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MULTIGENE ANALYSES OF MONOCOT RELATIONSHIPS: A SUMMARY

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ABSTRACT

We present an analysis of supra-familial relationships of monocots based on a combined matrix of nuclear 18S and partial 26S rDNA, plastid *atp*B, *mat*K, *ndh*F, and *rbc*L, and mitochondrial *atp*1 DNA sequences. Results are highly congruent with previous analyses and provide higher bootstrap support for nearly all relationships than in previously published analyses. Important changes to the results of previous work are a well-supported position of Petrosaviaceae as sister to all monocots above Acorales and Alismatales and much higher support for the commelinid clade. For the first time, the spine of the monocot tree has some bootstrap support, although support for paraphyly of liliids is still only low to moderate (79–82%). Dioscoreales and Pandanales are sister taxa (moderately supported, 87–92%), and Asparagales are weakly supported (79%) as sister to the commelinids. Analysis of just the four plastid genes reveals that addition of data from the other two genomes contributes to generally better support for most clades, particularly along the spine. A new collection reveals that previous material of *Petermannia* was misidentified, and now Petermanniaceae should no longer be considered a synonym of Colchicaceae. *Arachnitis* (Corsiaceae) falls into Liliales, but its exact position is not well supported. *Sciaphila* (Triuridaceae) falls with Pandanales. *Trithuria* (Hydatellaceae) falls in Poales near Eriocaulaceae, Mayacaceae, and Xyridaceae, but until a complete set of genes are produced for this taxon, its placement will remain problematic. Within the commelinid clade, Dasypogonaceae are sister to Poales and Arecales sister to the rest of the commelinids, but these relationships are only weakly supported.

Key words: Acorales, Alismatales, Arecales, Asparagales, Commelinales, commelinids, Dioscoreales, Liliales, mitochondrial genes, monocot phylogenetics, nuclear ribosomal genes, Pandanales, Petrosaviales, plastid genes, Poales, Zingiberales.

INTRODUCTION

In the time since the last major conference on monocots when results of a three-gene analysis were presented (Chase et al. 2000*b*), additional data have been collected representing two more plastid genes, *mat*K and *ndh*F, two mitochondrial genes, *atp*1 and *cob,* and a portion of an additional nuclear ribosomal gene, 26S rDNA (1200 bp at the 5′-end of the gene). We present in this paper results of a combined analysis of seven genes representing all three genomic compartments (including 18S rDNA, *atp*B, and *rbc*L, plus those listed above except for *cob,* results of which are described in Petersen et al. 2006).

Since the time of the first monocot conference at the Royal Botanic Gardens, Kew, in 1993 (Rudall et al. 1995), attention has been focused on establishing general relationships and developing a phylogenetic classification (APG 1998) for the monocots. The three conferences have been excellent in focusing attention on the gaps at one conference and filling many of them by the next. The second conference

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(Wilson and Morrison 2000) produced the first multigene analysis of the monocots (Chase et al. 2000*b*) and laid the framework for the work presented here. The major foci to be resolved by adding additional genes were the relationships of (i) the former liliid orders that Dahlgren et al. (1985) treated as Lilianae and (ii) higher-levels within the commelinids. Whereas Dahlgren et al. (1985) considered Lilianae to be monophyletic, DNA-based analyses have never recovered this topology (Chase et al. 1995*a*, 2000*b*) and instead have indicated that they are a grade relative to the commelinids, although this pattern has never been associated with bootstrap support greater than 50%, even with three genes. A combined analysis of *rbc*L and morphological data (Chase et al. 1995*b*) showed conversely that Lilianae were monophyletic, but again without robust internal support.

Within commelinids, ordinal relationships have been unclear relative to Dasypogonaceae. In Chase et al. (2000*b*), Zingiberales and Commelinales were sister taxa (with low support: 71% bootstrap), but all others were either unresolved in the strict consensus tree or not supported robustly by the bootstrap. It was hoped that by including additional data relationships of the liliid and commelinid orders and among the commelinids could be better assessed (we use these terms instead of lilioids and commelinoids to avoid confusion with subfamily names). Additional points of interest were to see how sequences from the mitochondrial genome compared with those found in the plastid and nuclear genes previously studied (18S rDNA, *atp*B, and *rbc*L) and how putatively more rapidly evolving regions such as plastid *mat*K and *ndh*F performed at the highest levels in the monocots. Previous work indicated that both these regions would do well (Givnish et al. 1999; Fuse and Tamura 2000). The addition of 26S rDNA seemed logical because combining plastid regions and 18S rDNA had increased internal support (Chase et al. 2000*a*; Soltis et al. 2000), but in other cases this region has not been particularly useful (Zanis et al. 2002).

The issue of congruence of different gene regions has been approached several different ways. Previous studies have demonstrated that although incongruence—as measured by, e.g., the partition homogeneity test—is present, direct combination provides greater resolution and higher bootstrap percentages (Soltis et al. 1998; Reeves et al. 2001). We will not address these issues in depth here, but Petersen et al. (2006) do examine this question with respect to the two mitochondrial genes, *atp*1 and *cob*. Davis et al. (2004) also discussed these issues with respect to *atp*1 and *rbc*L. Because the majority of genes analyzed here are plastid (four of the seven), we conducted a separate combined analysis of these to compare with the combined analysis of all genes. The evidence produced by directly combining these genes, in spite of the incongruence observed in the patterns when each gene or genomic compartment is analyzed separately, demonstrates increased internal support for most clades, which would be compatible with an hypothesis of sampling error (i.e., too few characters to obtain a clear answer) being responsible for different patterns when genes are analyzed individually rather than incongruence caused by different patterns of inheritance or different biases in their patterns of molecular evolution.

There are also undoubtedly extensive differences in lineage-specific rates in each of these regions (Gaut et al. 1992), which could perturb phylogenetic patterns. Nevertheless, these differences do not appear to present major problems, and the history of monocot molecular phylogenetics has been one of consistency of overall results and predictability when applied to other questions (e.g., relationships within Asparagales and telomere repeat variation; Adams et al. 2001). Thus in this paper, we will present only combined results and dissect the questions of molecular evolution and incongruence in greater detail in future publications.

MATERIALS AND METHODS

Species used as place-holders for this study are similar to those in previous papers (Chase et al. 1995*a*, 2000*b*). For the newly produced data (since Chase et al. 2000*b*), we have exchanged DNA samples among the participating labs so that each gene was amplified from the same genomic DNAs, but in a minority of cases this has not happened. We are in the process of producing additional new sequences for 18S rDNA and *atp*B so that we have parallel sampling for these genes as well, but for the purposes of this paper we have in some cases substituted other genera from the same families, all of which have been demonstrated in published analyses to be monophyletic. This same procedure was used in Soltis et al. (2000) and Qiu et al. (1999) and has been shown not to have a negative effect on results; estimates of familial relationships appear to be robust to such substitutions. Because this is a preliminary report, a full table of species names, vouchers, and GenBank accession numbers will be provided in a future paper to be published elsewhere, but the matrices and other voucher information can be obtained from the corresponding author (MWC; *m.chase@kew.org*). Methods of sequence production have varied greatly over time; primers and protocols can be found in studies of the individual genes. A description of the amplification procedures and primers for these genes can be found in the following references: 18S rDNA (Soltis and Soltis 1998), 26S rDNA (Zanis et al. 2002, but we used just 1200 bp at the 5′-end, which contained three loop regions considered among the most variable in the gene), *atp*A (Davis et al. 1998), *atp*B (Hoot et al. 1995), *mat*K (Johnson and Soltis 1994; Molvray et al. 2000; Cuénoud et al. 2002; Hilu et al. 2003), *ndh*F (Pires and Sytsma 2002; McPherson et al. 2003), and *rbc*L (Fay and Chase 1996).

The combined matrix consists of 141 taxa, 16 of which are outgroups selected from results of studies of basal nodes in the angiosperms (e.g., Qiu et al. 1999). *Amborella* Baill. (Amborellaceae) was specified as sister to the rest of the taxa (i.e., it is the ultimate outgroup). Monocot placeholders were selected on the basis of previous large-scale studies (Chase et al. 2000*b*) and include all families now recognized by APG (1998) except for Aponogetonaceae, Limnocharitaceae, Posidoniaceae, Ruppiaceae, and Scheuchzeriaceae (all small families of Alismatales). Some of the most problematic ingroup taxa are missing most genes because they are achlorophyllous, and this causes problems with estimating their relationships and/or bootstrap support for their positions. Therefore, we conducted two sets of analyses on the combined matrix of all genes, with and without these problem taxa: *Arachnitis* R. A. Philippi (Corsiaceae; missing all plas-

tid data), *Sciaphila* Blume (Triuridaceae; missing all plastid regions), *Thismia* Griff., and *Burmannia* L. (Burmanniaceae; the former missing *mat*K and *ndh*F and the latter missing only *ndh*F). For *Trithuria* Hook. f. (Hydatellaceae), which is photosynthetic, several attempts have been made to amplify other regions, but the DNA is of poor quality; thus far, we have sequenced it for only 18S and 26S rDNA, *atp*1 and *rbc*L. As mentioned above, we also analyzed an all-plastid combined matrix (*atp*B, *mat*K, *ndh*F, and *rbc*L), again with these problem taxa excluded.

Unlike *atp*B and *rbc*L, the two new plastid genes, *ndh*F and particularly *mat*K, have insertions and deletions (indels), but these were in all cases in triplets, consistent with their coding nature. However, in some regions of *mat*K, alignment was problematic because of large numbers of unique or rare indels, which meant that large amounts of missing data were present for the great majority of taxa. We therefore excluded these regions from the analyses because including them contributed nothing to patterns of relationships. We did not code indels as characters to be included in the analysis because it would be complicated for a matrix of this size, plus in no case did we identify indels marking groups that were not already well supported by the bootstrap.

We analyzed the combined matrix using heuristic searches with PAUP* vers. 4.0b10 (Swofford 2001) using the following strategy: 500 replicates of randomized taxa entries with subtree-pruning-regrafting (SPR) swapping and a tree limit of twenty trees per replicate to reduce the time spent on swapping on suboptimal islands of trees. A second round of analysis using these as starting trees was also conducted, and we did this with tree-bisection-reconnection (TBR) swapping to determine if this more thorough swapping algorithm found any additional trees, which it did not. For the plastid-only analysis, we found three islands of equally parsimonious trees (this was determined by using only a single shortest tree as a starting tree and finding that we only recovered 12 trees rather than all 36). We used bootstrapping to estimate internal support with 500 replicates of simple-taxon addition, again with a limit of 20 trees per replicate and SPR swapping. We report all bootstrap percentages greater than 50 that are consistent with the strict consensus tree. We show a single tree (the first one found) to illustrate branch lengths (DELTRAN optimization, due to problems with ACCTRAN optimization in PAUP* vers. 4.0b10) and indicate which groups are not found in the strict consensus tree with arrowheads.

RESULTS

First we will describe the results of the analysis without the five problem taxa (*Arachnitis, Burmannia, Sciaphila, Thismia,* and *Trithuria*) and then indicate where each of these is placed and the effect on bootstrap percentages. The combined matrix (excluding regions with mostly missing data and the five problem taxa) included 11,235 positions, of which 7389 positions were variable and 4777 (43%) were potentially informative. Comparisons of the contribution of each gene to this total will be presented in a future paper. The analysis found three shortest trees of 68,434 steps with a consistency index (CI; including all positions) of 0.54 and a retention index (RI) of 0.48.

We will not discuss outgroup relationships of the monocots in this paper because some important taxa (e.g., eudicots) are not included so that a robust assessment of overall outgroup relationships of the monocots is not appropriately sampled. To describe the tree topology, we will use sister-group language so that terms like "basal" can be avoided; nodes can be "basal," but clades cannot be. Furthermore, we will use family names, not genera, to describe terminals, even though in many cases only up to three genera represent large families such as Orchidaceae. Family limits are now well characterized within the monocots, so this use is not misleading. For comparative purposes, a summary of the bootstrap consensus trees from the Chase et al. (2000*b*) paper and this study are presented in Fig. 1. In Fig. 2, 3 we show one of the individual trees with bootstrap percentages (BP) indicated below the branches, branch lengths above, and the node not found in all three trees is marked by an arrowhead. The monocots are monophyletic (89 BP; Fig. 2), with Acoraceae (100 BP) sister to the rest (excluded from their sister clade with 100 BP; this convention for indicating sister group relationships will be used throughout this paper). Alismatales (100 BP) are then sister to the remainder of monocots exclusive of Acoraceae (100 BP), and within the former Araceae (100 BP) are sister (99 BP) to Tofieldiaceae (100 BP) plus the aquatic clade (100 BP). With this level of sampling, the aquatic clade forms two subclades (100 and 87 BP): (i) Cymodoceaceae sister (78 BP) to Juncaginaceae plus Zosteraceae/Potamogetonaceae (100 BP); and (ii) Hydrocharitaceae sister (<50 BP) to Butomaceae/Alismataceae.

Petrosaviaceae (100 BP) are sister (95 BP) to the other four liliid orders plus commelinids. At the next node, Dioscoreales/Pandanales (87 BP) are sister (BP 77) to Liliales (100 BP) plus Asparagales/commelinids (79 BP). Within Dioscoreales (99 BP), Nartheciaceae (100 BP) are sister (100 BP) to Dioscoreaceae. Pandanales are well supported (100 BP), with Velloziaceae (100 BP) sister (100 BP) to Stemonaceae (100 BP) plus Pandanaceae (100 BP)/Cyclanthaceae (100 BP).

Within Liliales, Campynemataceae are sister (<50 BP) to Melanthiaceae (92 BP); the rest of the order (<50 BP) is composed of two groups (61 and 100 BP): (i) Petermanniaceae sister (100) to Colchicaceae (100 BP) plus Alstroemeriaceae/Luzuriagaceae (96 BP) and (ii) Smilacaceae sister (59 BP) to Philesiaceae/Rhipogonaceae (100 BP) and Liliaceae (100 BP).

Within Asparagales (95 BP; Fig. 3), Orchidaceae (100 BP) are sister (90 BP) to the rest. At the next node, a clade (85 BP) with Blandfordiaceae sister (100 BP) to Asteliaceae plus Lanariaceae/Hypoxidaceae (100 BP) is sister to the rest (<50 BP). At the next node, Boryaceae (100 BP) are sister (100 BP) to the rest, followed by Tecophilaeaceae (54 BP), and a clade (<50 BP) in which Doryanthaceae are sister (99 BP) to Ixioliriaceae/Iridaceae. Xeronemataceae and Xanthorrhoeaceae s.l. (98 BP) are then successively sister (100, 97 BP) to a clade in which Alliaceae s.l. (85 BP) and Asparagaceae s.l. (53 BP) are sisters.

The larger commelinid clade is well supported (100 BP), and within it two major subclades occur (100 and 58 BP); Arecales (Arecaceae; 100 BP) are sister to the rest of the commelinids (<50 BP). In the first major subclade, Commelinales and Zingiberales are sisters (100 BP). Within Zin-

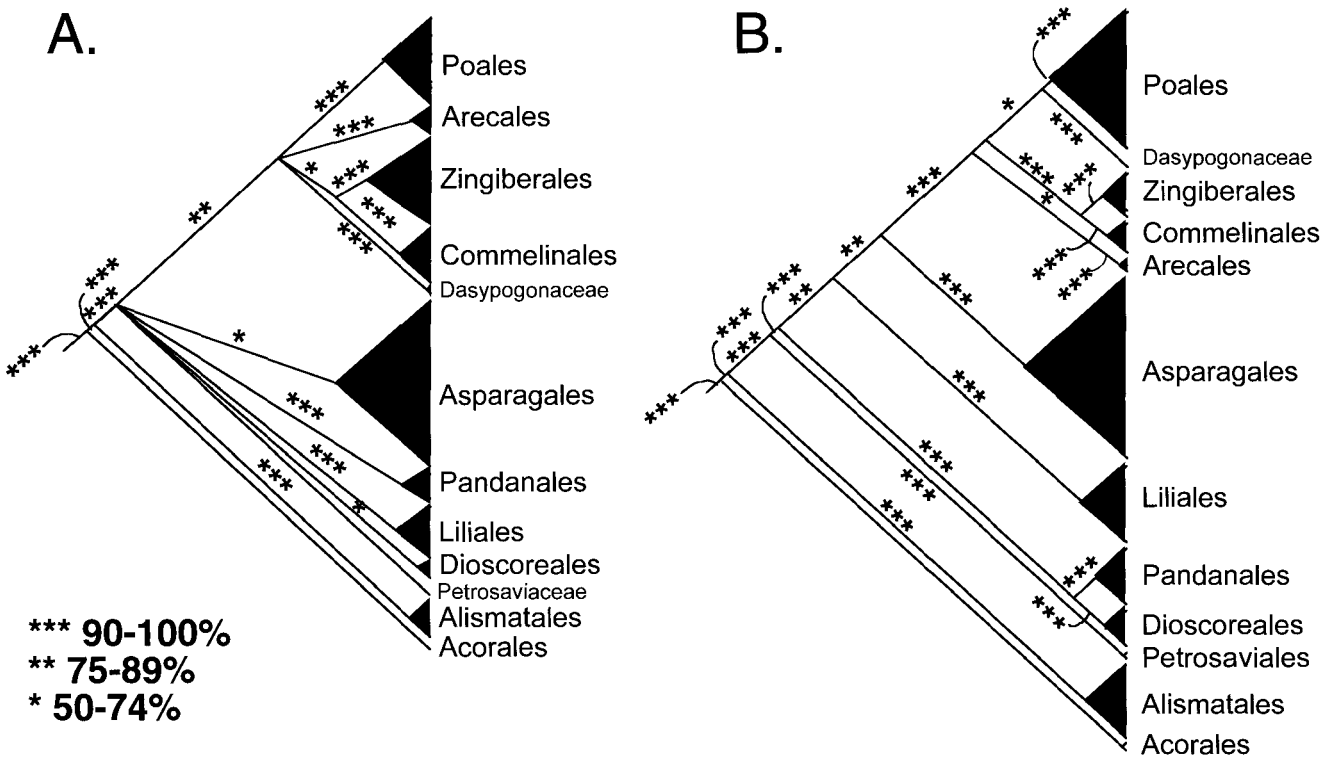

Fig. 1A–B.—Comparison between the bootstrap (50%) consensus trees produced by the (A) three-gene (modified from Chase et al. 2000*a*) and (B) seven-gene (this paper) analyses. Asterisks indicate general range of bootstrap percentages for each marked clade.

giberales (100 BP), Lowiaceae/Strelitziaceae (100 BP) are sister (59 BP) to the rest, to which Heliconiaceae and Musaceae are successively sisters (57 and 98 BP, respectively). Finally, a well-supported clade (98 BP) has Zingiberaceae/Costaceae (97 BP) sister to Cannaceae/Marantaceae (98 BP). In Commelinales (100 BP), there are two subclades (68 and 53 BP): (i) Commelinaceae (100 BP) sister to Hanguanaceae, and (ii) Philydraceae sister (77 BP) to Pontederiaceae/Haemodoraceae.

In the second major commelinid subclade (58 BP), Dasypogonaceae (100 BP) are sister (100 BP) to Poales. Within Poales, Bromeliaceae (100 BP) and Typhaceae (100 BP) are successive sisters to the rest (>50, 97 BP), within which Rapateaceae are sister to all others (82 BP). The remaining Poales form two clades, graminids and cyperids (90 and 76 BP, respectively). In the graminid clade, there are two subclades (69 and 85 BP): (i) Anarthriaceae sister (100 BP) to Centrolepidaceae plus Restionaceae (73 BP) and (ii) Flagellariaceae and Joinvilleaceae successively (100 and 55 BP, respectively) sister to Ecdeiocoleaceae/Poaceae.

In the cyperid clade, a clade (71 BP) with Eriocaulaceae/Xyridaceae is sister (60 BP) to the rest. The position of Mayacaceae is thus weakly supported, but it is sister (100 BP) to all other members of the cyperid clade (except Eriocaulaceae/Xyridaceae). Thurniaceae (100 BP) are sister (100 BP) to Juncaceae (100 BP)/Cyperaceae (100 BP).

In the combined matrix with the problem taxa included, there were nine trees of 69,689 steps with a CI = 0.53 and RI = 0.47. The strict consensus tree (not shown) is similar to that described above and shown in Fig. 2, except that Triuridaceae are sister to Velloziaceae in Pandanales (and the order has only 74 rather than 100 BP). Corsiaceae are embedded in Liliaceae (Liliales), and BP for the latter family drops to 83 (down from 100 BP). Hydatellaceae are embedded in Burmanniaceae (Dioscoreales), and BP for the order drops to less than 50 (down from 100 BP). Burmanniaceae (>50 BP) are sister to Dioscoreaceae. If Burmanniaceae are excluded from the analysis, then Hydatellaceae are sister to Mayacaceae. These taxa for which most of the gene regions are missing also have major effects on support far away from their positions (not shown); for example, in this analysis the commelinid clade dropped from 100 to 61 BP, Poales from 100 to 79 BP, and Liliales from 100 to 74 BP.

The combined plastid matrix consisted of 7019 characters, of which 5120 were variable and 3547 (50%) were potentially parsimony-informative. Analysis produced 36 trees of 54,671 steps with a CI = 0.56 and RI = 0.49. These 36 trees were in three islands of 12 trees each; starting with any one tree from each set of 12 only ends up with 12 trees (the definition of an island). The three islands vary in the relative positions of *Anemarrhena* Bunge relative to *Aphyllanthes* L., Alliaceae s.l., and the members of Themidaceae/Hyacinthaceae and Iridaceae/Ixioliriaceae relative to Doryanthaceae (all Asparagales). In island one, Doryanthaceae are sister to Iridaceae/Ixioliriaceae, *Anemarrhena* is sister to the rest of Agavaceae s.l. (Asparagaceae s.l.), and *Aphyllanthes* is sister to *Allium* L., which leaves Themidaceae/Hyacinthaceae a sister pair. In island two, Doryanthaceae are sister to the larger clade containing most of Asparagales, whereas *Aphyllanthes* is sister to *Brodiaea* Sm. (Themidaceae), and this pair is sister to *Anemarrhena*, leaving *Scilla* L. (Hyacinthaceae) as sister to Agavaceae s.l. In the second island, Alliaceae s.l. are intact. In

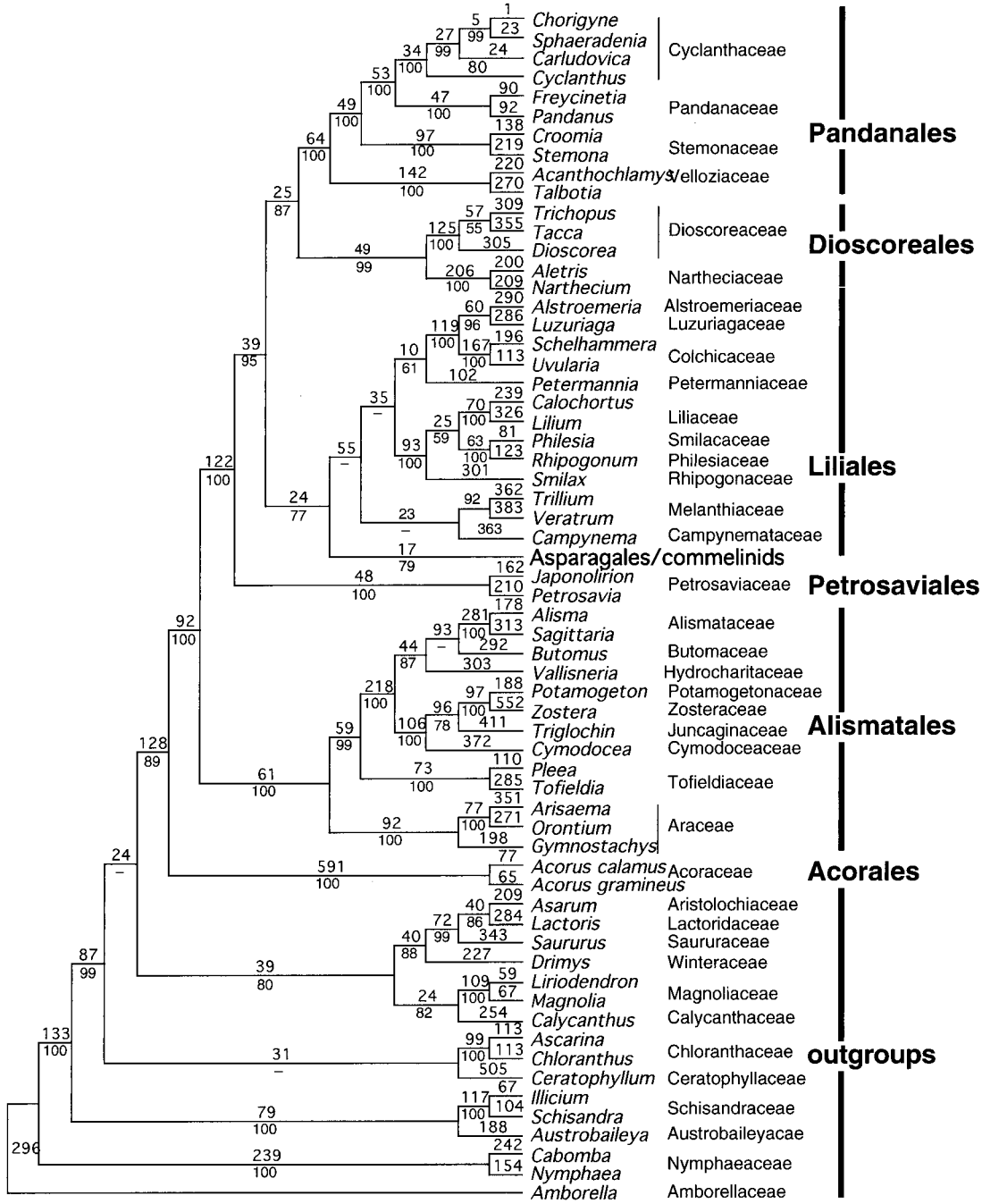

Fig. 2.—A single tree randomly selected from the three equally most-parsimonious trees produced from the combined matrix of all genes with the problem taxa removed (see text). The ten basalmost nodes along the spine of the tree are shown. Numbers above branches are estimated substitutions (DELTRAN optimization), and numbers below branches are bootstrap percentages. Clades not present in all trees are marked with an arrowhead.

the third island, *Anemarrhena* is again sister to Agavaceae s.l., and Doryanthaceae are sister to the larger clade, whereas *Scilla* is sister to *Aphyllanthes/Brodiaea*; Alliaceae s.l. are again intact. The strict consensus tree (see Fig. 4, 5) was nearly identical to that of the combined matrix of all genes except that relationships within Asparagaceae/Alliaceae (Asparagales) were less resolved, and Mayacaceae are sister to the rest of the cyperid clade rather than being sister to Cyperaceae/Juncaceae/Thurniaceae as in the combined analysis, but their position in the plastid tree received <50 BP. Brome-liaceae/Typhaceae are sister taxa (74 BP), whereas in the combined analysis of all data they are weakly supported as successive sisters to the order. Generally, BPs were lower in the plastid-only analysis than the analysis with all data, but in some cases BPs were more or less unchanged with the additional data; for example, along the spine of the tree from the basal node of the monocots up to the node of the commelinid clade, percentages in the plastid analysis were 96, 95, 100, 85, 80, 82, and 100 whereas for the combined analysis of all genes (without the problem taxa), they were 89, 100,

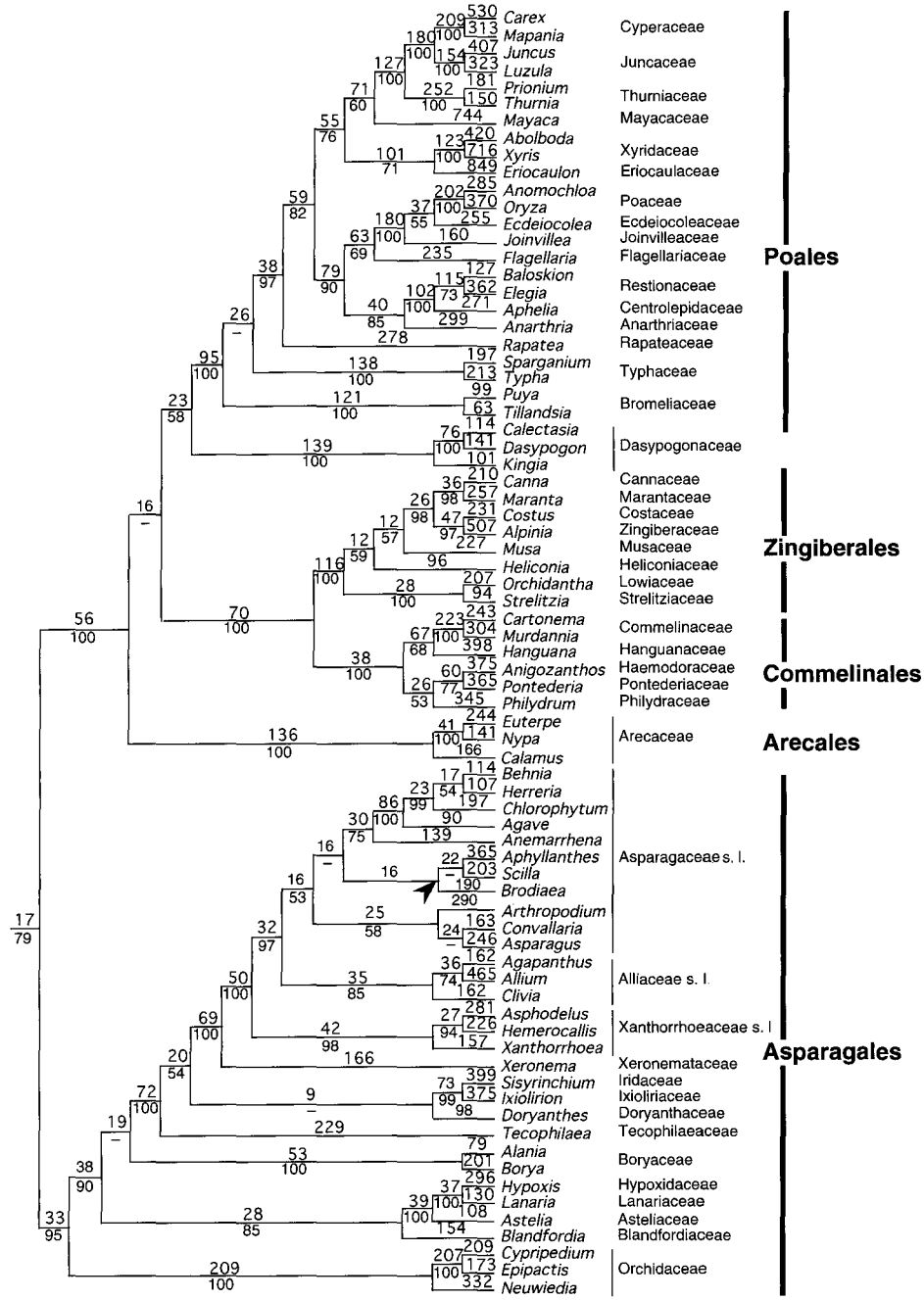

Fig. 3.—The same single tree as in Fig. 2 produced from the combined matrix of all genes with the problem taxa removed (see text). The Asparagales/commelinid clades are shown. Numbers above branches are estimated substitutions (DELTRAN optimization), and numbers below branches are bootstrap percentages. Clades not present in all trees are marked with an arrowhead.

100, 95, 77, 79, and 100. In a few other cases, support from the plastid combined matrix was somewhat higher, but never more than that for the node (along the spine, as above) at which Asparagales are sister to the commelinids (plastid 82 BP, all genes 79 BP).

DISCUSSION

Age and Relationships of Monocots to Other Angiosperms

Based on molecular clock approaches, monocots are the first major angiosperm clade to appear. Bremer (2002) dated their origin at 134 million years ago (mya), which is much older than their first appearance in the fossil record in the mid-Cretaceous (Gandolfo et al. 2002) and about the age of the oldest angiosperm fossils. Wikström et al. (2001) placed the origin at 140–155 mya, but their calibration point was outside the monocots, whereas that of Bremer (2002), which seems more reasonable in terms of the fossil record, was within. Our analyses here did not include one of the major clades of angiosperms, eudicots, and thus cannot be considered to be a robust assessment of higher-level angiosperm relationships. The data for such a study are available, but

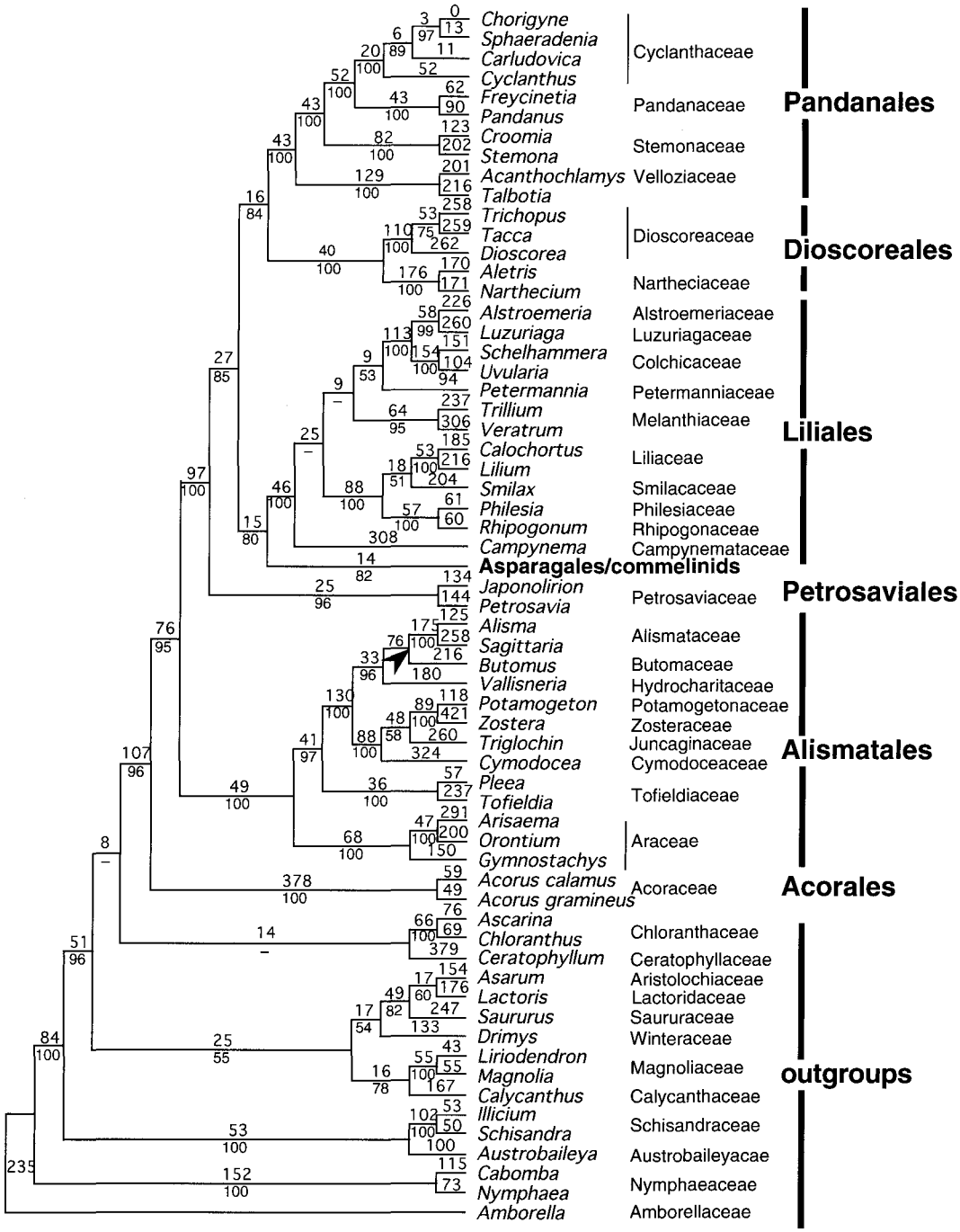

Fig. 4.—A single tree randomly selected from the 36 equally most-parsimonious trees produced from the combined matrix of all plastid genes with the problem taxa removed (see text). The ten basalmost nodes along the spine of the tree are shown. Numbers above branches are estimated substitutions (DELTRAN optimization), and numbers below branches are bootstrap percentages. Clades not present in all trees are marked with an arrowhead.

this is the focus of many other efforts, so we did not deem it important to include these data in this analysis. Duvall et al. (2006) found with Bayesian analyses of combined nuclear *PHY*C, plastid *ndh*F and *rbc*L and mitochondrial *atp*1 that monocots were sister with high posterior probabilities to the magnoliid clade (Canellales, Laurales, Magnoliales, and Piperales); Davis et al. (2004) using *atp*A and *rbc*L produced a similar result, but with low bootstrap support (<55 BP). Graham et al. (2006) using just plastid DNA, placed the monocots as sister to a clade composed of Ceratophyllaceae plus eudicots with 73 BP. Other analyses of higher-level relationships with angiosperms have varied as to which clade is sister to the monocots, and we do not understand how to compare bootstrap percentages to Bayesian posterior probabilities. Several studies have indicated that the latter are over-inflated estimates of confidence (Suzuki et al. 2002), so at present it remains unclear as to whether the Duvall et al. (2006) results are reliable.

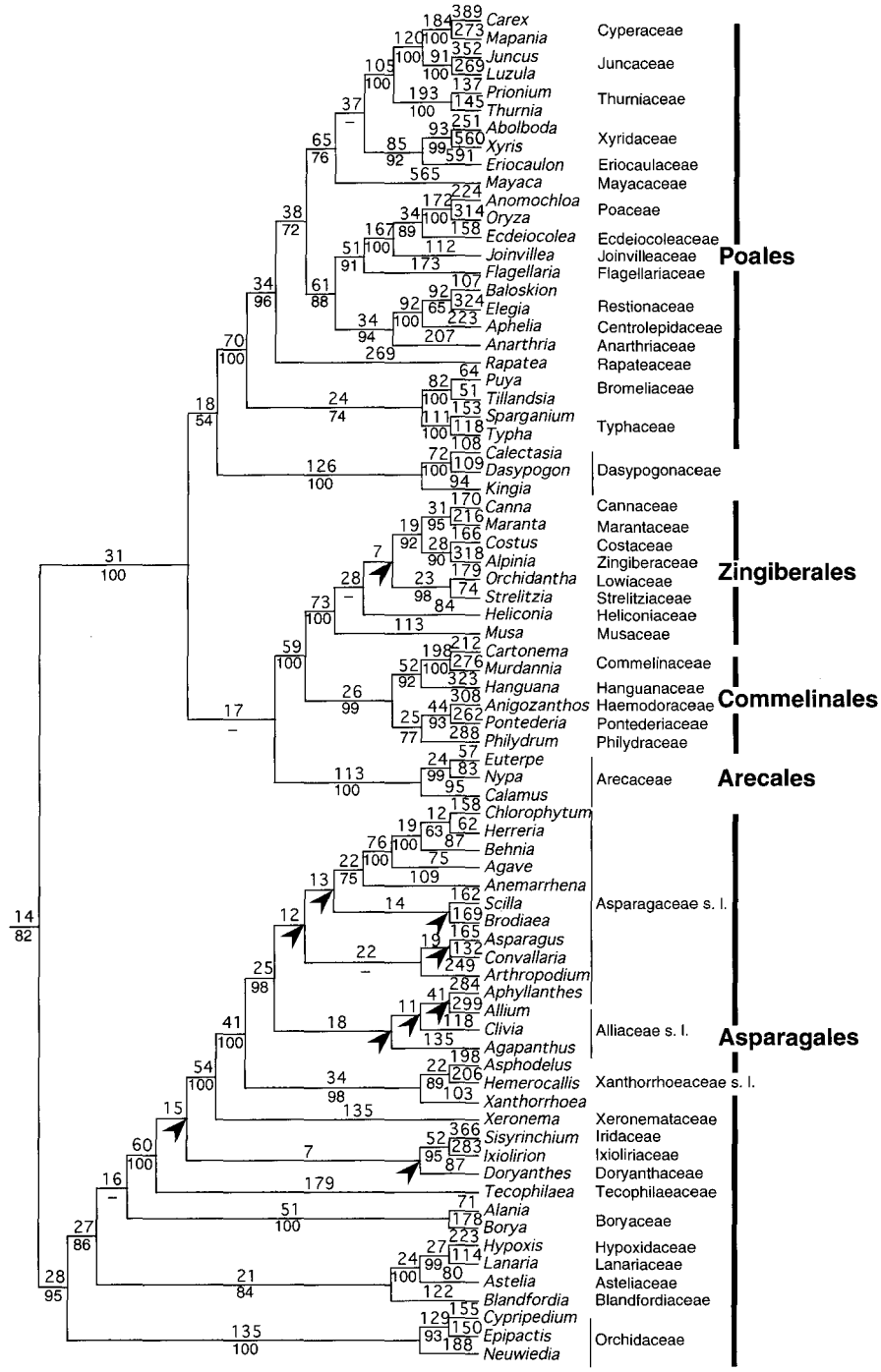

Fig. 5.—The same single tree as in Fig. 4 produced from the combined matrix of all plastid genes with the problem taxa removed (see text). The Asparagales/commelinid clades are shown. Numbers above branches are estimated substitutions (DELTRAN optimization), and numbers below branches are bootstrap percentages. Clades not present in all trees are marked with an arrowhead.

All Three Genomes Versus Plastid Only

The tree from all genes combined is clearly similar to the patterns observed in the plastid-only results, but at this stage there are too few data from the nuclear (one gene and only partial sequences for a second) and mitochondrial (one gene) genomes to say what the predominant patterns in these would be. Most clades with high bootstrap support (greater than 90%) in the mitochondrial (Davis et al. 2004) or 18S rDNA analyses (Soltis et al. 1998) do not contradict those in the combined plastid analysis presented here. The situation with the position of *Acorus* L. in the Davis et al. (2004) paper is complex; in the combined analysis of mitochondrial *atp*A and plastid *rbc*L *Acorus* is placed with high bootstrap/jackknife support (95–97%) as sister to the aquatic clade (Alismatales s.s.), which is an effect of *atp*A (*Acorus* is in this same position in the separate analysis of this gene, although it is weakly supported). Alismatales s.l. are also only weakly supported in Davis et al. (2004). These results are

difficult to interpret, and those same data are included here without decreasing support produced when the plastid data are analyzed alone. Separating sampling errors (too few data) from incompatible patterns, whether from biological or molecular causes, is notoriously difficult (Huelsenbeck et al. 1996), and until we have worked more with matrices of more genes from each of the genomic compartments, we will not be able to robustly address the reasons why individual genes do not produce identical patterns. At the least, it is clear that adding one mitochondrial and two nuclear genes to the four plastid genes does not produce a worse hypothesis of monocot relationships in terms of lower internal support, and the patterns obtained from all combined analyses of DNA data thus far are highly congruent with the results of other studies (but see Davis et al. 2004 for another perspective). Thus, although doubts may linger about whether direct combination of DNA data from different regions is an appropriate method of analysis, the results so far appear to be robust and predictive. Therefore, as long as the results of combined analysis with genes from all three genomes appear to be improvements over their predecessors, this route should continue to be followed. However, performing combined analyses should not prevent us from exploring the patterns produced by the individual compartments or the potential causes of deviations in pattern, as in Petersen et al. (2006). It is also clear that there are problems with the mitochondrial and nuclear genes as indicators of relationships for the achlorophyllous taxa (Burmanniaceae, Corsiaceae, and Triuridaceae) plus *Trithuria* (Hydatellaceae), the last of which is photosynthetic but which has also been problematic in other studies (Bremer 2002; Davis et al. 2004).

Multigene Analysis of Monocots in 2000 Versus 2005

The trees presented here (Fig. 1) resolve relationships of a number of the major monocot clades, and they provide stronger support for both of the two major foci that were unresolved in Chase et al. (2000*a*). The additional data produce trees in which the liliid orders continue to be paraphyletic. Petrosaviaceae are clearly sister (combined 100, 100 BP for the two encompassing nodes, plastid 100, 85 BP) to all orders except for Acorales and Alismatales. Pandanales and Dioscoreales are a clade with moderate support (87, 84 BP in the combined and plastid analyses). Likewise, Asparagales and the commelinids form a moderately supported clade (79, 82 BP). Adding additional genes appears to be required before a confident estimate of relationships for these clades is obtained, although with seven genes we appear to be approaching this point.

Graham et al. (2006) with ca. 14–15 kb of plastid DNA per taxon, found that the Asparagales/commelinid clade was strongly supported (96 BP). The relationships in Graham et al. (2006) are nearly identical to those found with four plastid genes here (Fig. 4, 5) and generally have similar levels of bootstrap support. Analyzing just plastid *ndh*F, Givnish et al. (2006) also found similar relationships, but of course with lower support than in Graham et al. (2006) and here. Support for Dioscoreales/Pandanales is lower (63 vs. 87 BP), as is that for the node of Liliales sister to Asparagales/commelinids (70 vs. 77 BP) and the positions of Arecales and Dasypogonaceae (both <50 BP). It should be noted that in Graham et al. (2006), the positions of Arecales and Dasypogonaceae are different from those obtained in this study (i.e., Arecales are sister to Poales with 33 BP rather than sister to all other commelinids with <50 BP; Dasypogonaceae are sister to Commelinales/Zingiberales with 38 BP rather than—as here—sister to Poales with 58 BP).

With respect to relationships of the orders within the commelinids, we see a similar pattern in the two analyses presented here (but note that Graham et al. [2006] did not get exactly these same relationships as noted above). Relationships here are resolved, but the two most crucial nodes, those placing Dasypogonaceae as sister to Poales and Arecales sister to all other commelinids are weakly supported (<50, 58 BP; Fig. 3, 5). Support for the Commelinales/Zingiberales clade is much higher than in Chase et al. (2000*b*; 100 vs. 71 BP), as is support for all of the orders except for Arecales, which was already 100 BP. Support for the commelinid clade is also improved, 100 vs. 77. Thus we see some major improvements in terms of increased support for the spine of the monocot tree, and there were substantial improvements in support for the positions of Petrosaviaceae, Dioscoreales/Pandanales and monophyly of the commelinids. The single most crucial node to higher-level relationships is that linking Liliales to Asparagales/commelinids; in the combined analysis of all genes, this node was only 77 BP vs. 80 BP in the plastid analysis (70 BP in Graham et al. 2006). More data and more extensive examination of the patterns present in the separate genomic compartments are now required to better assess confidence in this node.

It now appears appropriate to adopt Petrosaviales because they are sister to a clade composed of many orders. This is a formally stated prerequisite described in APG II (2003). The name is already available in the literature.

Alismatales.—The additional data have improved bootstrap support for the order, 100 here vs. 92 BP in Chase et al. (2000*b*). The position of Tofieldiaceae relative to Araceae and the aquatic families (Alismatanae sensu Dahlgren et al. 1985) is here strongly supported as sister to them both (100 BP), whereas there was less than 50 BP for the position previously. Two subclades within the aquatic families are moderately to strongly supported (100 and 87 BP), as in Les and Haynes (1995): (i) Alismataceae, Butomaceae, and Hydrocharitaceae (and perhaps Najadaceae and Limnocharitaceae, which were not included here) and (ii) Cymodoceaceae, Juncaginaceae, Potamogetonaceae, and Zosteraceae (and Aponogetonaceae, Posidoniaceae, Ruppiaceae, and Scheuchzeriaceae, also not included here).

Liliales.—In the shortest trees, Campynemataceae are sister to Melanthiaceae, in which they were previously included (Dahlgren et al. 1985). This pair of families is sister to all the remaining Liliales, but with less than 50 BP. The plastid-only trees (Fig. 4, 5) differ and resolve the positions of these taxa (Fig. 4), but this is <50 BP. Rhipogonaceae are strongly supported as sister to Philesiaceae, but the position of Smilacaceae is weakly supported relative to Liliaceae s.s. and Philesiaceae/Rhipogonaceae. One major difference between trees in this study and those of most previous analyses is the position of Petermanniaceae, which in Chase et al. (2000*b*) and Rudall et al. (2000) were embedded in Colchicaceae, rather than being sister to Colchicaceae and Alstroemeri-

aceae/Luzuriagaceae as here (Fig. 2, 4). The reason for this change is that we discovered that the material identified as *Petermannia* F. Muell. is in fact *Tripladenia* D. Don (both are vining taxa with broad, dicot-like leaves from southeastern Australia). Therefore, we recognize Petermanniaceae as a distinct family and not a synonym of Colchicaceae as in APG II (2003). The other major change is the addition of Corsiaceae to this clade. Their exact position is not clear, and the evidence is at this point based solely on the rDNA data; *Arachnitis* is sister to *Lilium* L. (result not shown), but this is weakly supported. See Fay et al. (2006) for a better-sampled analysis of Liliales.

Dioscoreales/Pandanales.—These two orders forming a moderately supported clade (87 BP) is a major shift from previous analyses. Nartheciaceae being sister to the rest of Dioscoreales is now strongly supported unlike their position in Chase et al. (2000*b*) and Caddick et al. (2002*a*), and there is morphological evidence to support this position (Caddick et al. 2002*b*). *Thismia* is missing 26S rDNA, *mat*K and *ndh*F, and *Burmannia* is missing *ndh*F, and there is a tendency for all the achlorophyllous taxa to attract each other in the *atp*1 tree (Petersen et al. 2006), which may lower bootstrap support for the phylogenetic patterns in Dioscoreales. APG II (2003) recognized a broader concept of Dioscoreaceae (including Taccaceae and Trichopodaceae) based on the results in Caddick et al. (2002*a*, *b*).

Relationships within Pandanales are little changed over previous studies, and the only major alteration is that Velloziaceae are now strongly supported as sister to the rest, although the position of Triuridaceae relative to Velloziaceae is not clear. We have only the rDNA and *atp*1 data upon which to base the placement of Triuridaceae.

Asparagales.—The relationship of Orchidaceae to the rest of Asparagales now seems clear; they are sister to the rest of the order both here (90, 86 BP) as well as in Graham et al. (2006; 76 BP) and Hilu et al. (2003; <50 BP). All analyses to date, except for that of Savolainen et al. (2000) in which they were unresolved, have positioned orchids in Asparagales. This result has much higher support than in Chase et al. (2000*b*; 56 BP). In Pires et al. (2006), support for Orchidaceae in Asparagales is moderate (88 BP).

The position of Boryaceae remains unclear relative to the rest of the order (except for the orchids) and the hypoxid clade (BP 85 in the combined analysis), which includes Blandfordiaceae, Lanariaceae, Asteliaceae, and Hypoxidaceae and is moderately supported. The last three families share a number of characters (Rudall et al. 1998) and could be combined into one family on the basis of these results. Blandfordiaceae are morphologically highly divergent from the rest of these, although based on DNA data they appear to be related to them. In Graham et al. (2006), Boryaceae are weakly supported as sister to the hypoxid clade (78 BP). The next clade up from Boryaceae has Tecophilaeaceae as sister (54 BP) to the rest, followed by a weakly supported (<50 BP) clade with Doryanthaceae sister to Ixioliriaceae/Iridaceae (99 BP). Although the relationship of Iridaceae to Ixioliriaceae here and in Hilu et al. (2003) is strongly supported, other studies (Graham et al. 2006; Pires et al. 2006) place Ixioliriaceae with Tecophilaeaceae, and the positions of all of these families require additional sampling to establish their interrelationships.

Support for the next clade (Xeronemataceae upward in Fig. 3, 5) is strong (100 BP). Within the clade sister to Xeronemataceae, Xanthorrhoeaceae s.l. (including Asphodelaceae and Hemerocallidaceae) are sister (100 BP) to that termed the "higher asparagoids" (Rudall et al. 1997), which APG II (2003) lumped into two families, Alliaceae s.l. (including Amaryllidaceae and Agapanthaceae, all with umbellate inflorescences enclosed by two large bracts) and Asparagaceae s.l. (including Agavaceae s.l., Aphyllanthaceae, Hyacinthaceae, Laxmanniaceae, Ruscaceae, and Themidaceae, which all have racemes except for the last that have umbellate inflorescences like Alliaceae but differ in lacking the two enclosing bracts). With the taxon sampling used here, *Aphyllanthes* L. causes problems, as documented previously in Fay et al. (2000) and McPherson et al. (in press). In the combined analysis of all data, *Aphyllanthes* fell with *Scilla* L. (but with BP <50; Fig. 3), but in the plastid combined analysis *Aphyllanthes* was one of the taxa involved in creating islands of equally most-parsimonious trees, so that in the strict consensus tree this part of the tree was highly unresolved (Fig. 5). With a greater sampling of genera, Pires et al. (2006) placed *Aphyllanthes* with *Lomandra* Labill. and *Sowerbaea* Sm. (Laxmanniaceae). McPherson et al. (in press) examined the problems associated with the placement of *Aphyllanthes*. To illustrate this effect, we removed *Aphyllanthes* here as well and did a bootstrap analysis of the combined data (results not shown), and the bootstrap percentages went up dramatically; for example, Alliaceae s.l. received 91 BP (it was less than 85 BP in the combined analysis here; Fig. 3), and Asparagaceae s.l. was 62 BP (vs. 53 BP in Fig. 3). A similar experiment was reported in Pires et al. (2006), in which Alliaceae s.l. received 100 BP and Asparagaceae s.l. 96 BP. Graham et al. (2006) omitted *Aphyllanthes* and obtained 100 and 97 BP for Alliaceae s.l. and Asparagaceae s.l., respectively. We will not discuss relationships within Alliaceae s.l. and Asparagaceae s.l. and refer readers to the better-sampled analyses of Pires et al. (2006).

Commelinids.—The commelinid clade has a long history of recognition (Dahlgren et al. 1985) and was present in the first large analyses of *rbc*L in the monocots (Chase et al. 1993, 1995*a*; Duvall et al. 1993), although it was poorly supported. In all analyses here they received 100 BP (Fig. 3, 5), as they also did in Graham et al. (2006). Within commelinids, inter-ordinal relationships are consistently resolved, but the positions of Dasypogonaceae and Arecales are not well supported. Our analyses and those of Graham et al. (2006) do not agree on the position of these two taxa; because of this inconsistency and poor support the former could yet end up being placed in either Poales or Arecales, so acceptance of Dasypogonales would be premature (the ordinal name already exists).

Within Poales, relationships are much clearer than in Chase et al. (2000*b*), perhaps partly due to the better sampling of this study. The relative positions of Bromeliaceae and Typhaceae remain weakly supported (*Sparganium* L. and *Typha* L. are sisters, 100 BP; recognition of Sparganiaceae in APG II 2003 was an accident and not intended). Graham et al. (2006) reverses their positions relative to our

results, but again without bootstrap support >50. Our analysis of plastid DNA and that of Givnish et al. (2006) (just plastid *ndh*F) make Bromeliaceae and Typhaceae sister taxa, but with weak support. Rapateaceae are then sister to the remainder of the order with moderate to strong support (97, 82 BP in the combined analysis; 96, 72 BP in the plastid analysis).

The remaining families are split into two large subclades: (i) the graminid clade with the restionid families (Anarthriaceae, Centrolepidaceae, and Restionaceae) sister to Poaceae plus Ecdeiocoleaceae, Flagellariaceae, and Joinvilleaceae, and (ii) the cyperid clade, which has Xyridaceae/Mayacaceae sister to Cyperaceae plus Eriocaulaceae, Juncaceae, and Thurniaceae. Hydatellaceae (results not shown) appear to be related to the Xyridaceae/Eriocaulaceae clade, although the large amount of missing data for *Trithuria* and spurious attraction with Burmanniaceae makes this assessment tentative. All these relationships are similar to those of other studies focusing just on the commelinid clade (Givnish et al. 1999; Bremer 2002). The position of Mayacaceae as sister to the cyperid clade is variably supported here (60, 100 BP), but in Graham et al. (2006) it is strongly supported (100, 100 BP).

Prospects for improvement.—The accumulating monocot data matrix will require the addition of yet more genes before relationships of Asparagales, commelinids, and Liliales to the other clades are all strongly supported. Noncoding plastid regions, such as the *trn*L intron and *trn*L–F intergenic spacer, which have worked well for estimating relationships at the basal nodes in the angiosperms (Borsch et al. 2003) will not work in the monocots as a whole because alignment is problematic (Fay et al. 2000), and many groups have large numbers of plastid microsatellite motifs that make sequencing these regions technically extremely difficult (Devey et al. 2006). Other plastid regions can be added to the matrix to help address the remaining issues (Graham et al. 2006), but it would be desirable to include nuclear protein-coding genes and additional mitochondrial genes in future work.

Plastid genes are either absent or highly divergent in achlorophyllous taxa such as some Burmanniaceae, Corsiaceae, and Triuridaceae, which presents problems for obtaining clear placements of such taxa in the monocot tree. We had hoped that mitochondrial genes would permit us to better assess relationships of these taxa, but highly heterogeneous rates among different lineages of monocots, including achlorophyllous species, makes this more difficult and less satisfactory than anticipated (Petersen et al. 2006).

Nuclear, low-copy protein-coding genes would be potentially valuable additions to the combined data matrices (such as *PHYC*; Mathews and Donoghue 1999; Duvall et al. 2006), but thus far most of these that have been tried appear to be routinely and reliably amplified from monocots. However, investigations with prospective loci are ongoing. With emerging EST collections from across the monocots and the complete genomic sequence of *Oryza* L., we may be able to identify some good candidates soon, as Fulton et al. (2002) have done in eudicots. Although we are reasonably confident that patterns obtained thus far with plastid genes, which have the greatest impact on topology, appear to be made clearer (i.e., have higher bootstrap percentages) by addition of genes from the other two genomes, there is at least an interest in having good representation from all three genomes so that we can use the phylogenetic framework of the combined analyses to make evaluations of molecular evolution for these loci more robust. Hybridization and horizontal transfer are not likely to greatly affect either monocot tree topologies or optimization of other data on trees. In the first case, this is because hybrids are formed by such closely related species (which are only little diverged in their DNA sequences) that the effect would be exceedingly small. In the second, this is because we already have evidence that the existing trees are predictive of other attributes for these taxa (e.g., Adams et al. 2001), which would not be the case if horizontal transfers of only one or a few genes had occurred. Use of plastid genes in monocot phylogenetics has been a great success and parallels that obtained for angiosperms as a whole, but we nonetheless look forward to seeing how additional mitochondrial and nuclear genes contribute to our knowledge of monocot phylogenetics.

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