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Phylogeny of the Asparagales based on three plastid and two mitochondrial genes[1]

Ole Seberg[2,9], Gitte Petersen[2], Jerrold I. Davis[3], J. Chris Pires[4], Dennis W. Stevenson[5], Mark W. Chase[6], Michael F. Fay[6], Dion S. Devey[6], Tina Jørgensen[2], Kenneth J. Sytsma[7], and Yohan Pillon[6,8]

[2]Botanical Garden, Natural History Museum of Denmark, Sølvgade 83, opg. S, DK-1353 Copenhagen K, Denmark; [3]Department of Plant Biology, 228 Plant Science, Cornell University, Ithaca, New York 14853 USA; [4]Biological Sciences, 105 Tucker Hall, University of Missouri-Columbia, Missouri 65211-7400 USA; [5]The New York Botanical Garden, Bronx, New York 10458 USA; [6]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK; [7]Department of Botany, University of Wisconsin, 430 Lincoln Drive, Madison, Wisconsin 53706-1381 USA

- *Premise of the study:* The Asparagales, with ca. 40% of all monocotyledons, include a host of commercially important ornamentals in families such as Orchidaceae, Alliaceae, and Iridaceae, and several important crop species in genera such as *Allium*, *Aloe*, *Asparagus*, *Crocus*, and *Vanilla.* Though the order is well defined, the number of recognized families, their circumscription, and relationships are somewhat controversial.
- *Methods:* Phylogenetic analyses of Asparagales were based on parsimony and maximum likelihood using nucleotide sequence variation in three plastid genes (*matK, ndhF*, and *rbcL*) and two mitochondrial genes (*atp1* and *cob*). Branch support was assessed using both jackknife analysis implementing strict-consensus (SC) and bootstrap analysis implementing frequency-within-replicates (FWR). The contribution of edited sites in the mitochondrial genes to topology and branch support was investigated.
- *Key results:* The topologies recovered largely agree with previous results, though some clades remain poorly resolved (e.g., Ruscaceae). When the edited sites were included in the analysis, the plastid and mitochondrial genes were highly incongruent. However, when the edited sites were removed, the two partitions became congruent.
- *Conclusions:* Some deeper nodes in the Asparagales tree remain poorly resolved or unresolved as do the relationships of certain monogeneric families (e.g., Aphyllanthaceae, Ixioliriaceae, Doryanthaceae), whereas support for many families increases. However, the increased support is dominated by plastid data, and the potential influence of mitochondrial and biparentially inherited single or low-copy nuclear genes should be investigated.

Key words: *Aphyllanthes*; Asparagales; edited sites**;** incongruence; maximum likelihood; parsimony; Ruscaceae.

The Families of the Monocotyledons by Dahlgren et al. (1985) may be considered the starting point of modern systematics of the monocotyledons—undoubtedly, one of the largest (~62 000 species) and economically most important named clades in the angiosperm tree of life. The classification of Dahlgren et al. (1985) was largely built on an earlier, meticulous analysis of mostly traditional, morphological/chemical characters by Dahlgren and Clifford (1982) combined with a peculiar pseudocladistic analysis of these data by Dahlgren and Rasmussen (1983, p. 350), which they named "reverse cladistics". In this reverse cladistics approach, all families and the four major groupings or complexes (Ariflorae-Triuridiflorae-Alismatiflorae, Liliales-Orchidales, Bromeliiflorae-Zingiberiflorae, and Commeliniflorae complexes), which were used as a framework for discussion, were a priori considered monophyletic. The method was primarily intended to show "how much homoplasy must be accepted if [the] current eclectic classification largely reflects phylogeny" (Dahlgren and Rasmussen, 1983, p. 350), rather than pretending to be a phylogenetic analysis in any modern sense of the word. Nonetheless, these character mappings are often erroneously interpreted as phylogenetic trees (Conran, 1995; De Mello-Silva, 2005; Linder and Rudall, 2005; Bogler et al., 2006). Takhtajan (1987) and Thorne (1992) published alternative classifications exclusively or primarily based on traditional characters, and Stevenson and Loconte (1995) also published a classification based upon a parsimony analysis of morphological characters; however, the classification of monocots by Dahlgren et al. remained the most widely accepted prior to the "molecular revolution". A more recent classification of the angiosperms by Thorne and Reveal (2007) is at odds with our current perception based on molecular phylogenetics and combined molecular and morphological analyses (Chase et al., 1995), not only at higher taxonomic levels, but also when it comes to circumscription of families, where several paraphyletic taxa are recognized, although some of these smaller families are well characterized morphologically.

Dahlgren et al. (1985) included Dioscoreales, Asparagales, Liliales, Burmanniales, and Melanthiales in their petaloid monocotyledons, Liliiflorae. However, both in this treatment and in the earlier treatment by Dahlgren and Rasmussen

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[8]Present address: Department of Biology, University of Hawai'i, Hilo, Hawai'i 96720 USA

[9]Author for correspondence (e-mail: oles@snm.ku.dk)

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(1983), the position of Dioscoreales, which were considered the most archaic group of monocots, remained obscure, whereas the other four groupings (see above) were considered individual clades without obvious relationships to each other. Today, the taxa included in the Burmanniales and Melanthiales are largely transferred to the Dioscoreales and Liliales, respectively.

Adhering to Huber's (1969) earlier ideas, Dahlgren et al. (1985) considered Asparagales a monophyletic group separate from Liliales. However, Dahlgren et al. emphasized that virtually none of the characters considered diagnostic for separating the Asparagales from the Liliales by Huber was of compelling phylogenetic significance because all showed more or less homoplasy. What largely remained as possible candidate synapomorphies were simple styles and phytomelan-encrusted seed coats, although even these characters have exceptions. The most conspicuous synapomorphy, the phytomelan-encrusted seed coats, was additionally supposed to be repeatedly gained and lost in connection with changes from dehiscent to indehiscent fruit types.

This unstable morphological foundation for the separation of Asparagales from Liliales (see also, e.g., Rudall et al., 2000a; Rudall, 2002) became even more evident as taxa from the Liliales of Dahlgren et al. (1985); for example, Iridaceae and Orchidaceae s.l., were shown to be more closely related to members of Asparagales than to the remaining Liliales by molecular data (Chase et al., 1993, 1995; Davis et al., 2004; Givnish et al., 2005; Pires et al., 2006; Fay et al., 2000). Thus, Asparagales were redefined not only by the inclusion of taxa transferred from Liliales, but also by the exclusion of taxa, e.g., Dasypogonaceae s.l., Hanguanaceae, Luzuriagaceae pro parte, and Philesiaceae. Additionally, some families previously accepted by Dahlgren et al. (1985), e.g., Anthericaceae and Alliaceae, were shown to be polyphyletic (Fay and Chase, 1996; Chase et al., 1995).

With the exception of an investigation by Savolainen et al. (2000), all subsequent analyses, using a variable number of taxa and different molecular markers, have recovered this recircumscribed Asparagales as monophyletic (Chase et al., 1993; Nadot et al., 1995; Soltis et al., 1997, 2000; Tamura et al., 2004; Givnish et al., 2005; Li and Zhou, 2007). This is reflected in the Angiosperm Phylogeny Group's (APG) series of classifications (APG, 1998; APG II, 2003; APG III, 2009; Chase et al., 2000a), and it is this delineation of the Asparagales that will be used in the present investigation. Thus circumscribed, Asparagales, with approximately 1100 genera and 26 000 species, includes more than one third of the monocotyledons. However, 880 of these genera and 22 075 of the species belong to Orchidaceae alone (Stevens, 2001).

The number and circumscription of families in Asparagales and their interrelationships have remained somewhat controversial. In their precladistic approach, Dahlgren et al. (1985) recognized 30 families, whereas Kubitzki (1998), influenced by recent developments in molecular systematics, recognized 33 often differently circumscribed families. The three APG classifications accept a diminishing number of families within Asparagales. Thus, the original APG recognized 29 families (APG, 1998; Chase et al., 2000a), APG II recognized 14, plus 11 optional [bracketed] families (APG II, 2003), and APG III recognized only 14 families (APG III, 2009). The optional families allowed in the APG II classification may be viewed both as a compromise between Dahlgren et al.'s (1985) classification of the lilioid monocots, with its many small, nonmonophyletic families and earlier classifications with few large families (see e.g., Melchior, 1964), and as an attempt to alleviate alleged pedagogical problems related to the preferred recognition of fewer but larger and more ungainly monophyletic families. In the APG III (2009) classification, the large monophyletic families are the only ones maintained, and a subfamilial classification is introduced (Chase et al., 2009) largely by lowering the taxonomic rank of the previously accepted, optional, bracketed families in the APG II to subfamilies, e.g., Alliaceae with the two bracketed families Agapanthaceae and Amaryllidaceae, becomes Amaryllidaceae (over Alliaceae, for reasons of nomenclatural conservation) with three subfamilies; Agapanthoideae, Allioideae, and Amaryllidoideae. However, it remains a moot point whether the difficult-to-recognize bracketed families of APG II are a worse or better choice than the equally difficult-to-recognize subfamilies of APG III.

A few attempts have been made to reconstruct the phylogeny within the Asparagales. The first was based on *rbcL* data and included 172 species of monocots (Chase et al., 1995) with an emphasis on Lilianae (= Liliiflorae; sensu Dahlgren et al. [1985]). In this analysis, the Asparagales was divided into two major groups; a paraphyletic "lower" and a monophyletic "higher asparagoids", morphologically recognizable by simultaneous microsporogenesis and inferior ovary and successive microsporogenesis and superior ovary, respectively (Rudall et al., 1997). These two informal groups, which have later been renamed "non-core" and "core asparagoids" by Kim et al. (2010), remain in subsequent analyses. In an analysis concentrating on the Asparagales, Fay et al. (2000) used four plastid regions (*rbcL*, *atpB*, the *trnL* intron, and the *trnL-F* intergenic spacer) and included representatives of all families recognized by APG (1998); in total, the analysis included sequences from 108 accessions, of which 90 were from the Asparagales and 18 from outgroup taxa. However, this analysis included only 64 terminals belonging to the Asparagales, and 14 belonging to outgroups. Several sequences from different taxa—usually species belonging to the same genera—were combined into "composite" terminals. Results of this analysis by Fay et al. (2000) and a later one by Pires et al. (2006), which was based on six plastid regions (*atpB*, *rbcL*, *trnL* intron, and *trnL-F* intergenic spacer, *ndhF*, and *matK*) and one mitochondrial gene (*atp1*) and 79 taxa, yielded results similar to those presented here, and both recovered the two major groups already suggested by Chase et al. (1995). A recent study used genome survey sequencing (GSS) to acquire organellar and rDNA sequences to further resolve the phylogenetic tree of 50 taxa within the core Asparagales. However, using 79 plastid protein-coding genes, five mitochondrial genes, and three rDNA loci Steele et al. (2012) largely recovered the same relationships as Pires et al. (2006) though often with stronger support.

This paper represents the most comprehensive taxon sampling of the Asparagales to date, with 138 taxa from Asparagales plus 15 outgroup taxa, as sampled for five genes from two genomic compartments, *atp1* and *cob* from the mitochondrial genome and *rbcL*, *matK*, and *ndhF* from the plastid genome. In addition to producing the most fully corroborated phylogenetic analysis of the Asparagales so far, we explore areas of conflict in phylogenetic signal between the two genomic partitions, which could, for example, be caused by edited sites or replacement of original gene copies with processed paralogs in the mitochondrial genes (Bowe and DePamphilis, 1996; Petersen et al., 2006a, b; Cuenca et al., in press).

MATERIALS AND METHODS

***Taxon sampling*—**Whenever possible, multiple genera were sampled for each family in Asparagales in APG II. The ingroup consisted of 138 taxa and an outgroup of 15 taxa, mainly from Pandanales, Dioscoreales, Petrosaviales, Liliales, and a number of taxa from the "commelinids" (Chase et al., 2006). The taxon sampling within Asparagales included the same 65 taxa used in Pires et al. (2006), except for six genera not included here. However, only two of the 14 outgroup taxa used by Pires et al. (2006) are the same as the ones used here. Most of the terminals (genera) were composite, i.e., sequences are combined from more than one species within a genus. Thus, 91 genera (59%) were represented by two to five species, while 55 genera (36%) were represented by a single species, and seven genera (5%) were represented by one to five undetermined species. Information about the species used, including authorities, etc. may be found in Appendix S1 (see Supplemental Data with the online version of this article). To facilitate comparison, we provisionally assigned the family names (including the bracketed families) used in APG II (2003) to the families in the Asparagales.

***Molecular methods*—**Many methods have been used to produce the sequences analyzed in this paper; we provide below one standard method. Total genomic DNA was extracted from dried leaves using the DNeasy plant extraction kit (Qiagen, Crawley, West Sussex, UK), following the manufacturer's instructions. PCR was performed under standard conditions, and the products were purified using the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions. In some cases, where PCR amplification yielded only faint bands, PCR-generated DNA fragments were cloned with a TOPO TA Cloning Kit (Invitrogen, Carlsbad, California, USA). Fragments were ligated into the pCR 2.1-TOPO vector and introduced into chemically competent *Escherichia coli* cells of strain DH5α-T1. Plasmid DNA then was extracted using a QIAprep Spin Miniprep Kit (Qiagen, Valencia, California, USA) and sequenced, either with the original amplification primers or with the M13 plasmid primers supplied with the cloning kit. Cycle sequencing was performed using the ABI PRISM Dye Terminator Cycle Ready Reaction kit with AmpliTaq DNA Polymerase, FS (Applied Biosystems, Wellesley, Massachusetts, USA), and the products were purified as described. Sequencing was conducted on an ABI 3130XL automated sequencer (Applied Biosystems), and sequence editing was done using Sequencher versions 4.6 to 4.8 (Gene Codes Corp., Ann Arbor, Michigan, USA).

For PCR amplification of *atp1*, primers atpAF1.5 and atpAB1.5 were preferentially used to amplify the entire fragment in one piece (Petersen et al., 2006a). Additional primers (atpA-F5, atpA-B4 [Davis et al., 1998], atpA43f, atpA393r, atpA418f, atpA558r, atpA633r, atpA652f, atpA1137r [Petersen et al., 2006a]) were used for sequencing and sometimes also for PCR amplification when the principal amplification failed. Alignment of the sequences was unproblematic, and the final alignment includes two 6-bp gap regions and three 3-bp gap regions.

For PCR amplification of *cob* primers JD1F and JD1R were preferentially used to amplify the entire fragment in one piece (Petersen et al., 2006a). Additional primers (TCOBF1, TCOBF2, TCOBF3, TCOBR1, TCOBR2, TCOBR3 [Petersen et al., 2006a]) were used for sequencing and sometimes also for PCR amplification when the principal amplification failed. Alignment of the sequences was entirely unproblematic as the sequences are of equal length.

Methods for production of *rbcL* sequences have varied over time, but a typical protocol is provided in Fay et al. (2000). For *matK* and *ndhF*, protocols are provided in Pires et al. (2006).

***Data analysis*—**In the parsimony analyses, all characters were weighted equally and treated as nonadditive. Gaps were treated as missing during tree search. Phylogenetic analyses of all data, the plastid, and mitochondrial data were conducted in the program PAUP* ver. 4.0b10 (Swofford, 2002), using the default options, except that 200 random addition sequences, holding 200 trees at each step were used. When running the mitochondrial data set alone, we excluded the three taxa for which no data could be collected, and only 500 trees were collected per replicate due to the very large number of equally parsimonious trees found. Runs of individual genes that could not be swapped to completion within a reasonable time period were repeated several times but were terminated when an excessive number of equally parsimonious trees were collected (see Table 1). The length of the most parsimonious trees derived from the combined data set was verified in the program TNT (ver. 1.1) (Goloboff et al., 2008) running a traditional search with 1000 replicates and 10 trees held per replicate. Even extensive manipulation with the parameter settings (sectorial search, ratchet, drift, and tree fusing) in "New Technology search" (Goloboff et al., 2008) did not change the consensus tree, and despite the inherent differences between the two programs in the manner in which zero length branches are collapsed (in PAUP* when their maximum length is zero and in TNT when their minimum length is zero, see e.g., Coddington and Scharff [1994]), the same four equally parsimonious trees were found. The strict consensus tree of the four trees is fully resolved except for two polytomies involving three and four taxa, respectively. Incongruence between the plastid and mitochondrial matrices was assessed with the incongruence length difference (ILD) test of Farris et al. (1994) run in PAUP* ("partition homogeneity test") with 1000 random replicates, holding five trees at each step, saving no more than 100 trees per run. The incongruence length difference (ILD) test was run both with and, following the suggestion of Lee (2001), without the uninformative sites included. The use of the ILD test is somewhat controversial (see, for example, Aagesen et al., 2005; Ramírez, 2006; Kjer et al., 2007; Quicke et al., 2007). However, we do not believe that any of the conditions that may create false positives (type I errors) or negatives (type II errors) apply to the present data set (see, also Hipp et al., 2004; Planet, 2006).

In the parsimony analysis, support for clades in the combined analysis was assessed using both jackknife and bootstrap analyses. In accordance with the suggestion of Davis and others (Soreng and Davis 1998; GPWG, 2001; Davis et al., 2004), we have chosen to use the SC ("strict-consensus") jackknife values as our principal measures of support, but have also indicated the FWR ("frequency-within-replicates") bootstrap values for the purpose of easy comparison; see also Simmons and Freudenstein (2011). The SC jackknife analysis was done in TNT using the default collapsing rule, a removal rate of e^{-1} (= 0.36), and 1000 replicates. In TNT, jackknife values below 1% are not retrievable. The FWR bootstrap analysis was run in PAUP* using the default collapsing rule, running 1000 random replicates, using full heuristic search, holding five trees at each step, but saving no more than 100 trees in each replicate. In PAUP* bootstrap percentages below 50 and above five were derived from the table of bipartitions; percentages below five are not retrievable. For assessing the contribution of the plastid and mitochondrial data to the overall tree, both total Bremer support (BS) including the whole data set and partitioned BS, with the data separated into the two logical partitions, was calculated in the program TreeRot (ver. 3) (Sorenson and Franzosa, 2007) using either the default setting (BS) or with the number of trees saved limited to 500 (partitioned BS); all other settings corresponded to those in the standard runs. It was checked whether total BS = $\sum$ partitioned BS, and in the few cases where discrepancies were found, they were resolved by using the converse constraint method (Baker and DeSalle, 1997; Baker et al., 1998). The actual BS values are not indicated, but nodes that are resolved by both or either of the two data partitions are marked in Fig. 1A–C. The taxonomic implications of APG III (2009) for certain groups of interest, e.g., Aphyllanthaceae and Ruscaceae as defined by APG II (2003), are discussed in detail.

To test the possible effect of edited sites in the mitochondrial genes on the combined phylogenetic hypothesis, we performed an analysis after excluding all predicted edited sites identified by the program PREP-Mt (Mower, 2005). An ILD test and a jackknife analysis, as already described, were also run with the edited sites excluded.

A maximum likelihood analysis of the complete data set was done at the Cyperinfrastructure for Phylogenetic Analysis (CIPRES; www.phylo.org) running RAxML-HPC BlackBox ver. 7.2.8 (Stamatakis, 2006) and the beta interface. Thus, the input data were the same as in the combined parsimony analysis and, where run as two partitions, plastid and mitochondrial sequences, respectively. Default options were used, except that the GTRGAMMA model was applied to each partition individually in the basic analysis and in bootstrapping. A set of 1000 bootstrap replicates was run instead of using the build in stopping criteria in the program (Stamatakis et al., 2008; Pattengale et al., 2010). The bootstap percentages were summarized as a majority-rule consensus tree in PAUP*. The ML and bootstrap tree was manipulated in the program FigTree ver. 1.3.1 (tree.bio.ed.ac.uk/software/figtree).

RESULTS

With 153 terminals and five genes, the matrix potentially includes 765 sequences. However, a number of sequences could not be produced: *atp1* and *cob* from *Agrostocrinum*, *Rhuacophila*, and *Thelionema*; *ndhF* from *Petrosavia*, *Eucharis*, *Geosiris*, *Apostasia*, and *Phalaenopsis*; and *matK* from *Agrostocrinum, Alania*, *Geitonoplesium*, *Muscari*, and *Acanthocarpus*. Thus, the final matrix consists of 150 sequences of *atp1* and *cob*, 148 *ndhF*

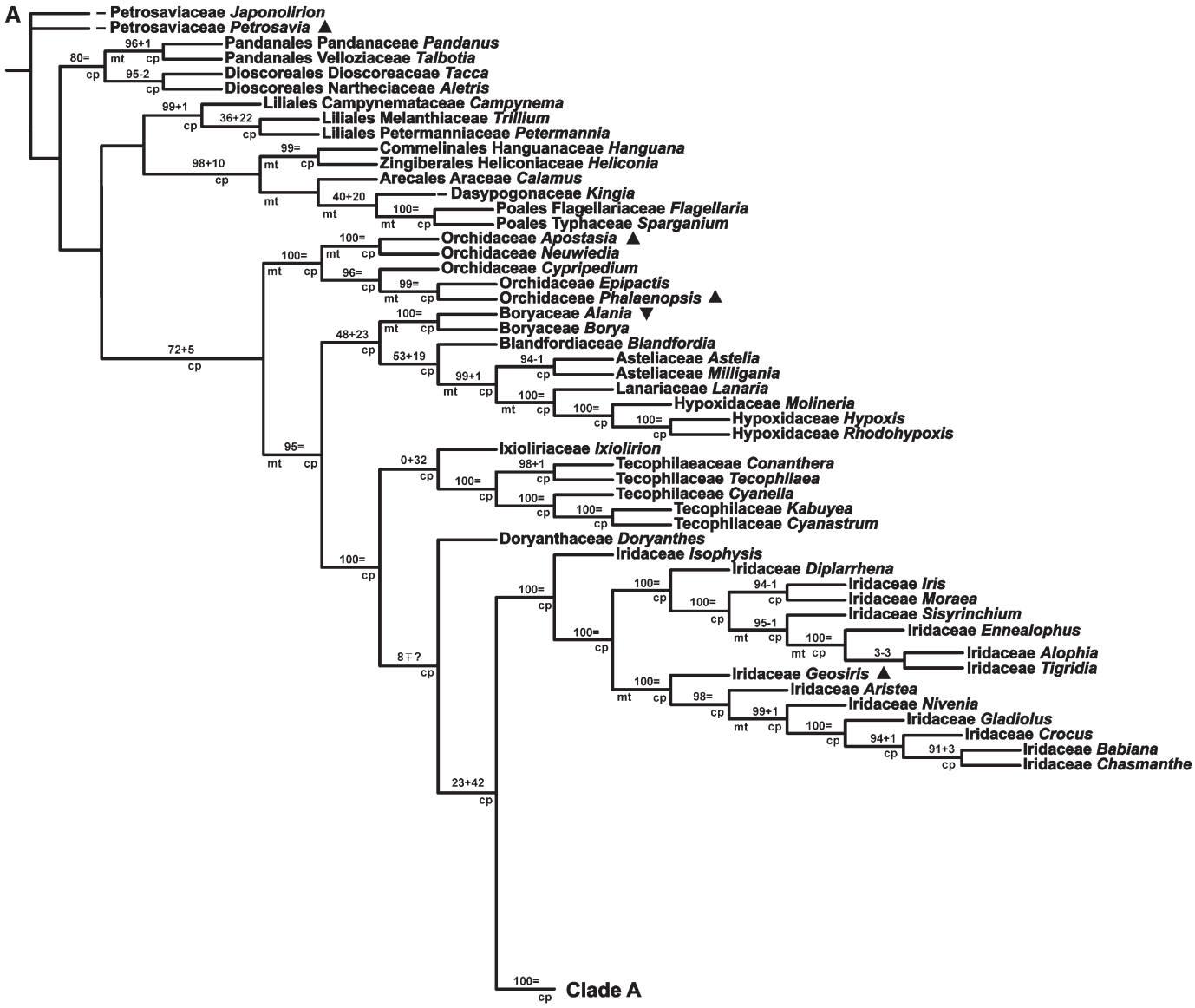

Fig. 1. (A–C) Strict consensus of four most-parsimonious trees for 153 monocotyledon terminals. Each terminal in the ingroup is preceded by the family name; outrgroup terminals are preceded by the order and family names. In a few instances, the terminals are not assigned to an order in APG II (2003) or APG III (2009); this is indicated by a dash (for further details, see online Appendix S1). A number of terminals lack both *cob* and *atp1* (*Agrostocrinum*, *Rhuacophila*, and *Thelionema*) and are marked by a solid circle; a few lack *ndhF* (*Apostasia*, *Eucharis*, *Geosiris*, *Petrosavia*, and *Phalaenopsis)* or *matK* (*Acanthocarpus*, *Agrostocrinum*, *Alania*, *Geitonoplesium*, and *Muscari*) and are marked by a solid triangle, pointing upward and downward, respectively. The first number above each branch is the strict-consensus jackknife percentage. The frequency-within-replicates bootstrap percentage for each group is indicated just afterward by its relationship to the jackknife analysis, either by an equal sign (if the scores are equal) or by a plus or minus sign and a second number (e.g., 53+19 denotes a jackknife percentage of 53 and a bootstrap percentage of 72). Letters below branches mark groups that are resolved by separate analysis of the plastid (cp; *matK*, *ndhF*, and *rbcL*) or mitochondrial (mt; *cob* and *atp1*) subsets of the matrix.

sequences, 153 *rbcL* sequences, and 148 *matK* sequences. Of the 749 sequences, 313 (42%) have been generated for this study, 89 (12%) are *atp1* sequences, 116 (15%) are *cob* sequences, 9 (1%) are *ndhF*, 34 (5%) are *rbcL*, and 65 (9%) are *matK* sequences. The rest 436 (58%) are from GenBank or other investigators (for further details, see Appendix S1 in Supplemental Data with the online version of this article).

Because there were different success rates in amplifying the 5′- and 3′-ends of the genes, all sequences were trimmed to avoid too many missing characters. The two mitochondrial genes plus *rbcL* were easily aligned, with only a few trivial indels. However, the 3′-end of *ndhF* and both the 3′- and the 5′- ends of *matK* were more difficult to align. An alignment of the sequences was conducted by eye and the total alignment, which also indicates the trimmed leading and trailing edges, has been deposited in TreeBaseII (http://purl.org/phylo/treebase/phylows/study/TB2:S12348). The final matrix thus includes 7588 characters of which 2694 (35.5%) are potentially parsimony informative, and of these 313 (11.6%) are from the mitochondrial data set.

Analysis of the complete matrix resulted in four equally parsimonious trees of length 16669, with a CI of 0.29 (always excluding nonparsimony informative characters) and an RI of

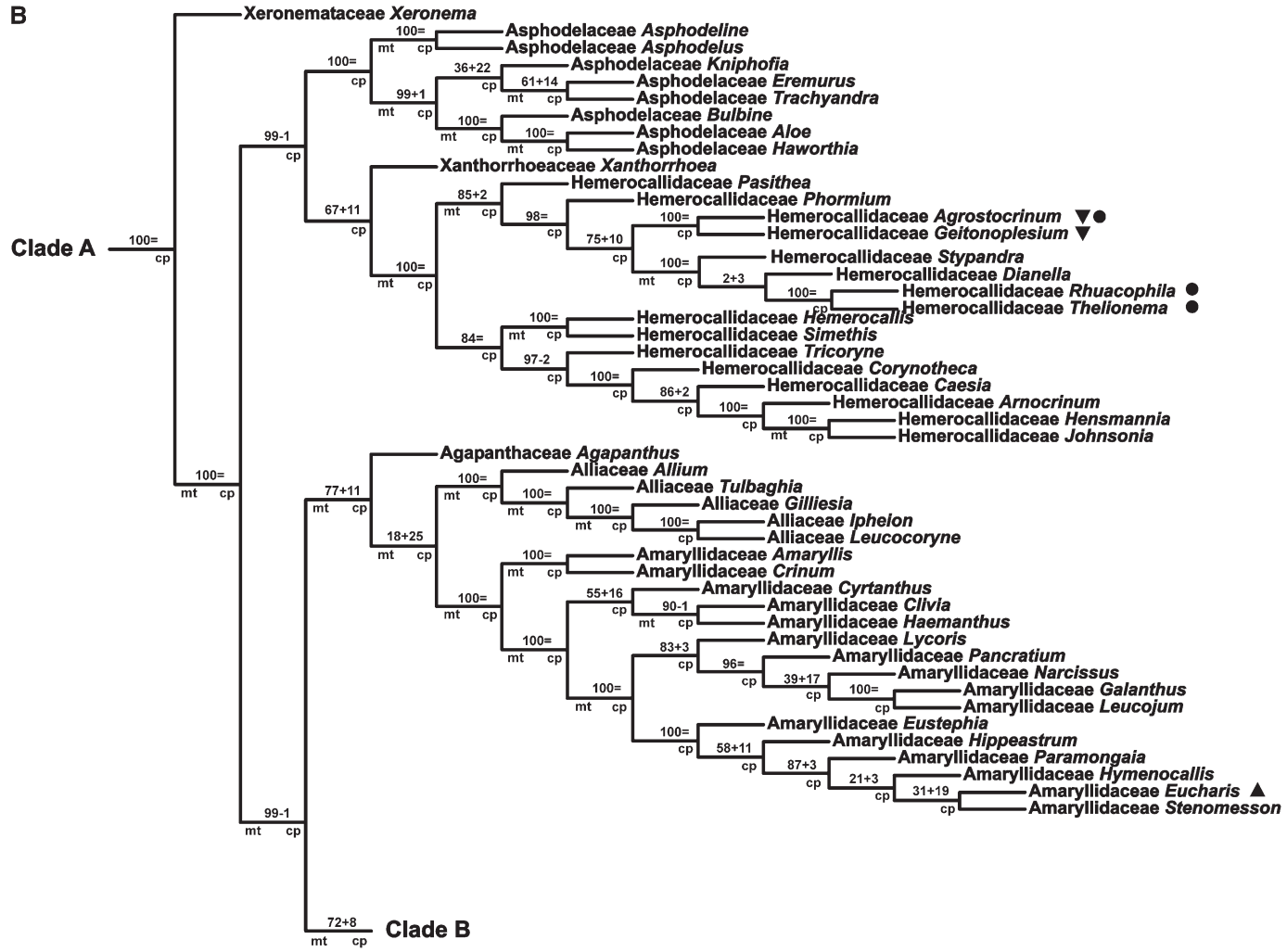

Fig. 1. Continued.

0.64. There are 147 resolved nodes in the consensus tree; of these, 60 nodes are supported by both data partitions, whereas 83 and 4 are supported only by the plastid or mitochondrial data sets, respectively (Fig.1A–C). The statistics for the total data set, each major data partition (mitochondrial and plastid sequences), plus each individual data set may be found in Table 1. The incongruence length difference (D_{xy}) is 90 steps, and as none of the 1000 random partition replicates yielded a length difference of this magnitude, the two data partitions must be considered incongruent, with a p-value <0.001.

Removal of the 12 taxa for which we have been unable to obtain one or more sequences from the matrix has no influence on tree topology, but increases the number of trees to eight, decreases tree length from 16 699 to 15 766 steps, and insignificantly changes the retention index and consistency index (from 0.642 to 0.639 and from 0.290 to 0.295, respectively).

The program PREP-Mt predicts the presence of seven edited sites in *atp1* (varying from 0 to 7 among individual taxa) and 24 in *cob* (varying from 0 to 21 among individual taxa). Excluding these sites from combined phylogenetic analysis results in eight equally parsimonious trees (L = 16 471, CI = 0.29, RI = 0.64) and a strict consensus tree identical to the one calculated from the analysis including all sites. By exclusion of the edited sites the mitochondrial and plastid data partitions become congruent with a p-value = 0.724 and the actual incongruence length difference (D_{xy}) is only 35 steps. When the uninformative characters are excluded prior to analysis, the two partitions remain incongruent ($P < 0.01$) and become congruent if the edited sites are removed ($P = 0.665$).

Using the combined data set, both the parsimony analysis and the maximum likelihood analysis recover all families in both the APG II (including the optional, bracketed families) and APG III systems as monophyletic, except that both have the monotypic, optional family Hesperocallidaceae included in Agavaceae, and the Ruscaceae is rendered paraphyletic by the position of *Eriospermum* as sister group to Asparagaceae s.s. in the maximum likelihood analysis. Otherwise, the main difference between the parsimony analysis and maximum likelihood analysis is restricted to areas weakly or unsupported in both analyses. Thus, relationships within and between a few families are different. At the family level, this applies most notably to the Ruscaceae, which is largely unsupported in the maximum likelihood analysis, and to the relationships between families, e.g., between Tecophilaeaceae, Doryanthaceae, Ixioliriaceae, and a clade corresponding to Asparagaceae plus Alliaceae sensu APG III that is unresolved on the maximum likelihood tree (see

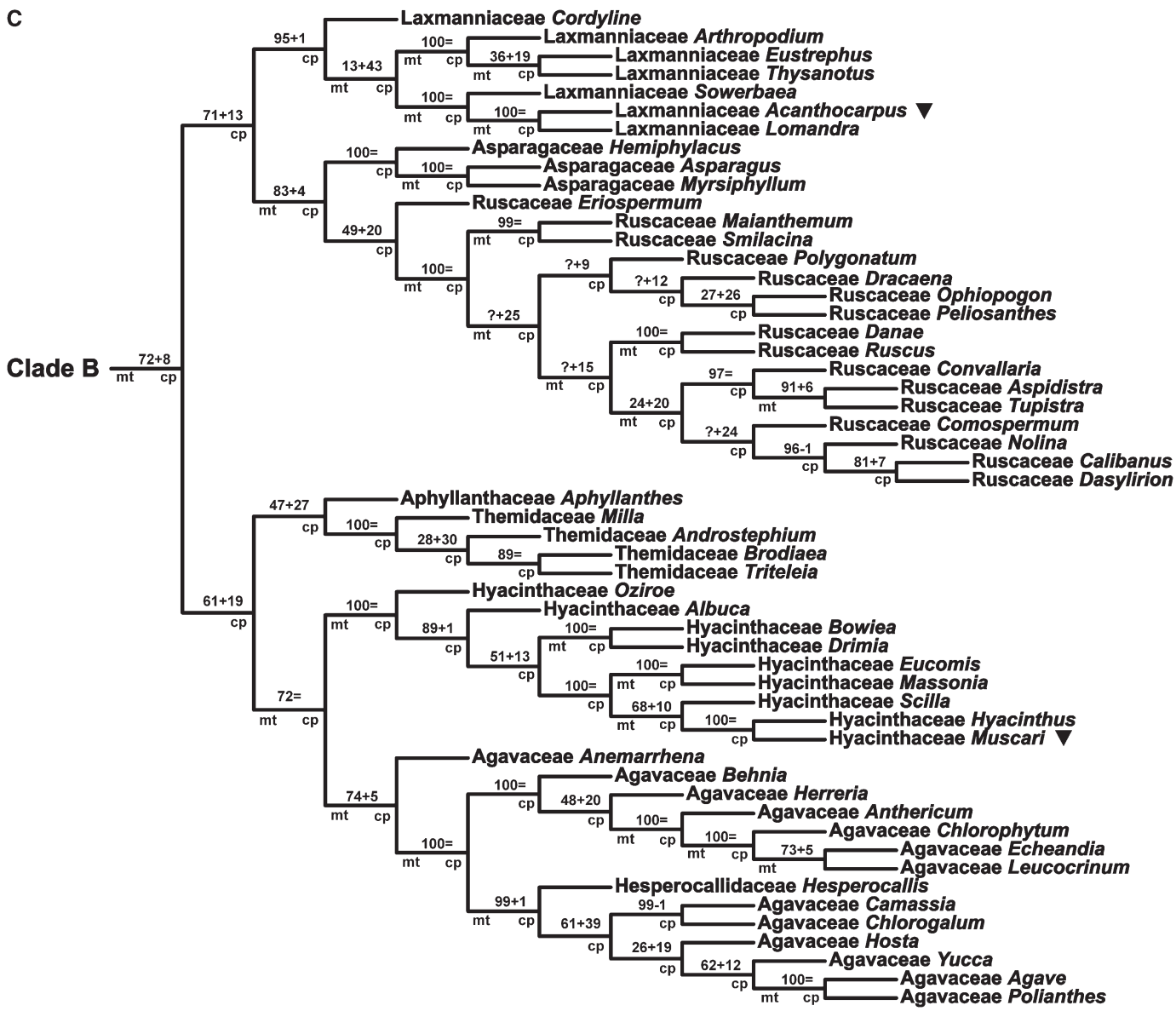

Fig. 1. Continued.

Fig. 2A–C). In view of the minor differences between the parsimony- and maximum-likelihood-based trees, the following discussion is restricted to the parsimony analysis unless otherwise indicated.

Of the 14 families accepted by APG III, six are monogeneric and only represented by a single taxon in the present analysis. Of the remaining nine families, five are supported by both plastid and mitochondrial data (Amaryllidaceae, Asparagaceae, Boryaceae, Orchidaceae, and Tecophilaeaceae), one by plastid data only (Hypoxidaceae), and in two instances the plastid data support and the mitochondrial data reject the relationships (Asteliaceae and Iridaceae).

If the mitochondrial data set is run on its own (excluding the taxa for which no sequence is available, namely *Agrostocrinum*, *Rhuacophila*, and *Thelionema*), the tree becomes much less resolved as only 82 nodes are recovered in comparison to the 147 nodes on the combined tree, corresponding to 56% of the nodes (see Appendix S2 in online Supplemental Data). However, nearly all families recognized in APG II remain monophyletic though their internal relationships are changed or more frequently become less resolved. The only exceptions are (1) Tecophilaeaceae and (2) Asteliaceae, which are totally unresolved, (3) Laxmanniaceae, which becomes paraphyletic with respect to Amaryllidaceae plus Asphodelaceae, and (4) Amaryllidaceae, which becomes paraphyletic with respect to Asphodelaceae. Furthermore, Themidaceae becomes sister to Hyacinthaceae. Additionally, the relationships of the Orchidaceae with respect to a number of outgroup taxa and the remaining part of the Asparagales becomes unclear, too. The relationships of several typically monogeneric families are changed. Thus, Agapanthaceae is sister to Alliaceae; Ixioliriaceae to Iridaceae; Aphyllanthaceae to Laxmanniaceae, Asphodelaceae plus Amaryllidaceae; and Lanariaceae to Ixioliriaceae, Hypoxidaceae, and Iridaceae. The same happens to taxa that are placed as sister group to the rest of certain families, e.g., both *Anemarrhena*, which is included in Agavaceae, and *Eriospermum*, which is included in Ruscaceae, change position.

TABLE 1. Sequence characteristics and tree statistics for the total data set, each major data partition (mitochondrial and plastid sequences), and each individual data.

Statistic	*atp1*	*cob*	mtDNA	*rbcL*	*ndhF*	*matK*	cpDNA	All data
Aligned length	1162	1089	2251	1374	2208	1755	5337	7588
Inf. characters	193	120	313	432	979	970	2381	2694
% inf.	17	11	14	31	44	55	45	36%
% of total	7	4	12	16	36	36	88	
Min. length	290	157	447	707	1841	1842	4390	4837
Tree length	647	402	1088	2790	6583	6027	15521	16699
No. of trees	>300000	>350000	>300000	>300000	>300000	10524	2048	4
CI	0.45	0.39	0.41	0.25	0.28	0.31	0.28	0.29
RI	0.81	0.83	0.81	0.58	0.64	0.64	0.63	0.64

Notes: aligned length = length of sequences used with leading and trailing edges trimmed; inf. characters = number of parsimony informative sites; % inf. = number of parsimony informative sites as a percentage of each partition or gene; % of total = number of parsimony informative sites in each partition or gene as a percentage of the total number of informative sites; min. length = theoretically minimum length of a tree of each partition or gene; trees = number of equally parsimonious trees for each partition or gene; CI = consistency index, excluding uninformative character; RI = retention index.

If the plastid data set is run on its own, the tree becomes only marginally less resolved than the one obtained by the combined analysis, as 138 nodes are recovered in comparison to the 147 nodes on the combined tree, corresponding to 94% of the nodes (see online Appendix S3). Neglecting relationships among the outgroup taxa, the relationships between *Agapanthus*, Alliaceae, and Amaryllidaceae become unresolved, as does a single node in each of Laxmanniaceae, Hyacinthaceae, and Anthericaceae. Most severely affected are the Ruscaceae, in which five nodes collapse into a polytomy.

To describe branch support (Fig. 1A–C) consistently, we used the terminology of Chase et al. (2000a) for the SC jackknife and the FWR bootstrap proportions. Thus, a branch is considered strongly supported if it has SC jacknife and FWR bootstrap proportions of 85–100%, moderately supported if 75–84%, and weakly supported if 50–74%.

The combined data provide only moderate support for the Asparagales sensu APG III (jackknife = 72% and bootstrap = 77%). Within the order, the Orchidaceae are sister to the remaining families with strong jackknife and bootstrap support (both = 100%), and a series of smaller families, Boryaceae, Blandfordiaceae, Asteliaceae, Lanariaceae, and Hypoxidaceae constitute a strongly supported clade sister to all other families except Orchidaceae. Most of these small families and their relationships are strongly supported, with the exception of the relationships between Boryaceae and Blandfordiaceae and the other families, which are either not supported or only weakly supported. The sister clade to this clade of five small families is strongly supported (both jackknife and bootstrap = 100%) and corresponds to the "higher asparagoids" (Chase et al., 1995) or "core asparagoids" (Kim et al., 2010), but within this clade the relationships between Ixioliriaceae, Tecophilaeaceae, Doryanthaceae, Iridaceae and a clade consisting of the remaining families (Clade A, Fig. 1) are not supported, though both this latter clade, and Tecophilaeaceae and Iridaceae are themselves strongly supported (both with jackknife and bootstrap = 100%). Within Clade A, Xeronemataceae are sister to the remaining families with strong support, and a clade consisting of Xanthorrhoeaceae sensu APG III, in turn, a strongly supported sister group to clades consisting of Amaryllidaceae and Asparagaceae sensu APG III. Xanthorrhoeaceae themselves are strongly supported, whereas Amaryllidaceae and Asparagaceae sensu APG III are moderately (jackknife = 77%, bootstrap = 88%) to strongly supported (jackknife = 72%, bootstrap = 80%), respectively.

DISCUSSION

***Asparagales*—**Using *rbcL* data, Chase et al. (1995) conducted the first relatively densely sampled phylogenetic analysis that included most families of the Asparagales in the sense of Dahlgren et al. (1985), represented by 83 species. This analysis, which the authors considered only preliminary (Chase et al., 1995), does clearly show, despite several methodological shortcomings, the rather artificial nature of several of Dahlgren et al.'s families. Representatives of a number of the families recognized by Dahlgren et al., e.g., Anthericaceae, Asphodelaceae, Funkiaceae, and Hyacinthaceae, are scattered across the tree, and Iridaceae and Orchidaceae, which usually were placed in Liliales, are members of Asparagales. However, many relationships are in stark contrast to the ones found in subsequent analyses, including the present one.

Employing the APG (1998) circumscription of the Asparagales and using genera as terminals ("composite" sequences; see above), and four plastid regions, Fay et al. (2000) produced a more comprehensive analysis of the order. Notwithstanding alignment problems and the inconsistency of successive weighting (Goloboff, 1993), the single tree they recovered has a structure that is almost completely compatible with the one found in the present analysis. Apart from minor rearrangements within families, the most obvious deviations are Aphyllanthaceae as sister to Hyacinthaceae, and Xanthorrhoeaceae as sister to Asphodelaceae, though these resolutions both have low support.

In the present analyses, Xanthorrhoeaceae are sister to Hemerocallidaceae and with Asphodelaceae as their sister group, whereas in Chase et al. (2006) and Givnish et al. (2005), the two other possible resolutions of this trifurcation are found. In agreement with Fay et al. (2000) and Pires et al. (2006), but in contrast to Chase et al. (2006), who found Ixioliriaceae as sister group to Iridaceae, Ixioliriaceae are here found as sister to Tecophilaeaceae, but without support.

The resolution of the Asparagales found here (Fig. 1A–C) is similar to that recovered by Pires et al. (2006), except that in the present tree Aphyllanthaceae are sister to Themidaceae, Aphyllanthaceae plus Themidaceae are sister to Hyacinthaceae plus Agavaceae, and Laxmanniaceae are sister to Asparagaceae plus Ruscaceae. However, the alternative resolutions found by Pires et al. (2006) have low or no bootstrap (<50%) support.

Generally speaking, since Pires et al. (2006), we have made relatively little progress in resolving many of the controversial relationships within the Asparagales. Some deeper nodes in the

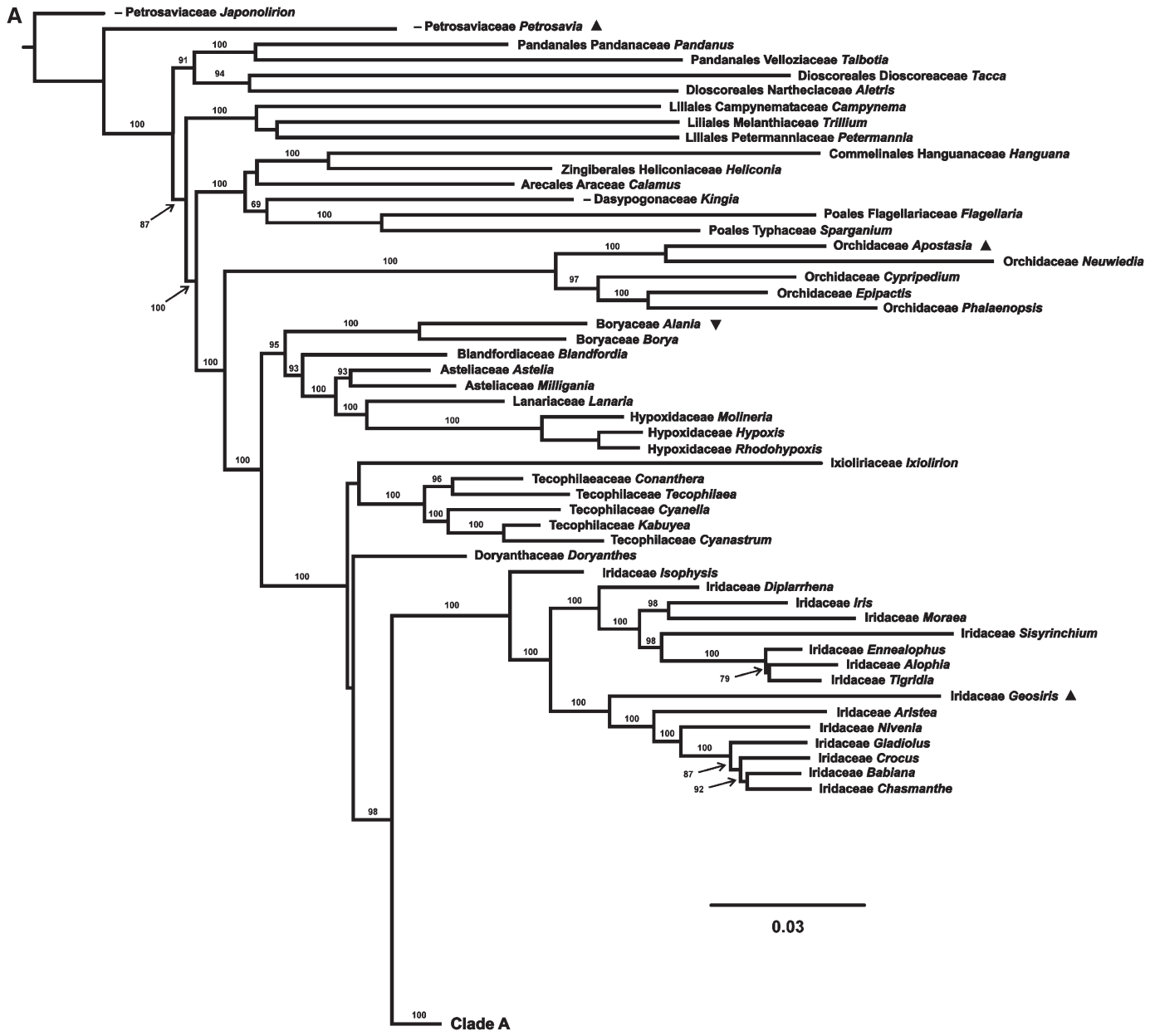

Fig. 2. (A–C) Maximum likelihood tree for 153 monocotyledon terminals. Each terminal in the ingroup is preceded by the family name; outgroup terminals are preceded by the order and family names. In a few instances, the terminals are not assigned to an order in APG II (2003) or APG III (2009); this is indicated by a dash (for further details, see online Appendix S1). A number of terminals lack both *cob* and *atp1* (*Agrostocrinum*, *Rhuacophila*, and *Thelionema*) and are marked by a solid circle; a few lack *ndhF* (*Apostasia*, *Eucharis*, *Geosiris*, *Petrosavia,*and *Phalaenopsis)* or *matK* (*Acanthocarpus*, *Agrostocrinum*, *Alania*, *Geitonoplesium*, and *Muscari*) and are marked by a solid triangle, pointing upward and downward, respectively. The number above each branch is the bootstrap percentage.

Asparagales tree still remain poorly or unresolved as does the relationships of certain monogeneric families (e.g., Ixioliriaceae, Doryanthaceae), whereas the support for the placement of many families is growing. In this respect, the position of *Aphyllanthes* and the relationships within Ruscaceae s.l. are particularly recalcitrant.

***Aphyllanthaceae*—**The monotypic *Aphyllanthes* has been characterized as a problematic taxon with an uncertain and labile phylogenetic position by a number of authors (Fay et al., 2000; Chase et al., 2006; Graham et al., 2006; Pires et al., 2006). Using a rather limited taxon sampling within the Asparagales, Givnish et al. (2005), Graham et al. (2006), and Chase et al. (2006) recovered it as sister group to *Laxmannia* (Laxmanniaceae), *Scilla* (Hyacinthaceae), and *Brodiaea* (Themidaceae), or to *Chlorophytum* and *Yucca* (both Agavaceae), respectively, but without support (bootstrap support ≤ 50%). Having a much denser taxon sampling in their studies of the Asparagales Fay et al. (2000) and Pires et al. (2006) recovered *Aphyllanthes* as sister group to Hyacinthaceae or Laxmanniaceae, respectively, again without support. Steele et al. (2012) found *Aphyllanthes* as sister to Agavaceae but with weak bootstrap support

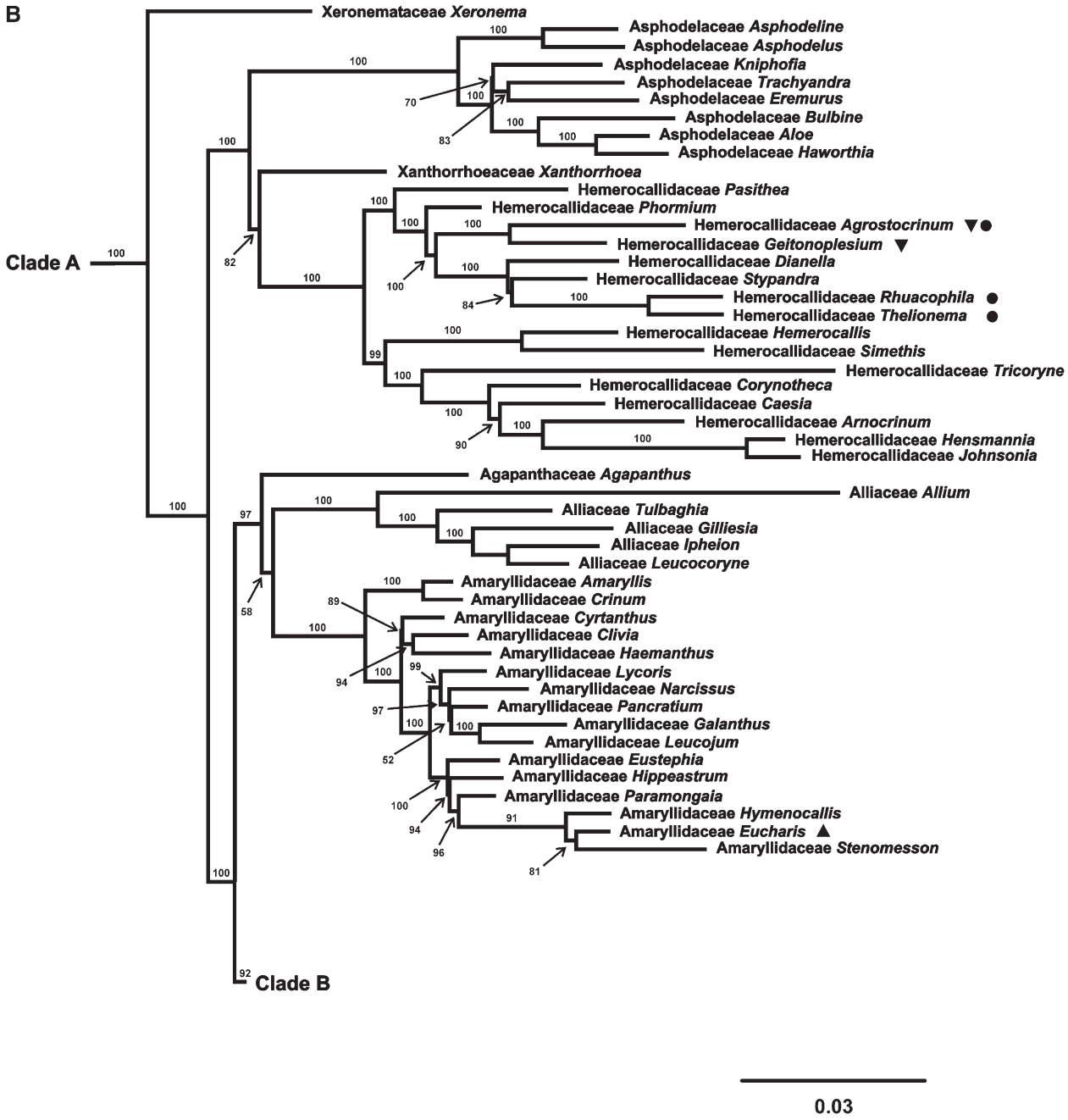

Fig. 2. Continued.

in their parsimony (60% FWR bootstrap) and maximum likelihood (71%) analyses.

In the present parsimony analysis, *Aphyllanthes* is sister to Themidaceae (Fig. 1C), moderately supported by the FWR bootstrap and not supported by the SC jackknife analyses, and sister group to the Agavaceae plus Hesperocallidaceae in the maximum likelihood analysis (Fig. 2C), still without bootstrap support. However, in the parsimony analysis Aphyllanthaceae, Agavaceae, Hesperocallidaceae, and Themidaceae constitute a weakly (SC jackknife) to moderately (FWR bootstrap) supported clade. If *Aphyllanthes* is excluded from the parsimony analysis, the number of equally parsimonious trees increases to eight, length drops dramatically (from 16 699 to 16 408), CI remains unchanged, and there is a slight increase in RI (from 0.64 to 0.65). However, the overall relationships in the consensus tree remain unchanged except for two very weakly supported branches that collapse. If *Aphyllanthes* is excluded from the maximum likelihood analysis, the tree topology remains unchanged, too. Fay et al. (2000) attributed the uncertain position of *Aphyllanthes* to long-branch attraction. However, plastid regions are generally slowly evolving and mitochondrial regions even more so, and both *Allium* (Alliaceae) and *Tricoryne* (Hemerocallidaceae) are on considerably longer branches apparently without causing similar problems. In general, the primary

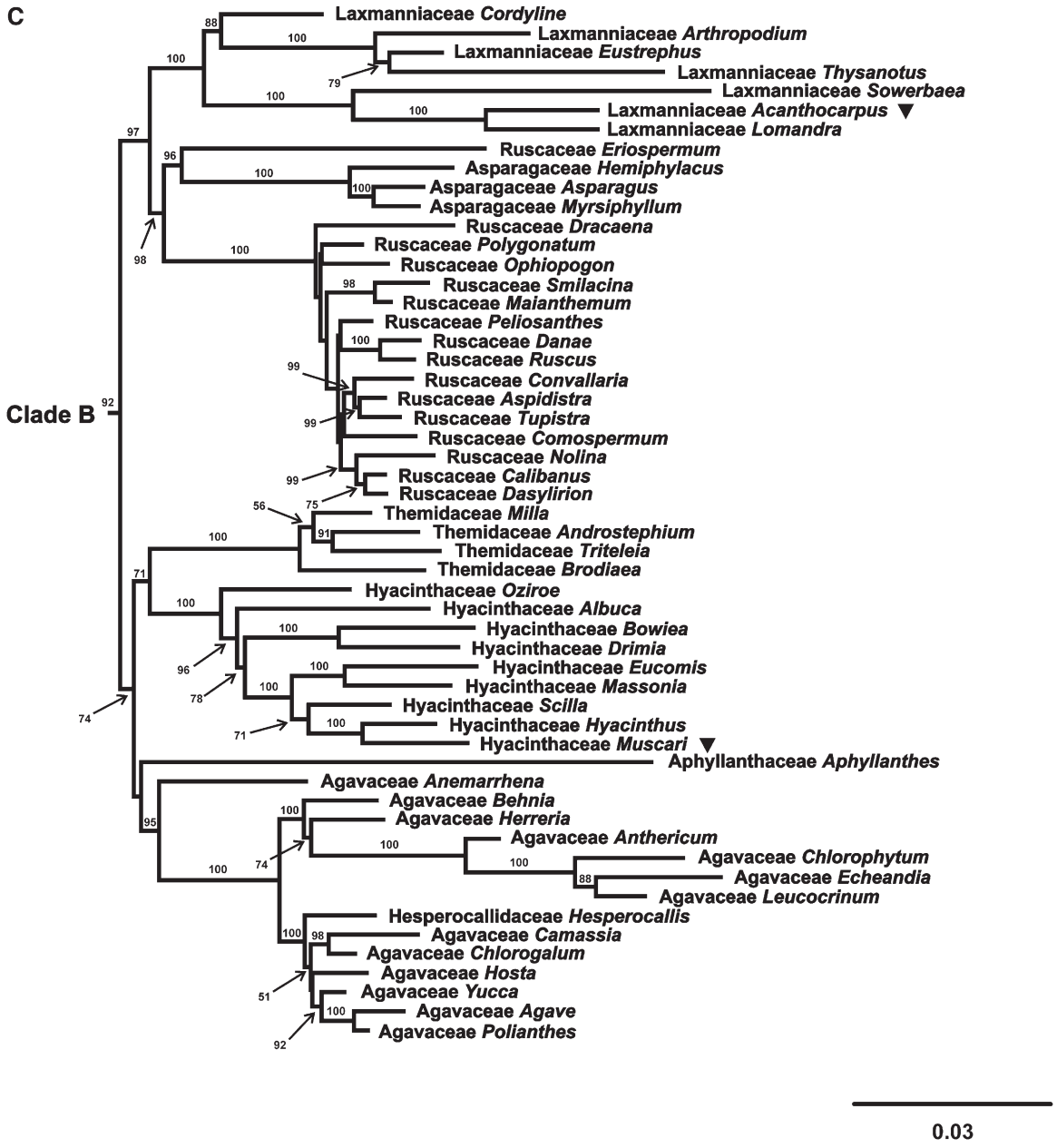

Fig. 2. Continued.

effect of *Aphyllanthes* appears to be instability in the relationships among a series of closely related families (Agavaceae, Hyacinthaceae, and Themidaceae), and removal of *Aphyllanthes* prior to analysis does increase SC jackknife support for the relationships between these three families (data not shown). However, the taxon sampling in the Asparagales in the papers of Fay et al. (2000), Givnish et al. (2005), Chase et al. (2006), and Graham et al. (2006) is too limited to form a strong opinion on the possible causes. Even in the papers dealing specifically with the Asparagales (Fay et al., 2000; Pires et al., 2006; the present study), the support for any of the conflicting relationships between these three families and Aphyllanthaceae is either nonexistent or at best moderate.

Ruscaceae s.l.—One of the most weakly supported areas in the parsimony tree is the topology within Ruscaceae s.l. (= sensu APG II, 2003), although the group itself is well supported (bootstrap and jackknife = 100%). Within Ruscaceae s.l., most internal branches have bootstrap or jackknife support below 50%. Low support values has been found in other analyses (Pires et al., 2006; Rudall et al., 2000b; Kim et al., 2010) and is the main argument for lumping the genera into one large family in the APG II (2003) and APG III (2009) systems. The low support is generally caused by lack of characters, not by conflict among data partitions or differences in RNA editing patterns among taxa. This corresponds largely to the results of the maximum likelihood analysis though the

Ruscaceae is only monophyletic if Asparagaceae s.s. is included (see Fig. 2C).

If the Convallariaceae s.s. (e.g., sensu Conran and Tamura 1998) is constrained as monophyletic (to include *Maianthemum*, *Smilacina*, *Polygonatum*, *Ophiopogon*, *Peliosanthes*, *Convallaria*, *Aspidistra*, and *Tupistra*), and the matrix rerun, the expected increase in tree length (tree length increases by seven steps) is observed, but CI and RI remain unchanged, although the number of equally parsimonious trees increases to eight. If the edited sites are removed during analyses, the number of equally parsimonious trees is unchanged (four), tree length changes from 16699 to 16477, and a small increase at the third significant digit (data not shown) in both CI and RI is seen. The overall tree topology does not change at all; changes are restricted to Ruscaceae s.l. only, and the changes do make it possible also to recognize families such as, e.g., Nolinaceae and Ruscaceae s.s. (and several monogeneric families). This is not entirely surprising as both Nolinaceae and Ruscaceae s.s. have very high bootstrap and jackknife support (from 95% to 100%) on the unconstrained tree. This actually makes it possible to accept several previously recognized families sunk into synonymy in APG II and III.

Results of the analyses by Rudall et al. (2000b) of what largely corresponds to the Ruscaceae sensu APG II (2003) based on morphology and molecular data (*rbcL* and *trnL-F*) are mainly in disagreement with ours. Agreement is mostly found in smaller clades like Nolinaceae and Ruscaceae s.s., and the combined data sets and the molecular data set have a monophyletic Ruscaceae, whereas the monogeneric Eriospermaceae is excluded in the morphological analysis. Yamashita and Tamura (2000), using two plastid regions separately (*rbcL* and the *trnK* intron; viz. including *matK*), did not recover Convallariaceae s.s. as monophyletic, but found both tribes Polygonateae and Ophiopogoneae monophyletic, whereas tribes Aspidistreae and Convallarieae constitute a clade in which neither tribe is monophyletic. The monophyly of this combined clade is also supported by the present analysis.

A recent analysis aimed at determining relationships within the Ruscaceae s.l. by Kim et al. (2010) used two plastid genes (*rbcL* and *matK*) and one nuclear gene (18S rDNA) and included a more complete taxon sampling at the generic level than used here (the only genus not included is *Heteropolygonatum*). The data were analyzed using both parsimony (and successive weighting) and Bayesian inference. The strongly supported clades usually have high posterior probabilities, but the overall tree topologies within Ruscaceae s.l. differ, and the discussion below refers to the single tree obtained by successive weighting initiated from the set of 5721 equally parsimonious trees. Several clades corresponding to the previously narrowly circumscribed families are strongly supported, with bootstrap between 99 and 100%; Ruscaceae s.s., Dracaenaceae, Nolinaceae, and Eriospermaceae. Additionally, Eriospermaceae are strongly supported (bootstrap = 100%) as sister group to the remaining Ruscaceae s.l. However, Convallariaceae s.s. are nonmonophyletic and include very few well supported clades. In fact, only five clades have a bootstrap value greater than 65%, one of which corresponds to the Ophiopogoneae and one that corresponds to Aspidistreae and part of Convallarieae.

Support for Asparagaceae s.l., which includes the Ruscaceae s.l., as suggested by both APG II (2003) and APG III (2009), is not particularly strong, as this large family has only moderate jackknife (72%) and bootstrap support (80%), though the relationship is supported by both plastid and mitochondrial data. However, Steele et al. (2012) found stronger bootstrap support for Asparagaceae s.l. (100%).

Philosophically, there is no doubt that the shortest tree should be recognized as the preferred tree. However, the taxonomic consequences of a given topology may vary. Due to the low support for many nodes, and no matter the chosen taxonomic level, the expanded Ruscaceae preferred by APG II (2003) may be an ephemeral option (and by implication this may apply to other broadly defined families in the Asparagaceae of APG III [2009]). One could have chosen to accept the previously recognized smaller families, at least, until unequivocal evidence points to the contrary. [As proposed by APG III (2009) and as supported by analyses based on plastid DNA (e.g., Chase et al., 2000b; Pires et al., 2006) and on plastid and nuclear DNA (Kim et al., 2010), coauthors M. W. Chase and M. F. Fay prefer the use of Asparagaceae s.l. and Amaryllidaceae s.l. over the recognition of so many small families as here, in spite of the low support found in some previous studies. There is substantial evidence that plastid DNA is a reliable source of data for constructing relationships and corresponds well with results from other sources (e.g., low-copy nuclear DNA; as in Górniak et al. (2010)), who produced for Orchidaceae almost identical but more strongly supported results than matrices of several plastid DNA regions, as in e.g., Cameron (2004).]

***Comments on other clades within the Asparagales*—**Despite having a much smaller taxon sampling, we recovered many other relationships in our broad-scale analysis of Asparagales that are largely similar to those found in more comprehensive studies of individual families. Thus, the analyses by Meerow et al. (1999, 2010) and by Ito et al. (1999), all focused on Amaryllidaceae, invariably showed a mostly African clade, Amaryllideae, as sister to the rest of the family, as in the present analysis. Similarly, the sister group relationship between the remaining African clades, Cyrtantheae and Haemantheae (plus the Australasian tribe Calostemmateae, not included in this study), found in subsequent analyses (Meerow and Snijman 2006; Meerow et al., 2000, 2010), is also recovered here.

Where there is agreement in taxon sampling and substantial bootstrap support for the relationships, as in the overall structure found within Hyacinthaceae in the analysis by Pfosser and Speta (1999), our results are similar except for the first two sister group relationships where the branching order is different.

The results obtained here are similar to the single tree found by using successive weighting by Chase et al. (2000b) in their analysis of the Asphodelaceae, although the relationships among *Kniphofia*, *Eremurus*, and *Trachyandra* are different—a deviant relationship that is partly reflected in their chosen parsimony tree, which is poorly supported.

In the present analysis, the main part of the Iridaceae falls in two clades roughly corresponding to the subfamily Iridoideae and the subfamilies Ixioideae plus Nivenioideae, with the monotypic subfamily Isophysidoideae as their sister group. This relationship corresponds exactly to that found by Reeves et al. (2001) in their study of the Iridaceae, as does the relationships within the subfamilies (the present analysis includes only representatives from the tribe Ixieae of the Ixioideae).

Our taxon sampling in the Orchidaceae is small, but nonetheless it agrees with the results of most analyses, molecular (Chase et al., 2003) as well as morphological (Freudenstein and Rasmussen, 1999), in supporting the close relationship between *Neuwiedia* and *Apostasia*, which are both included in the Apostasioideae, and usually sister to the remaining orchids.

A comparably close similarity between the present results and the phylogenetic trees found by Fay and Chase (1996) for the Themidaceae is evident if their tree is converted into a strict consensus tree. However, this similarity breaks down in the analysis by Pires et al. (2001) in which *Brodiaea* is no longer monophyletic and *Androstephium* is the sister group of *Triteleia*, not *Brodiaea*.

When it comes to the first branching sister groups (following Orchidaceae) of Asparagales, i.e., Boryaceae, Blandfordiaceae, Hypoxidaceae, Lanariaceae, and Asteliaceae, the tree presented here (Fig. 1A), to the extent that the taxon sampling agrees, is identical in structure to the single tree obtained by combining the morphological and molecular data in the analysis by Rudall et al. (1998).

The phylogenetic hypothesis of Bogler and Simpson (1995, 1996) for the Agavaceae is difficult to compare with the one found in the present paper, primarily because the family is circumscribed differently by those authors, and the part that is the same suffers from *Yucca* being nonmonophyletic.

The effect of RNA editing—Combined analysis of the plastid and mitochondrial data partitions results in a better-resolved strict consensus tree than those of the individual data partitions. The different family-level relationships suggested by the mitochondrial genes analyzed alone may to some extent be caused by the sequence pattern of edited sites. Certain families (i.e., Laxmanniaceae, Amaryllidaceae, and Asphodelaceae) may be held together by the almost exclusive presence of T's instead of C's at the edited sites of both *cob* and *atp1*. These sequences, which thus require little or no editing, may have an origin as processed paralogs, which can create problems for phylogeny reconstruction (Bowe and DePamphilis, 1996; Petersen et al., 2006a, b). However, in the combined phylogenetic analyses, which in terms of the topology of the resulting strict consensus trees is unaffected by the inclusion of edited sites, the potentially misleading characters are too few to overturn the pattern in nonedited sites from the mitochondrial and plastid genes. The sequence pattern and changes in evolutionary rates of the edited sites in the mitochondrial genes may, however, affect levels of branch support—in particular in the contribution from the mitochondrial data partition. Thus, when the analysis of both data partitions is run with the edited sites excluded a significant drop in SC jackknife percentages is observed (from 95 to 55%) for Laxmanniaceae, whereas other very well-supported clades (100% jackknife support) are virtually unaffected (e.g., Amaryllidaceae and Asphodelaceae; data not shown). However, there is no consistent, overall pattern to these changes. Support for some clades is even increased by removal of the edited sites. This is in agreement with Duvall et al. (2008) who observed similar unpredictable changes in congruence when edited sites were removed prior to phylogenetic analysis.

In considering this point, it should be noted that the aforementioned analyses are either restricted to or dominated by data from the plastid genome. Few analyses have used data from the mitochondrial genome, and where data from the nuclear genome have been used, they have almost always been from multicopy arrays, like ITS or noncoding ribosomal genes (see however, Chase et al., 2000a; Górniak et al., 2010), which potentially may undermine the present classification. In the present analysis, the plastid and mitochondrial data are incongruent, and only 43% of the nodes obtained by the combined analysis are supported by the mitochondrial data set, whereas 97% are supported by the plastid data. However, if the edited sites are removed from the analysis, the two partitions become congruent.

Despite the fact that uniparentally inherited characters are ideally suited for current phylogenetic methods, which almost invariably presuppose a dichotomous history, two overarching issues might limit the validity of the gene tree reconstructed by a data set dominated by organellar data (and impair our ability to turn it into a species tree). These are (either individually or in combination): (1) the largely uniparental inheritance of the organellar genomes and (2) the widespread occurrence of allopolyploidy (paleopolyploidy (Jiao et al., 2011) as well as more recent polyploidy). Adding to these problems are molecular processes like RNA editing and retroprocessing and the possibility of HGT (horizontal gene transfer) and EGT (endosymbiontic gene transfer).

It is a well-established fact that organelle-based gene trees, in most angiosperms usually tracking the maternal line (Corriveau and Coleman, 1988; Mogensen, 1996), need not reflect the "true" species tree; and the deeper in time allopolyploidization took place, the less likely we are to resolve the event, and the greater the consequences are for our understanding of phylogeny. Producing more organellar data is not likely to help us resolve this conundrum, as all data belong to the same linkage group and are likely to trace the same evolutionary history. The presence of RNA editing, which takes place both in plastids and mitochondria, and retroprocessing, which currently is only known in mitochondria, has been used to evaluate the utility of plastid vs. mitochondrial gene trees. Although this may occasionally be well justified, one must bear in mind that both processes often are clade-specific (Cuenca et al., 2010) and hence indicative of phylogenetic relationship.

Leaving aside the matter that most available phylogenetic methods are insensitive to ancient reticulations, it is still noteworthy that the nuclear genome behaves differently from the organellar genomes, even in balanced polyploids, e.g., the ones in which both genomes are of the same size and structure. Shortly after polyploidization the nuclear genome is—in principle—likely to reflect its own "combined" history faithfully, but as time goes by the phylogenetic signal might be increasingly blurred by processes like gene conversion, concerted evolution, and gene silencing (Wendel, 2000). If these processes affect the combined nuclear genomes randomly, they are bound to harbor an increasingly unpredictable imprint from each parental genome as time progresses. Nevertheless, if there is an inherited bias in the process, e.g., the information in one parental genome is more likely to change than the other, or the changes are driven by directional forces (e.g., selection), the likelihood of obtaining a correct, unbiased estimate of the species phylogeny decreases even further.

At least theoretically, sequencing the whole plastid or mitochondrial genome is likely to lead to support for the same gene tree (unless the mitochondria include more nuclear or plastid sequences than native mitochondrial sequence). Sequencing whole nuclear genomes may not help us at all (Soltis et al., 2004). In a recent paper, Górniak et al. (2010, p. 785) indicated that "[d]ue to their biparental inheritance, nuclear DNA regions give also information about hybridization, a phenomenon of major importance in the evolution of angiosperms." However, this is only correct if one can find all copies of the nuclear genes in the supposed hybrid (Petersen and Seberg, 2004; Petersen et al., 2006c, 2008, 2011), which may or may not be the case. A priori, the fate of any gene copy in an ancient or recent allopolyploid is unpredictable. In the example described by Górniak et al. (2010), only one copy

of a nuclear encoded gene is found in each taxon, and the possibility of hybridization cannot be excluded.

It is not clear what should be expected when a dichotomous branching pattern is forced upon an inherently reticulate pattern, or perhaps more importantly, what happens to more or less differentiated sets of genomes combined in an allopolyploid. In the best of all worlds, we would expect a balanced polyploid to force an unresolved node, a trifurcation, involving the two parents and their possible descendant—but this is unlikely to occur (see e.g., McDade, 1990, 1992). However, the plastid data may be used as a baseline against which the incongruence between nuclear genes can be assessed.

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