparagales are here represented by no less than 407 sequences from 27 families. The order is weakly supported (59.5%) and interrelationships among the families are largely unresolved. Liliales with 58 sequences are supported by a jackknife frequency of 67.3%. The order comprises four strongly supported branches, (1) Alstroemeriaceae, Colchicaceae, Luzuriagaceae (in the strict sense comprising Luzuriaga only) and part of Uvulariaceae including the type genus, (2) Melanthiaceae (excluding Aletris of the tribe Narthecieae and Pleea of Tofieldieae=Tofieldiaceae) and Trilliaceae, (3) Calochortaceae, Liliaceae, Philesiaceae, Smilacaceae and several other genera of Uvulariaceae, and (4) Campynemataceae. The first three correspond to the Liliales subgroups a, b, and c of CHASE & al. (1995a) and they are here supported by the jackknife values of 100%, 85.2%, and 96.4%, respectively. At the family level, there is apparently considerable reclassification to be expected within Asparagales and Liliales, nine families of which are indicated to be non-monophyletic by our jackknife analysis (Appendix 3; CHASE & al. 1996, RUDALL & CHASE 1996). These orders comprise also several families well supported as monophyletic. Examples of such families represented by at least ten sequences are Asphodelaceae, Orchidaceae and Themidaceae (Appendix 4).

Except for the large monocot groups shown in Fig. 2 and discussed above, the jackknife analysis does not support any other supraordinal groups of monocots. The subclass *Commelinidae*, for example, is not recognized. There are, however, some suprafamilial groups not corresponding to currently recognized orders or subclasses (Appendix 5). One such group comprises *Cyclanthaceae*, *Pandanaceae*, *Stemonaceae* and *Velloziaceae* (*Stemonales* sensu CHASE & al. 1995b), together supported as monophyletic with a jackknife frequency of 93.0%. Within this group, a sister group relationship between *Cyclanthaceae* and *Pandanaceae* (*Pandanales*) is supported by 98.5%.

Relationships within the eudicots are not well resolved; the eudicot branch of the jackknife tree comprises a polytomy of 48 branches (Fig. 2). Several of these branches consist of single sequences or small groups of various phylogenetically archaic eudicots and asterids. The polytomy also contains several branches representing orders of these groups. In addition to the orders, there are three branches leading to other large, monophyletic groups, viz. the subclasses *Caryophyllidae* (93 sequences), *Rosidae* (642 sequences) and that part of *Asteridae* sometimes referred to as 'asterid I' (CHASE & al. 1993; 267 sequences). *Caryophyllidae* are strongly supported as monophyletic (99.8%), asterid I only moderately so (69.4%), and *Rosidae* are very weakly supported (53.9%).

Among the phylogenetically archaic eudicots (i.e. excluding rosids and asterids), the three orders *Ranunculales* s. 1. (including *Papaveraceae*; 88.4%), *Santalales* (99.4%), and *Trochodendrales* (100%) are well supported as monophyletic. *Caryophyllales* has since long been recognized on its morphological and chemical features, and it is well supported also by the *rbcL* data (95.8%). The two families *Polygonaceae* and *Plumbaginaceae* are usually associated with *Caryophyllales*, and today often classified together with *Caryophyllales* in a subclass *Caryophyllidae* (e.g. CRONQUIST 1981). Molecular data have added several

families to this subclass, outside *Caryophyllales* but alongside *Polygonaceae* and *Plumbaginaceae* (ALBERT & al. 1992, CHASE & al. 1993; cf. Appendix 5). The latter two families are strongly supported as sister groups (99.8%). Two other small families of *Caryophyllidae*, *Asteropeiaceae* and *Physenaceae*, together form a well supported sister group of *Caryophyllales* (97.8%; MORTON & al. 1997a).

The rosids or Rosidae are a weakly supported clade in the jackknife tree (53.9%). Of the 642 sequences, 494 form a weakly supported subclade (55.3%), roughly corresponding to the 'rosid I' of CHASE & al. (1993; cf. Fig. 2 and Appendix 5), except that *Geraniaceae* and some related families are not included (cf. PRICE & PALMER 1993). Several large orders of rosids are recognized, Capparales (94.3%; RODMAN & al. 1993), Myrtales (99.9%; CONTI & al. 1996), Fagales/Juglandales (89.3%), Sapindales (98.2%; FERNANDO & al. 1995), Celastrales (93.7%; cf. SAVOLAINEN & al. 1997), Cucurbitales (94.6%), Urticales (75.1%), and Malpighiales/Violales (72.7%). Urticales form a group together with the families Barbeyaceae, Elaeagnaceae, Rhamnaceae and Rosaceae (77.1%), but interrelationships among these five taxa are unresolved or only weakly supported. Malpighiales/Violales are here taken in a very wide sense and include 29 families (Appendix 5) represented by 199 sequences in the jackknife tree. Among the families are the problematic Flacourtiaceae, which according to our analysis seem to be the most polyphyletic of all traditionally recognized flowering plant families. In the jackknife tree, the 20 Flacourtiaceae sequences appear as 18 separate branches, two of them even outside Malpighiales/Violales. Salicaceae are nested in a portion of *Flacourtiaceae*, supported by a jackknife frequency of 95.4%. This is consistent with the general view that *Flacourtiaceae* is a "garbage can" family.

Malvales are only weakly supported (50.4%), but the core of the order, with the four families *Bombacaceae*, *Malvaceae*, *Tiliaceae* and *Sterculiaceae*, is a well supported monophyletic group (98.2%). The extended concept of *Malvales* includes several small families (Appendix 5; cf. ALVERSON & al. 1998, BAYER & al. 1998). *Fabales* are here taken to include not only *Fabaceae* s. l. but also *Polygalaceae*, *Surianaceae* (*Fernando* & al. 1993) and *Xanthophyllaceae*. This clade of four families is weakly supported by 59.5% of the replicates, but their sister group is a single sequence of the genus *Quillaja* (traditionally in *Rosaceae*), and with *Quillaja* included, the *Fabales* clade is well supported by 95.6% replicates. As observed by MORGAN & al. (1994), *Quillaja* is not a member of *Rosaceae*. Apparently, it could be classified in a family of its own.

One unnamed, well supported rosid clade of eight families (97.4%) corresponds to what could be defined as an extended order *Oxalidales*, with *Cephalotaceae*, *Connaraceae*, *Cunoniaceae*, *Davidsoniaceae*, *Elaeocarpaceae*, *Eucryphiaceae*, *Oxalidaceae* and *Tremandraceae*. Two well supported pairs of sister families are *Krameriaceae/Zygophyllaceae* (96.3%; SHEAHAN & CHASE 1996) and *Geraniaceae/Hypseocharitaceae* (99.6%; PRICE & PALMER 1993). Another unnamed clade, possibly related to *Geraniaceae* (PRICE & PALMER 1993), consists of *Francoaceae*, *Greyiaceae*, *Ledocarpaceae*, *Melianthaceae* and *Vivianiaceae* (83.6%).

The asterids are not recognized as a monophyletic group, although their possible monophyly is not contradicted by the jackknife tree. They comprise 25

branches in the unresolved polytomy of eudicots. Of these branches, 20 represent single sequences or small groups usually classified as asterids, four are larger groups at the ordinal level, and one branch corresponds to the 'asterid I' of CHASE & al. (1993; OLMSTEAD & al. 1993), here represented by 267 sequences and supported by a jackknife frequency of 69.4%. This group commonly includes three major orders, *Gentianales* (BREMER 1996), *Lamiales/Scrophulariales* (OLMSTEAD & REEVES 1995) and *Solanales*. The first two are well supported, by 99.4 and 96.7%, respectively, but *Solanales* are not recognized in the tree. Instead, they are represented by three separate branches within the same polytomy of the asterid I clade, (1) *Boraginaceae*, (2) *Convolvulaceae* and *Solanaceae* as sister groups (95.2%) and (3) a clade comprising *Montiniaceae*, *Hydrophyllaceae* and *Sphenocleaceae* (59.9%).

The other well supported orders of asterids are *Apiales/Araliales* (92.7%; PlUNKETT & al. 1996), *Asterales/Campanulales* (85.3%; GUSTAFSSON & al. 1996, GUSTAFSSON & BREMER 1997), *Dipsacales* (BACKLUND & BREMER 1998), and *Ericales* s. l. including *Ebenales*, *Primulales* and some *Theales* (85.2%, MORTON & al. 1997b). This latter group includes many different families (cf. Appendix 5) traditionally classified in different orders, for example, *Ebenaceae* (MORTON & al. 1997b), *Ericaceae* (KRON & CHASE 1993), *Lecythidaceae* (MORTON & al. 1997c), *Primulaceae* and *Theaceae*. Two well supported subgroups within *Ericales* are a clade of *Balsaminaceae*, *Marcgraviaceae*, *Pellicieraceae* and *Tetrameristicaceae* (retained at the 100 % level), and the three families usually classified in a separate order *Primulales*, viz. *Primulaceae*, *Myrsinaceae* and *Theophrastaceae* (99.1%; ANDERBERG & al. 1998).

There are few interfamilial relationships among the 20 branches of asterid sequences outside the orders discussed above. One family pair is the *Aucubaceae* and *Garryaceae* clade, found in 98.3% of the replicates. Another group is the so called '*Ilex* clade' (OLMSTEAD & al. 1993, MORGAN & SOLTIS 1993), consisting of *Aquifoliaceae*, *Helwingiaceae*, and *Phyllonomaceae* (92.8%).

Phylogenetic reconstruction and the *rbcL* gene. The *rbcL* gene proves to be extremely useful for phylogenetic reconstruction. It is not always informative, but may be so at widely different levels of the taxonomic hierarchy. Variation in the gene may support very old, major groups such as streptophytes, land plants, seed plants and flowering plants. It may also resolve relationships within families and even within genera. Included in the data matrix are, for example, 22 species of *Nothofagus (Nothofagaceae)* and 12 species of *Drosera (Droseraceae)*, which are resolved into 11 and 9 supported clades, respectively. In other groups at various taxonomic levels, the *rbcL* gene is phylogenetically uninformative. This seems to be the case, for example, with respect to the problems of resolving the basal clades of major groups such as ferns, flowering plants and eudicots.

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Appendix 1. The 184 flowering plant families represented by single sequences only.

Archaic angiosperms	Tofieldiaceae	Bataceae
	Typhaceae	Begoniaceae
Amborellaceae	Zannichelliaceae	Bretschneideraceae
Austrobaileyaceae	Zosteraceae	Brexiaceae
Barclayaceae		Caricaceae
Ceratophyllaceae	Eudicots	Caryocaraceae
Degeneriaceae		Cephalotaceae
Eupomatiaceae	Achatocarpaceae	Cneoraceae
Himantandraceae	Aextoxicaceae	Coriariaceae
Idiospermaceae	Amaranthaceae	Corynocarpaceae
Illiciaceae	Ancistrocladaceae	Crossosomataceae
Lactoridaceae	Asteropeiaceae	Davidsoniaceae
Monimiaceae	Basellaceae	Diegodendraceae
Myristicaceae	Buxaceae	Eucryphiaceae
Schizandraceae	Cercidiphyllaceae	Francoaceae
	Daphniphyllaceae	Geissolomataceae
Monocots	Didiereaceae	Greyiaceae
	Dioncophyllaceae	Gyrostemonaceae
Acoraceae	Eupteleaceae	Heteropyxidaceae
Aphyllanthaceae	Frankeniaceae	Hippocrateaceae
Aponogetonaceae	Grossulariaceae	Hugoniaceae
Blandfordiaceae	Gunneraceae	Hypseocharitaceae
Burmanniaceae	Iteaceae	Irvingiaceae
Butomaceae	Leeaceae	Kirkiaceae
Calochortaceae	Menispermaceae	Koeberliniaceae
Campynemataceae	Misodendraceae	Krameriaceae
Cannaceae	Molluginaceae	Lacistemataceae
Cymodoceaceae	Myrothamnaceae	Ledocarpaceae
Dioscoreaceae	Nepenthaceae	Leitneriaceae
Doryanthaceae	Olacaceae	Lepuropetalaceae
Dracaenaceae	Opiliaceae	Lophopyxidaceae
Eriocaulaceae	Penthoraceae	Lythraceae
Eriospermaceae	Physenaceae	Malesherbiaceae
Flagellariaceae	Platanaceae	Medusagynaceae
Haemodoraceae	Portulacaceae	Melianthaceae
Hanguanaceae	Pterostemonaceae	Memecylaceae
Heliconiaceae	Rhabdodendraceae	Moringaceae
Hydrocharitaceae	Simmondsiaceae	Muntingiaceae
Ixioliriaceae	Tamaricaceae	Neuradaceae
Lemnaceae	Tetracarpaeaceae	Nitrariaceae
Lowiaceae	Tetracentraceae	Oliniaceae
Najadaceae	Trochodendraceae	Paracryphiaceae
Potamogetonaceae		Parnassiaceae
Rapateaceae	Eudicots – rosids	Penaeaceae
Restionaceae		Plagiopteridaceae
Ruppiaceae	Akaniaceae	Psiloxylaceae
Ruscaceae	Alzateaceae	Punicaceae
Scheuchzeriaceae	Balanopaceae	Resedaceae
Sparganiaceae	Barbeyaceae	Rhynchocalycaceae

Appendix 1 (continued)		
Salvadoraceae	Buddlejaceae	Myoporaceae
Sarcolaenaceae	Byblidaceae	Myrsinaceae
Scyphostegiaceae	Callitrichaceae	Oncothecaceae
Sphaerosephalaceae	Cardiopteridaceae	Pellicieraceae
Ticodendraceae	Carpodetaceae	Phellinaceae
Tiliaceae	Cyclocheilaceae	Phrymataceae
Tovariaceae	Desfontainiaceae	Phyllonomaceae
Trapaceae	Donatiaceae	Roridulaceae
Tremandraceae	Eremosynaceae	Sphenocleaceae
Trigoniaceae	Eucommiaceae	Tetrachondraceae
Tropaeolaceae	Garryaceae	Tetrameristaceae
Turneraceae	Griseliniaceae	Vahliaceae
Vivianiaceae	Grubbiaceae	Valerianaceae
Xanthophyllaceae	Helwingiaceae	
	Hippuridaceae	
Eudicots – asterids	Hydrophyllaceae	
	Hydrostachyaceae	
Aucubaceae	Melanophyllaceae	

Montiniaceae

Appendix 2. The 30 flowering plant families not supported as clades in the analysis, yet possibly monophyletic (grouping the sequences for each family into a single clade is not contradicted by the tree). The first figure is the number of sequences, the second is the number of clades in the tree.

Archaic angiosperms	Eremolepidaceae 2/2	Sterculiaceae 4/4
	Hamamelidaceae 7/3	
Nymphaeaceae 4/2	Santalaceae 2/2	Eudicots – asterids
	Viscaceae 3/3	
Monocots		Acanthaceae 12/4
	Eudicots – rosids	Bignoniaceae 3/3
Colchicaceae 10/2		Dipsacaceae 2/2
Convallariaceae 9/7	Anacardiaceae 9/3	Escalloniaceae 2/2
Iridaceae 37/2	Bombacaceae 2/2	Hydrangeaceae 5/3
Marantaceae 3/2	Cunoniaceae 3/2	Nyssaceae 3/3
Phormiaceae 14/7	Euphorbiaceae 89/5	Pedaliaceae 3/3
	Myrtaceae 5/4	Styracaceae 7/2
Eudicots	Oxalidaceae 2/2	Theaceae 6/2
	Rhamnaceae 30/3	Verbenaceae 6/2
Droseraceae 14/2	Rutaceae 9/4	

276

Avicenniaceae

Simultaneous parsimony jackknife analysis

Appendix 3. The 29 flowering plant families not supported as clades in the analysis and indicated to be non-monophyletic (some sequences of each family form clades with sequences from other families). The first figure is the number of sequences, the second is the number of clades in the tree.

Archaic angiosperms	Nolinaceae 4/3	Elaeocarpaceae 5/4
	Smilacaceae 2/2	Flacourtiaceae 20/18
Aristolochiaceae 3/2	Uvulariaceae 16/3	Ixonanthaceae 3/2
		Simaroubaceae 7/5
Monocots	Eudicots	Staphyleaceae 2/2
		Ulmaceae 7/4
Agavaceae 9/9	Aizoaceae 3/2	
Amaryllidaceae 21/6	Chenopodiaceae 3/2	Eudicots – asterids
Araceae 9/8	Loranthaceae 5/2	
Asteliaceae 2/2	Phytolaccaceae 3/2	Apiaceae 58/7
Hyacinthaceae 54/3		Araliaceae 22/10
Juncaceae 8/5	Eudicots – rosids	Cornaceae 13/4
Luzuriagaceae 3/2		Scrophulariaceae 6/4
Melanthiaceae 11/3	Capparaceae 3/3	
Musaceae 2/2	Celastraceae 17/11	

Appendix 4. The 158 flowering plant families supported as clades in the analysis. The first figure is the number of sequences included, the second is the frequency of jackknife replicates in which the family appears as a clade. Figures in bold are comparatively high support values (>80% of jackknife replicates) for families represented by at least 10 sequences.

Bromeliaceae 6/100

Archaic angiosperms

Annonaceae 35/**99.5** Cabombaceae 2/99.4 Calycanthaceae 3/99.5 Canellaceae 2/100 Chloranthaceae 3/94.4 Hernandiaceae 2/80.9 Lauraceae 4/58.8 Magnoliaceae 10/64.5 Piperaceae 2/100 Saururaceae 2/93.8 Winteraceae 3/100

Monocots

Alismataceae 3/100 Alliaceae 16/76.3 Alstroemeriaceae 3/100 Anthericaceae 6/100 Arecaceae 8/99.7 Asparagaceae 2/100 Asphodelaceae 14/**100** Boryaceae 2/96.0 Commelinaceae 4/99.9 Costaceae 3/67.5 Cyanastraceae 3/79.5 Cyclanthaceae 2/98.0 *Cyperaceae* 73/88.1 Dasypogonaceae 3/99.5 Hypoxidaceae 5/83.8 Joinvilleaceae 2/100 Liliaceae 9/57.3 Lomandraceae 10/51.7 Orchidaceae 163/99.8 Pandanaceae 2/100 Philesiaceae 2/59.3 Philydraceae 2/100 Poaceae 71/100 Pontederiaceae 14/100 Stemonaceae 2/99.5 Strelitziaceae 3/51.3 Taccaceae 2/100 Tecophilaeaceae 6/99.3 Themidaceae 15/93.5 Trilliaceae 5/100 Velloziaceae 3/100

Xanthorrhoeaceae 2/100 Zingiberaceae 4/95.7

Eudicots

Berberidaceae 3/73.2 Cactaceae 3/96.2 Caryophyllaceae 4/100 Crassulaceae 4/96.9 Dilleniaceae 4/100 Haloragaceae 2/98.0 Lardizabalaceae 5/98.7 Nelumbonaceae 2/100 Nyctaginaceae 2/93.9 Paeoniaceae 2/100 Papaveraceae 3/76.8 Plumbaginaceae 40/100 Polygonaceae 4/99.9 Proteaceae 3/100 Ranunculaceae 5/94.5 Sabiaceae 2/89.3 Saxifragaceae 24/61.8 Stegnospermataceae 2/99.9 *Vitaceae* 12/62.5

Appendix 4 (continued) Eudicots – rosids

Achariaceae 2/88.3 Betulaceae 6/66.4 Brassicaceae 2/99.7 Burseraceae 3/98.5 Cannabaceae 2/100 Casuarinaceae 5/99.9 Chrysobalanaceae 2/100 Clusiaceae 5/63.1 Combretaceae 4/100 Connaraceae 2/100 Cucurbitaceae 3/100 Datiscaceae 2/99.7 Dichapetalaceae 3/99.0 Dipterocarpaceae 2/99.6 Elaeagnaceae 3/100 Erythroxylaceae 2/99.9 Fabaceae 76/91.5 Fagaceae 8/66.5 Geraniaceae 13/100 Huaceae 2/100 Humiriaceae 2/81.3 Juglandaceae 3/100 Limnanthaceae 2/100 Linaceae 4/100 Malpighiaceae 17/100 Malvaceae 3/99.3 Melastomataceae 4/100 Meliaceae 8/80.8 Moraceae 3/60.2 Myricaceae 2/99.9 Nothofagaceae 22/100 Ochnaceae 2/99.9

Onagraceae 9/97.6 Pandaceae 2/99.7 Passifloraceae 2/82.6 Peganaceae 2/100 Polygalaceae 3/99.8 Ouiinaceae 3/99.7 Rhizophoraceae 2/99.9 Rosaceae 42/**99.9** Salicaceae 14/81.0 Sapindaceae s.l. 7/99.9 Stackhousiaceae 2/86.3 Surianaceae 5/99.3 Tetramelaceae 2/96.3 Thymelaeaceae 5/97.6 Urticaceae 4/97.1 Violaceae 15/100 Vochysiaceae 3/99.9 Zygophyllaceae 17/77.9

Eudicots – asterids

Actinidiaceae 3/99.5 Adoxaceae s.1. 4/88.3 Alseuosmiaceae 3/100 Apocynaceae s.1. 25/**80.1** Aquifoliaceae 4/99.6 Argophyllaceae 2/100 Asteraceae 26/**85.4** Balsaminaceae 3/100 Boraginaceae 4/99.8 Bruniaceae 2/100 Calyceraceae 3/58.2 Campanulaceae 9/100 Caprifoliaceae 2/98.1 Clethraceae 2/99.3 Convolvulaceae 2/100 Cyrillaceae 2/98.4 Diapensiaceae 2/97.2 Ebenaceae 6/98.0 Ericaceae s.l. 24/89.0 Fouquieriaceae 2/100 Gelsemiaceae 3/86.3 Gentianaceae 8/97.4 Gesneriaceae 2/73.8 Globulariaceae 2/83.6 Goodeniaceae 13/77.0 Lamiaceae 45/91.7 Lecythidaceae 8/95.5 Lentibulariaceae 2/59.3 Loasaceae 6/96.4 Loganiaceae 4/99.8 Marcgraviaceae 2/95.6 Menvanthaceae 5/50.3 Oleaceae 4/65.8 Pentaphragmataceae 2/100 Pittosporaceae 5/100 Polemoniaceae 3/99.9 Primulaceae 6/100 Rubiaceae 103/91.7 Sapotaceae 8/99.1 Sarraceniaceae 3/99.4 Solanaceae 17/100 Stilbaceae 3/89.7 Stylidiaceae 2/99.3 Symplocaceae 3/100 Theophrastaceae 2/99.5

Simultaneous parsimony jackknife analysis

Appendix 5. Selected clades of more than one flowering plant family supported by the analysis. The first figure is the number of sequences included, the second is the frequency of replicates in which the group appears as a clade. Figures in bold are comparatively high support values (>80% of jackknife replicates) for groups represented by at least 10 sequences. A dash indicates an unsupported family that does not appear as a clade within the group.

Archaic angiosperms

Magnoliales 49/54.0 Annonaceae 35/**99.5** Degeneriaceae 1 Eupomatiaceae 1 Himantandraceae 1 Myristicaceae 1 Magnoliaceae 10/64.5

Laurales 11/75.8 Calycanthaceae 3/99.5 Hernandiaceae 2/80.9 Idiospermaceae 1 Lauraceae 4/58.8 Monimiaceae 1

Unnamed 4/53.0 Aristolochiaceae 3/-Lactoridaceae 1

Piperales 4/100 Piperaceae 2/100 Saururaceae 2/93.8

Unnamed 11/64.2 Amborellaceae 1 Austrobaileyaceae 1 Illiciales 2/97.0 Nymphaeales 7/100

Unnamed 3/95.7 Austrobaileyaceae 1 Illiciales 2/97.0

Illiciales 2/97.0 Illiciaceae 1 Schizandraceae 1

Unnamed 8/71.2 Amborellaceae 1 Nymphaeales 7/100

Nymphaeales 7/100

Barclayaceae 1
Cabombaceae 2/99.4
Nymphaeaceae 4/-

Monocots

All monocots except *Acoraceae* 721/66.9

All monocots except *Acoraceae* and next clade 697/68.8

Unnamed 24/55.1 Alismatales 13/**91.6** Arales 10/79.1 Tofieldiaceae 1

Alismatales 13/**91.6** Alismataceae 3/100 Aponogetonaceae 1 Butomaceae 1 Cymodoceaceae 1 Hydrocharitaceae 1 Najadaceae 1 Potamogetonaceae 1 Ruppiaceae 1 Scheuchzeriaceae 1 Zannichelliaceae 1 Zosteraceae 1

Arales 10/79.1 Araceae 9/-Lemnaceae 1

Asparagales 407/59.5 Agavaceae 9/-Alliaceae 16/76.3 Amaryllidaceae 20/-Anthericaceae 6/100 Aphyllanthaceae 1 Asparagaceae 2/100 Asphodelaceae 14/**100** Asteliaceae 2/-Blandfordiaceae 1 Boryaceae 2/100 Convallariaceae 9/-Doryanthaceae 1 Dracaenaceae 1 Eriospermaceae 1 Hyacinthaceae 54/-Hypoxidaceae 5/83.8 Iridaceae 37/-Ixioliriaceae 1 Luzuriagaceae p.p. 2/100 Lomandraceae 10/51.7 Nolinaceae 4/-Orchidaceae 163/99.8 Phormiaceae 14/-Ruscaceae 1 Tecophilaeaceae 6/99.3 Themidaceae 15/93.5 Xanthorrhoeaceae 2/100

Liliales 58/67.3 Alstroemeriaceae 3/100 Calochortaceae 1 Campynemataceae 1 Colchicaceae 10/-Liliaceae 9/57.3 Luzuriagaceae 1 Melanthiaceae 8/-Philesiaceae 2/59.3 Smilacaceae 2/-Trilliaceae 5/100 Uvulariaceae 16/-

Unnamed in Liliales 20/100 Alstroemeriaceae 3/100 Colchicaceae 10/-Luzuriagaceae 1 Uvulariaceae p.p. 6/72.4

Unnamed in Liliales 13/85.2 Melanthiaceae 8/-Trilliaceae 5/100

Unnamed in *Liliales* 24/**96.4** *Calochortaceae* 1

M. Källersjö & al.:

Aizoaceae 3/-

Appendix 5 (continued) *Liliaceae* 9/57.3

Philesiaceae 2/59.3 Smilacaceae 2/-Uvulariaceae p.p. 10/-

Stemonales/Pandanales 9/93.0 Cyclanthaceae 2/98.0 Pandanaceae 2/100 Stemonaceae 2/99.5 Velloziaceae 3/100

Unnamed in Stemonales/ Pandanales 6/50.4 Cyclanthaceae 2/98.0 Pandanaceae 2/100 Stemonaceae 2/99.5

Pandanales 4/98.5 Cyclanthaceae 2/98.0 Pandanaceae 2/100

Unnamed 3/100 Dioscoreaceae 1 Taccaceae 2/100

Unnamed 18/68.8 Commelinaceae 4/99.9 Pontederiaceae 14/100

Unnamed 81/791 Cyperaceae 73/**88.1** Juncaceae 8/-

Unnamed 74/**83.5** Joinvilleaceae 2/100 Poaceae 71/**100** Restionaceae 1

Typhales 2/63.8 Sparganiaceae 1 Typhaceae 1

Zingiberales 18/**98.1** Cannaceae 1 Costaceae 3/53.0 Heliconiaceae 1 Lowiaceae 1 Marantaceae 3/-Musaceae 2/- Strelitziaceae 3/-Zingiberaceae 4/91.0

Eudicots

Unnamed 8/61.4 Crassulaceae 4/96.9 Haloragaceae 2/98.0 Penthoraceae 1 Tetracarpaeaceae 1

Unnamed 2/811 Gunneraceae 1 Myrothamnaceae 1

Unnamed 2/98.3 Iteaceae 1 Pterostemonaceae 1

Unnamed 12/100 Leeaceae 1 Vitaceae 12/62.5

Caryophyllidae 93/**99.8** Ancistrocladaceae 1 Asteropeiaceae 1 Dioncophyllaceae 1 Droseraceae 14/-Frankeniaceae 1 Physenaceae 1 Plumbaginaceae 40/**100** Polygonaceae 4/99.9 Rhabdodendraceae 1 Simmondsiaceae 1 Tamaricaceae 1 Caryophyllales 26/**95.8**

Unnamed in Caryophyllidae 44/**99.8** Plumbaginaceae 40/**100** Polygonaceae 4/99.9

Unnamed in Caryophyllidae 28/**97.8** Asteropeiaceae 1 Physenaceae 1 Caryophyllales 26/**95.8**

Caryophyllales 26/**95.8** Achatocarpaceae 1

Amaranthaceae 1 Basellaceae 1 Cactaceae 3/96.2 Caryophyllaceae 4/100 Chenopodiaceae 3/-Didiereaceae 1 Molluginaceae 1 Nyctaginaceae 2/93.9 Phytolaccaceae 3/-Portulacaceae 1 Stegnospermataceae 2/99.9 Unnamed in Caryophyllales 9/80.8 Achatocarpaceae 1 Amaranthaceae 1 Caryophyllaceae 4/100 Chenopodiaceae 3/-Unnamed in *Caryophyllales*

17/**94.5** Aizoaceae 3/-Basellaceae 1 Cactaceae 3/96.2 Didiereaceae 1 Mulluginaceae 1 Nyctaginaceae 2/93.9 Phytolaccaceae 3/-Portulacaceae 1 Stegnospermataceae 2/99.9

Ranunculales/Papaverales 18/**88.4** Berberidaceae 3/66.0 Eupteleaceae 1 Lardizabalaceae 5/97.8 Menispermaceae 1 Papaveraceae 3/55.1 Ranunculaceae 5/83.5

Santalales 15/**99.4** Eremolepidaceae 2/-Olacaceae 1 Opiliaceae 1 Loranthaceae 5/-Misodendraceae 1 Santalaceae 2/-Viscaceae 3/-

280

Appendix 5 (continued) Trochodendrales 2/100 Tetracentraceae 1 Trochodendraceae 1 **Eudicots – rosids** Rosidae 642/53.9 Crossosomataceae 1 Francoaceae 1 Geissolomataceae 1 Geraniaceae 13/100 Greviaceae 1 Hypseocharitaceae 1 Ledocarpaceae 1 Melianthaceae 1 Simaroubaceae p.p. 2/100 Staphyleaceae 2/-Vivianiaceae 1 Capparales 18/94.3 Malvales 22/50.4 Myrtales 35/99.9 Sapindales 48/98.2 Rosid I 494/55.3 Unnamed 5/83.6 Francoaceae 1 Greyiaceae 1 Ledocarpaceae 1 Melianthaceae 1 Vivianiaceae 1 Unnamed 14/99.6 Geraniaceae 13/100 Hypseocharitaceae 1 Capparales 18/94.3 Akaniaceae 1 Bataceae 1 Brassicaceae 2/99.7 Capparaceae 3/-Bretschneideraceae 1 Caricaceae 1 Gyrostemonaceae 1 Koeberliniaceae 1 Limnanthaceae 2/100 Moringaceae 1 Resedaceae 1 Salvadoraceae 1 Tovariaceae 1 Tropaeolaceae 1

Malvales 22/50.4 Bombacaceae 2/-Diegodendraceae 1 Dipterocarpaceae 2/99.6 Malvaceae 3/99.3 Muntingiaceae 1 Neuradaceae 1 Sarcolaenaceae 1 Sphaerosephalaceae 1 Sterculiaceae 4/-Thymelaeaceae 5/97.6 Tiliaceae 1

Unnamed in *Malvales* 9/98.2 Bombacaceae 2/-Malvaceae 3/99.3 Tiliaceae 1 Sterculiaceae 4/-

Myrtales 35/99.9 Alzateaceae 1 Combretaceae 4/100 Heteropyxidaceae 1 Lythraceae 1 Melastomataceae 4/100 Memecylaceae 1 Myrtaceae 5/-Oliniaceae 1 Onagraceae 9/97.6 Penaeaceae 1 Psiloxylaceae 1 Punicaceae 1 Rhynchocalycaceae 1 Trapaceae 1 Vochysiaceae 3/99.9

Unnamed in Myrtales 12/**93.9** Lythraceae 1 Onagraceae 9/97.6 Punicaceae 1 Trapaceae 1

Sapindales 48/**98.2** Anacardiaceae 9/-Burseraceae 3/98.5 Cneoraceae 1 Kirkiaceae 1 Leitneriaceae 1 Nitrariaceae 1 Meliaceae 8/80.8 Paracryphiaceae 1 Peganaceae 2/100 Rutaceae 9/-Sapindaceae s.l. 7/99.9 Simaroubaceae p.p. 5/-

Unnamed in Sapindales 25/**87.9** Cneoraceae 1 Leitneriaceae 1 Meliaceae 8/80.8 Paracryphiaceae 1 Rutaceae 9/-Simaroubaceae p.p. 5/-

Rosid I 494/55.3 Barbeyaceae 1 Cephalotaceae 1 Connaraceae 2/100 Cunoniaceae 3/-Davidsoniaceae 1 Elaeagnaceae 3/100 Elaeocarpaceae 5/-Eucryphiaceae 1 Huaceae 2/100 Krameriaceae 1 Oxalidaceae 2/-Rhamnaceae 30/-Rosaceae 42/99.9 Tremandraceae 1 Zygophyllaceae 17/77.9 Celastrales 24/93.7 Cucurbitales 10/94.6 Fabales 85/59.5 Fagales/Juglandales 47/89.3 Urticales 16/75.1 Violales 199/72.7

Unnamed 16/97.4 Cephalotaceae 1 Connaraceae 2/100 Cunoniaceae 3/-Davidsoniaceae 1 Elaeocarpaceae 5/-Eucryphiaceae 1 Oxalidaceae 2/-Tremandraceae 1 Appendix 5 (continued) Unnamed 12/**93.4** Cephalotaceae 1 Cunoniaceae 3/-Davidsoniaceae 1 Elaeocarpaceae 5/-Eucryphiaceae 1 Tremandraceae 1

Unnamed 5/991 Cunoniaceae 3/-Davidsoniaceae 1 Eucryphiaceae 1

Unnamed 4/94.9 Connaraceae 2/100 Oxalidaceae 2/-

Unnamed 18/**96.3** *Krameriaceae* 1 *Zygophyllaceae* 17/77.9

Celastrales 24/**93.7** Brexiaceae 1 Celastraceae 17/-Hippocrateaceae 1 Lepuropetalaceae 1 Parnassiaceae 1 Plagiopteridaceae 1 Stackhousiaceae 2/86.3

Cucurbitales 10/**94.6** Begoniaceae 1 Coriariaceae 1 Corynocarpaceae 1 Cucurbitaceae 3/100 Datiscaceae 2/99.7 Tetramelaceae 2/96.3

Unnamed 86/**95.6** Quillaja 1 Fabales 85/59.5

Fabales 85/59.5 Fabaceae s.l. 76/**91.5** Polygalaceae 3/99.8 Surianaceae 5/99.3 Xanthophyllaceae 1 Fagales/Juglandales 47/**89.3** Betulaceae 6/66.4 Casuarinaceae 5/99.9 Fagaceae 8/66.5 Juglandaceae 3/100 Myricaceae 2/99.9 Nothofagaceae 22/**100** Ticodendraceae 1

Unnamed 92/77.1 Barbeyaceae 1 Elaeagnaceae 3/100 Rhamnaceae 30/-Rosaceae 42/**99.9** Urticales 16/75.1

Unnamed 50/51.6

Barbeyaceae 1 Elaeagnaceae 3/100 Rhamnaceae 30/-Urticales 16/75.1

Urticales 16/75.1 Cannabaceae 2/100 Moraceae 3/60.2 Ulmaceae p.p. 7/-Urticaceae 4/97.1

Malpighiales/Violales 199/72.7 Achariaceae 2/88.3 Balanopaceae 1 Carvocaraceae 1 Chrvsobalanaceae 2/100 Clusiaceae 5/63.1 Dichapetalaceae 3/99.0 Erythroxylaceae 2/99.9 Euphorbiaceae 89/-Flacourtiaceae p.p. 18/-Hugoniaceae 1 Humiriaceae 2/81.3 Irvingiaceae 1 Ixonanthaceae 3/-Lacistemaceae 1 Linaceae 4/100 Lophopyxidaceae 1

Malesherbiaceae 1 Malpighiaceae 17/100 Medusagynaceae 1 Ochnaceae 2/99.9 Pandaceae 2/99.7 Passifloraceae 2/82.6 Quiinaceae 3/99.7 Rhizophoraceae 2/99.9 Salicaceae 14/81.0 Scyphostegiaceae 1 Trigoniaceae 1 Violaceae 15/100

Unnamed in Malpighiales/ Violales 4/80.9 Erythroxylaceae 2/99.9 Rhizophoraceae 2/99.9

Unnamed in Malpighiales/ Violales 24/95.4 Flacourtiaceae p.p. 9/-Salicaceae 14/81.0 Scyphostegiaceae 1

Unnamed in Malpighiales/ Violales 5/100 Hugoniaceae 1 Linaceae 4/100

Unnamed in Malpighiales/ Violales 4/100 Malesherbiaceae 1 Passifloraceae 2/82.6 Turneraceae 1

Unnamed in Malpighiales/ Violales 6/99.7 Medusagynaceae 1 Ochnaceae 2/99.9 Quiinaceae 3/99.7

Eudicots - asterids

Unnamed 6/92.8 Aquifoliaceae 4/98.9 Helwingiaceae 1 Phyllonomaceae 1

282

Appendix 5 (continued)	
Unnamed 2/98.3 Aucubaceae 1 Garryaceae 1	Ericales/Primulales 96/ 85.2 Actinidiaceae 3/99.5 Balsaminaceae 3/100 Clethraceae 2/99.3
Apiales/Araliales 87/92.7 Apiaceae 58/- Araliaceae 22/- Griseliniaceae 1 Melanophyllaceae 1 Pittosporaceae 5/100 Asterales/Campanulales 68/ 85.3 Alseuosmiaceae 3/100 Argophyllaceae 2/100 Asteraceae 26/85.4 Calyceraceae 3/58.2	Cyrillaceae 2/98.4 Diapensiaceae 2/97.2 Ebenaceae 6/98.0 Ericaceae s.l. 24/ 89.0 Fouquieriaceae 2/100 Lecythidaceae 8/99.5 Marcgraviaceae 2/95.6 Pellicieraceae 1 Polemoniaceae 3/99.9 Roridulaceae 1 Sapotaceae 8/99.1 Sarraceniaceae 3/99.4 Styracaceae 7/-
Campanulaceae 9/100 Carpodetaceae 1 Donatiaceae 1 Goodeniaceae 13/77.0 Menyanthaceae 5/50.3	Symplocaceae 3/100 Tetrameristaceae 1 Theaceae 6/- Primulales 9/99.1
Pentaphragmataceae 2/100 Phellinaceae 1 Stylidiaceae 2/99.3	Unnamed in Ericales s.l. 7/100 Balsaminaceae 3/100 Marcgraviaceae 2/96.4 Pellicieraceae 1
Unnamed in Asterales 47/ 68.3	Tetrameristicaceae 1
Asteraceae 26/ 85.4 Calyceraceae 3/58.2 Goodeniaceae 13/77.0 Menyanthaceae 5/50.3	Primulales 9/99.1 Myrsinaceae 1 Primulaceae 6/99.6 Theophrastaceae 2/98.4
Unnamed in Asterales 42/ 95.3 Asteraceae 26/85.4 Calyceraceae 3/58.2 Goodeniaceae 13/77.0 Dipsacales 5/96.0 Caprifoliaceae 2/98.1 Dipsacaceae 2/- Valorianaceae 1	Asteridae 1 267/69.4 Boraginaceae 4/99.8 Convolvulaceae 2/100 Hydrophyllaceae 1 Montiniaceae 1 Solanaceae 17/ 100 Sphenocleaceae 1 Vahliaceae 1

Lamiales/Scrophulariales 97/**96.7**

Unnamed 19/**95.2** Convolvulaceae 2/100 Solanaceae 17/**100**

Unnamed 3/59.9 Hydrophyllaceae 1 Montiniaceae 1 Sphenocleaceae 1

Unnamed 2/85.2 Hydrophyllaceae 1 Sphenocleaceae 1

Gentianales 143/**99.4** Apocynaceae s.l. 25/**80.1** Gelsemiaceae 3/86.3 Gentianaceae 8/97.4 Loganiaceae 4/99.8 Rubiaceae 103/**91.7**

Lamiales/Scrophulariales 97/**96.7** Acanthaceae 12/-Avicenniaceae 1 Bignoniaceae 3/-Buddlejaceae 1 Byblidaceae 1 Callitrichaceae 1 Cyclocheilaceae 1 Gesneriaceae 2/73.8 Globulariaceae 2/83.6 Hippuridaceae 1 Lamiaceae 45/91.7 Lentibulariaceae 2/59.3 Myoporaceae 1 Oleaceae 4/65.8 Pedaliaceae 3/-Phrymataceae 1 Scrophulariaceae 6/-Stilbaceae 3/89.7 Tetrachondraceae 1 Verbenaceae 6/-

Appendix 6. Commonly recognized flowering plant families not represented in the analysis.

Basal angiosperms		Mendonciaceae
	Biebersteiniaceae	Mitrastemonaceae
Gomortegaceae	Bixaceae	Morinaceae
Trimeniaceae	Brunelliaceae	Nesogenaceae
	Carlemanniaceae	Pentadiplandraceae
Monocots	Circaeasteraceae	Pentaphylacaceae
	Cistaceae	Peridiscaceae
Anarthriaceae	Cochlospermaceae	Plantaginaceae
Centrolepidaceae	Columelliaceae	Plocospermataceae
Corsiaceae	Crypteroniaceae	Podostemaceae
Ecdeiocoleaceae	Cuscutaceae	Polyosmataceae
Hydatellaceae	Cynomoriaceae	Pottingeriaceae
Hydrocharitaceae	Dialypetalanthaceae	Ptaeroxylaceae
Juncaginaceae	Didymelaceae	Rafflesiaceae
Lilaeaceae	Dipentodonaceae	Rhoipteleaceae
Limnocharitaceae	Dirachmaceae	Rousseaceae
Mayacaceae	Duckeodendraceae	Saccifoliaceae
Posidoniaceae	Elatinaceae	Sargentodoxaceae
Thurniaceae	Goetzeaceae	Sonneratiaceae
Trichopodaceae	Hoplestigmataceae	Sphenostemonaceae
Triuridaceae	Hydnoraceae	Stachyuraceae
Xyridaceae	Icacinaceae	Symphoremataceae
	Julianiaceae	Tepuianthaceae
Eudicots	Lennoaceae	Tetradiclidaceae
	Lepidobotryaceae	Torricelliaceae
Anisophylleaceae	Lissocarpaceae	Tribelaceae
Aralidiaceae	Martyniaceae	
Balanophoraceae	Medusandraceae	

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