

PHYLOGENETICS OF LILIALES: SUMMARIZED EVIDENCE FROM COMBINED ANALYSES OF FIVE PLASTID AND ONE MITOCHONDRIAL LOCI

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ABSTRACT

In order to investigate interfamilial relationships of Liliales we analyzed a combined matrix of plastid *rbcL*, *trnL* intron, *trnL*–*F* intergenic spacer, *matK*, and *ndhF*, and mitochondrial *atp1* DNA sequences. The results are generally congruent with previous broad analyses and provide higher bootstrap support for many relationships. Important changes relative to previous studies are the recognition of Petermanniaceae distinct from Colchicaceae and the tentative inclusion of Corsiaceae in the order. This brings the number of families in the order from nine to eleven. The additional data presented here strengthen the case for including Uvulariaceae in Colchicaceae and Calochortaceae in Liliaceae.

Key words: Calochortaceae, Colchicaceae, Corsiaceae, Liliales, molecular phylogeny, Petermanniaceae, Uvulariaceae.

INTRODUCTION

In recent classifications (Chase et al. 2000; Angiosperm Phylogeny Group II [APG II 2003]), the order Liliales consists of nine families: Alstroemeriaceae, Campynemataceae, Colchicaceae, Liliaceae, Luzuriagaceae, Melanthiaceae, Philesiaceae, Rhipogonaceae, and Smilacaceae. This ordinal circumscription is generally similar to that of Dahlgren, Clifford, and Yeo (1985) but with some marked contrasts, notably the exclusion of Iridaceae and Orchidaceae (both in Asparagales in Chase et al. 2000; APG II 2003). The background to and circumscription of the order Liliales are discussed in detail elsewhere (Fay and Chase 2000; Rudall et al. 2000). Colchicaceae (including Uvulariaceae), Liliaceae (including Calochortaceae sensu Tamura [1998]); see Discussion) and Melanthiaceae (including Trilliaceae and Xerophyllaceae, but excluding Nartheciaceae, Petrosaviaceae, and Tofieldiaceae) are at variance with previous classifications, e.g., Dahlgren et al. (1985). Genera included in the 11 families of Liliales as circumscribed here are listed in Table 1, as well as their different placements in earlier systems. Two additions at the family level to Liliales are made: Corsiaceae and Petermanniaceae (see below).

In this paper, we discuss the interrelationships of these 11 families on the basis of combined analyses of plastid *trnL* intron and *trnL*–*F* intergenic spacer (together known as the *trnL*–*F* region), *rbcL*, *ndhF*, and *matK*, and the mitochondrial *atp1*. In addition, we focus in more detail on relationships within Liliaceae, following on from the studies of Fay and Chase (2000) and Rønsted et al. (2005).

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MATERIALS AND METHODS

Species used as placeholders for this study are similar to those in previous papers (Chase et al. 1995, 2000; Rudall et al. 2000; Rønsted et al. 2005). For newly produced data (since Chase et al. 2000 and Rudall et al. 2000), we exchanged DNA samples (notably true *Petermannia*) between the participating laboratories so that each locus was amplified from the same genomic DNA in most cases. Species used are listed in Table 2. Methods of sequence production have varied greatly over time; primers and protocols can be found in studies of the individual loci (summarized in Chase et al. 2006, with the addition of Taberlet et al. (1991) for the *trnL*–*F* region).

The combined matrix contained 36 taxa, including *Pandanus* L. f. and *Stemona* Lour. as outgroups based on the results of Chase et al. (2000, 2006). We analyzed the combined matrix using heuristic searches with PAUP* vers. 4.0b10 (Swofford 2002) using the following strategy: 500 replicates of randomized taxon entry with subtree-pruning-regrafting (SPR) swapping and a tree limit of 20 trees per replicate to reduce the time spent swapping on suboptimal islands of trees. In a second round of analysis we used these as starting trees with tree-bisection-reconnection (TBR) swapping to find any additional trees. We then used bootstrapping to estimate internal support with 500 replicates of simple taxon addition, again with a limit of 20 trees per replicate. DELTRAN optimization is used to illustrate branch lengths, due to problems with ACCTRAN optimization in PAUP* vers. 4.0b10. We report all bootstrap percentages (% BS) >50. The analyses were repeated excluding *Arachnitis* and *Petermannia* to evaluate potential problems due to missing data.

RESULTS

The aligned matrix contained 9141 characters, of which 1128 were excluded (mostly in the *trnL*–*F* region due to

Table 1. The families and genera of Liliales as recognized in this study. Where placements differ from four previous classifications, the alternative placement is given. The placement of Corsiaceae in the order is tentative at this stage. Genera included in this study are marked with an asterisk [*].

Family	Genus	Hutchinson 1959	Dahlgren et al. 1985	Brummitt 1992	Wilson and Morrison 2000; Chase et al. 2000 ^a
Alstroemeriaceae	<i>Alstroemeria</i> L.*	Alstroemeriaceae–Alstroemeriaceae			
	<i>Bomarea</i> Mirb.*	Alstroemeriaceae–Alstroemeriaceae			
Campynemataceae	<i>Leontochir</i> Phil.*	Alstroemeriaceae–Alstroemeriaceae			
	<i>Campynema</i> Labill.*	Haemodorales–Hypoxidaceae	Melanthiales–Campynemataceae	Melanthiaceae	
	<i>Campynemanthle</i> Baill.*	Haemodorales–Hypoxidaceae	Melanthiales–Campynemataceae	Melanthiaceae	
	<i>Androcymbium</i> Willd.*	Liliaceae–Iphigenieae			
	<i>Baeometra</i> Salisb.	Liliaceae–Anguillariteae			
	<i>Bulbocodium</i> L.	Liliaceae–Colchiceae	? ^c		
	<i>Burchardia</i> R. Br.	Liliaceae–Iphigenieae			
	<i>Campiorrhiza</i> Hutch.	Liliaceae–Iphigenieae			
	<i>Colchicum</i> L.*	Liliaceae–Colchiceae			
	<i>Disporum</i> Salisb.	Liliaceae–Polygonateae			
Colchicaceae ^b	<i>Gloriosa</i> L.	Liliaceae–Uvularieae	Uvulariaceae–Uvularieae	Convallariaceae	
	<i>Hexacyrtis</i> Dinter	Liliaceae–Uvularieae			
	<i>Iphigenia</i> Kunth*	Liliaceae–Iphigenieae			
	<i>Kuntheria</i> J. G. Conran & H. T. Clifford	n/a ^d	n/a	Convallariaceae	
	<i>Littonia</i> Hook.	Liliaceae–Uvularieae			
	<i>Merendera</i> Ram.	Liliaceae–Colchiceae			
	<i>Neodregea</i> C. H. Wright	Liliaceae–Anguillariteae			
	<i>Onixotis</i> Raf.*	Liliaceae–Anguillariteae (as <i>Diplo-</i> <i>dax</i>)			
	<i>Ornithoglossum</i> Salisb.	Liliaceae–Iphigenieae			
	<i>Sandersonia</i> Hook.	Liliaceae–Uvularieae or Tricyrti- deae			
	<i>Schelhammera</i> R. Br.	Liliaceae–Uvularieae			
	Corsiaceae	<i>Tripladentia</i> D. Don	Liliaceae–Uvularieae (as <i>Kreysi-</i> <i>gia</i>)	Uvulariaceae–Uvularieae Uvulariaceae–Uvularieae	Convallariaceae Convallariaceae
<i>Uvularia</i> L.*		Liliaceae–Uvularieae	Uvulariaceae–Uvularieae	Convallariaceae	
<i>Wurmbea</i> Thunb.		Liliaceae–Anguillariteae			
<i>Arachnitis</i> Phil.*		Burmanniales–Corsiaceae	Burmanniales–Corsiaceae		Dioscoreales–Corsiaceae
<i>Corsia</i> Becc.		Burmanniales–Corsiaceae	Burmanniales–Corsiaceae		Dioscoreales–Corsiaceae
<i>Corstlopsis</i> D. X. Zhang, R. M. Saunders, & C. M. Hu		n/a	n/a	n/a	n/a
<i>Amara</i> Honda*		Liliaceae–Tulipeae			
<i>Calochortus</i> Pursh*		Liliaceae–Tulipeae	Calochortaceae		
<i>Cardiocrinum</i> (Endl.) Lindl.*		Liliaceae–Tulipeae (as <i>Lilium</i>)			
<i>Clintonia</i> Dougl. ex Lindl.*		Liliaceae–Polygonateae	Uvulariaceae–Uvularieae	Convallariaceae	
<i>Erythronium</i> L.		Liliaceae–Tulipeae			
<i>Fritillaria</i> L.*		Liliaceae–Tulipeae			
Liliaceae	<i>Gagea</i> Salisb.*	Liliaceae–Tulipeae			
	<i>Lilium</i> L.*	Liliaceae–Tulipeae			
	<i>Lloydia</i> Salisb. ex Rehb.	Liliaceae–Tulipeae			

Table 1. Continued.

Family	Genus	Hutchinson 1959	Dahlgren et al. 1985	Brummitt 1992	Wilson and Morrison 2000; Chase et al. 2000 ^a
	<i>Medeola</i> Gronov. ex L.*	Trilliaceae	Liliaceae, Trilliaceae or Uvulariaceae?	Convallariaceae	
	<i>Notholirion</i> Wall. ex Voigt & Boiss.	Liliaceae–Tulipeae			
	<i>Prosartes</i> D. Don ^b	Liliaceae–Polygonateae	Uvulariaceae–Uvulariaceae	Convallariaceae	
	<i>Scoliotopus</i> Torr.*	Trilliaceae	Trilliaceae or Uvulariaceae?	Trilliaceae	
	<i>Streptopus</i> Michx.*	Liliaceae–Polygonateae	Uvulariaceae–Uvulariaceae	Convallariaceae	Colchicaceae
	<i>Tricyrtis</i> Wall.*	Liliaceae–Tricyrtideae	Uvulariaceae–Tricyrtideae	Convallariaceae	
	<i>Tulipa</i> L.*	Liliaceae–Tulipeae			
Luzuriagaceae	<i>Drymophila</i> R. Br.	Liliaceae–Polygonateae	Asparagales–Luzuriagaceae	Convallariaceae	
	<i>Luzuriaga</i> Ruiz & Pav.*	Alstroemeriales–Philesiaceae	Asparagales–Luzuriagaceae	Philesiaceae	
Melanthiaceae ^f	<i>Amianthium</i> A. Gray	Liliaceae–Veratreae	Melanthiales–Melanthiaceae		
	<i>Antictea</i> Kunth				
	<i>Chamaelirium</i> Willd.*	Liliaceae–Helonideae	Melanthiales–Melanthiaceae		
	<i>Chionographis</i> Maxim.	Liliaceae–Helonideae	Melanthiales–Melanthiaceae		
	<i>Daiswa</i> Raf.	Trilliaceae	Dioscoreales–Trilliaceae	Trilliaceae	
	<i>Helonias</i> L.	Liliaceae–Helonideae	Melanthiales–Melanthiaceae		
	<i>Kinugasa</i> Tatew. & Suto	Trilliaceae?	Dioscoreales–Trilliaceae	Trilliaceae	
	<i>Paris</i> L.	Trilliaceae	Dioscoreales–Trilliaceae	Trilliaceae	
	<i>Pseudotrillium</i> S. B. Farmer				
	<i>Schoenocaulon</i> A. Gray	Liliaceae–Veratreae	Melanthiales–Melanthiaceae		
	<i>Stenanthium</i> (A. Gray) Kunth	Liliaceae–Veratreae	Melanthiales–Melanthiaceae		
	<i>Toxicoscordion</i> Rydb.* ^g				
	<i>Trillidium</i> Kunth				
	<i>Trillium</i> L.*	Trilliaceae	Dioscoreales–Trilliaceae		
	<i>Veratrum</i> L.*	Liliaceae–Veratreae	Melanthiales–Melanthiaceae		
	<i>Xerophyllum</i> Michx.*	Liliaceae–Nartheceteae	Melanthiales–Melanthiaceae		
	<i>Zigadenus</i> Michx.	Liliaceae–Veratreae	Melanthiales–Melanthiaceae		
Petermanniaceae	<i>Petermannia</i> F. Muell.*	Alstroemeriales–Petermanniaceae	Dioscoreales–Petermanniaceae		Colchicaceae
Philesiaceae	<i>Lapageria</i> Ruiz & Pav.*	Alstroemeriales–Philesiaceae	Asparagales–Philesiaceae		
	<i>Philesia</i> Comm. ex Juss.*	Alstroemeriales–Philesiaceae	Asparagales–Philesiaceae		
Rhipogonaceae	<i>Rhipogonum</i> Forst.*	Liliales–Smitlacaceae	Dioscoreales–Smitlacaceae		
Smitlacaceae	<i>Smitax</i> L.*	Liliales–Smitlacaceae	Dioscoreales–Smitlacaceae		

^a Placements are given in the index of Wilson and Morrison 2000. The families recognized are those listed in Chase et al. 2000.

^b Vinnersten and Reeves (2003) showed that several genera of Colchicaceae as currently circumscribed are not monophyletic. Notably, *Bulbocodium*, *Colchicum*, and *Merendera* are embedded in *Androcymbium*, whereas *Gloriosa* and *Littoria* are intermingled, and *Onixotis* is possibly paraphyletic to *Wurmbea*. Thus, a taxonomic revision of the family at the generic level is necessary.

^c Dahlgren et al. (1985) did not mention *Burchardia*, and it is not possible to place it in their system.

^d n/a indicates that the genus was described after this taxonomic treatment.

^e *Prosartes* was considered in most previous systems to be a synonym of *Disporum* (Colchicaceae). However, North American species are members of Liliaceae, whereas Asian species are members of Colchicaceae. *Prosartes* applies to the North American species.

^f Genera as recognized by Zomlefer et al. 2005.

^g Previously treated as *Zigadenus* p.p.

Table 2. Taxa included in this study with voucher information. All loci were sequenced for the same species, except as indicated in the final column. If no locus is given in the final column, then this species was the default.

Family	Species	Locus	
Alstroemeriaceae	<i>Alstroemeria</i> L. sp.		
	<i>Bomarea hirtella</i> Herb.		
	<i>Leontochir ovallei</i> Phil.		
Campynemataceae	<i>Campynema lineare</i> Labill.		
Colchicaceae	<i>Androcymbium ciliolatum</i> Schltr. & K. Krause		
	<i>Androcymbium europaeum</i> C. Richt.	matK	
	<i>Colchicum speciosum</i> Stev.		
	<i>Iphigenia indica</i> A. Gray		
	<i>Onixotis triquetra</i> (L. f.) D. J. Maberley		
	<i>Uvularia perfoliata</i> L.		
	<i>Uvularia sessilifolia</i> L.	ndhF	
	<i>Arachnitis uniflora</i> Phil.		
	Liliaceae	<i>Tulipa (Amana) erythronioides</i> Baker	
		<i>Calochortus albus</i> Dougl. ex Benth.	ndhF
<i>Calochortus minimus</i> Ownbey			
<i>Cardiocrinum giganteum</i> Makino			
<i>Clintonia borealis</i> Raf.		matK	
<i>Clintonia umbellata</i> Torr.			
<i>Fritillaria meleagris</i> L.		ndhF	
<i>Fritillaria persica</i> L.		matK	
<i>Fritillaria raddeana</i> Regel			
<i>Gagea wilczekii</i> Braun-Blanquet & Maire			
<i>Lilium superbum</i> L.			
<i>Medeola virginiana</i> L.			
<i>Prosartes lanuginosa</i> D. Don		rbcL	
<i>Prosartes smithii</i> (Hook.) Utech, Shinwari & Kawano			
<i>Scoliopus bigelowii</i> Torr.			
<i>Streptopus amplexifolius</i> DC.			
<i>Tricyrtis affinia</i> Makino	ndhF		
<i>Tricyrtis latifolia</i> Maxim.			
<i>Tulipa kolpakowskiana</i> Regel			
<i>Tulipa pulchella</i> Boiss. ex Baker	ndhF		
<i>Tulipa systola</i> Stapf	matK		
Luzuriagaceae	<i>Luzuriaga radicans</i> Ruiz & Pav.		
Melanthiaceae	<i>Chamaelirium luteum</i> (L.) A. Gray		
	<i>Toxicoscordion fremontii</i> Rydb.		
	<i>Trillium erectum</i> L.		
	<i>Trillium grandiflorum</i> Salisb.	matK	
	<i>Veratrum stamineum</i> Maxim.	matK	
	<i>Veratrum viride</i> Ait.		
	<i>Xerophyllum tenax</i> (Pursh) Nutt.		
Pandanaceae	<i>Pandanus vandermeeschii</i> Balf. f.		
Petermanniaceae	<i>Petermannia cirrosa</i> F. Muell.		
Philesiaceae	<i>Lapageria rosea</i> Ruiz & Pav.		
	<i>Philesia buxiflora</i> Lam. ex Poir.		
Rhipogonaceae	<i>Rhipogonum elseyanum</i> F. Muell.		
Smilacaceae	<i>Smilax china</i> Vell.	matK	
	<i>Smilax glauca</i> Walter		
Stemonaceae	<i>Stemona japonica</i> (Blume) Miq.	matK	
	<i>Stemona tuberosa</i> Lour.		

problems with alignment). Both analyses (including *Arachnitis* and *Petermannia* [Fig. 1], and excluding these taxa, results not shown) gave the same topologies for the families of Liliales included in common, with only minor variation in bootstrap support on some branches. Here we present the data from the analysis with all taxa included. The analysis resulted in two trees (tree length 5736 steps, consistency index 0.60, retention index 0.60). Liliales were strongly supported (100% BS). The only difference between the trees pertained to the relative positions of Campynemataceae (here represented by *Campynema*) and *Arachnitis* as the first branch/es in the order, with *Arachnitis* and *Campynema*: (1) as successive sisters to, or (2) together as sister to the rest of the order. We show result (1), marking the branch that collapses in the strict consensus with an asterisk (Fig. 1).

The rest of the order (exclusive of *Arachnitis* and *Campynema*) was moderately supported (70% BS) as monophyletic. Melanthiaceae were then sister to the remaining families of Liliales, but with bootstrap support <50%. The other families fell in two clades. The first clade, with weak support (64% BS), was (Petermanniaceae (Colchicaceae (Alstroemeriaceae + Luzuriagaceae))). However, the branches defining the relationships between the four families within this clade all gained strong support (98–100% BS). The second, with strong support (99% BS), was ((Philesiaceae + Rhipogonaceae) (Smilacaceae + Liliaceae)). Bootstrap support was strong for the branches defining the sister group relationship between Smilacaceae and Liliaceae (94% BS) and the monophyly of both Liliaceae and Philesiaceae (both 100% BS). The sister group relationship between Philesiaceae and Rhipogonaceae was only weakly supported (54% BS).

In the families for which we sampled more than two genera, most relationships among the genera gained strong bootstrap support. In Melanthiaceae, two clades were recovered: (*Veratrum* + *Toxicoscordion*) (100% BS) and (*Chamaelirium* + *Xerophyllum*) (89% BS for the clade, 100% BS for the internal branch). In Colchicaceae, the topology was (*Uvularia* ((*Iphigenia* + *Onixotis*) (*Androcymbium* + *Colchicum*))), with all branches with 100% BS. In Alstroemeriaceae, *Alstroemeria* was weakly supported (57% BS) as sister to (*Bomarea* + *Leontochir*). In Liliaceae, clades gaining strong support were: (A) (*Amana* + *Tulipa*) (100% BS); (B) (*Cardiocrinum* (*Fritillaria* + *Lilium*)) (both branches 100% BS); (C) (*Clintonia* + *Medeola*) (100% BS); (D) (((A + *Gagea*) B) C) (100% BS, although the internal branches were only moderately supported, each with 74% BS); and (E) (*Streptopus* (*Prosartes* + *Scoliopus*)) (both branches 100% BS). *Tricyrtis* fell as sister to D and *Calochortus* as sister to E, but both with BS <50%. Clades A–E are indicated on Fig. 1.

DISCUSSION

Relationships of Liliales

The data presented here do not allow us to address the issue of the placement of Liliales with respect to other monocot orders. However, in the analyses of Chase et al. (2006), Liliales are weakly to moderately supported as sister to Asparagales + commelinids (86% BS in the plastid analy-

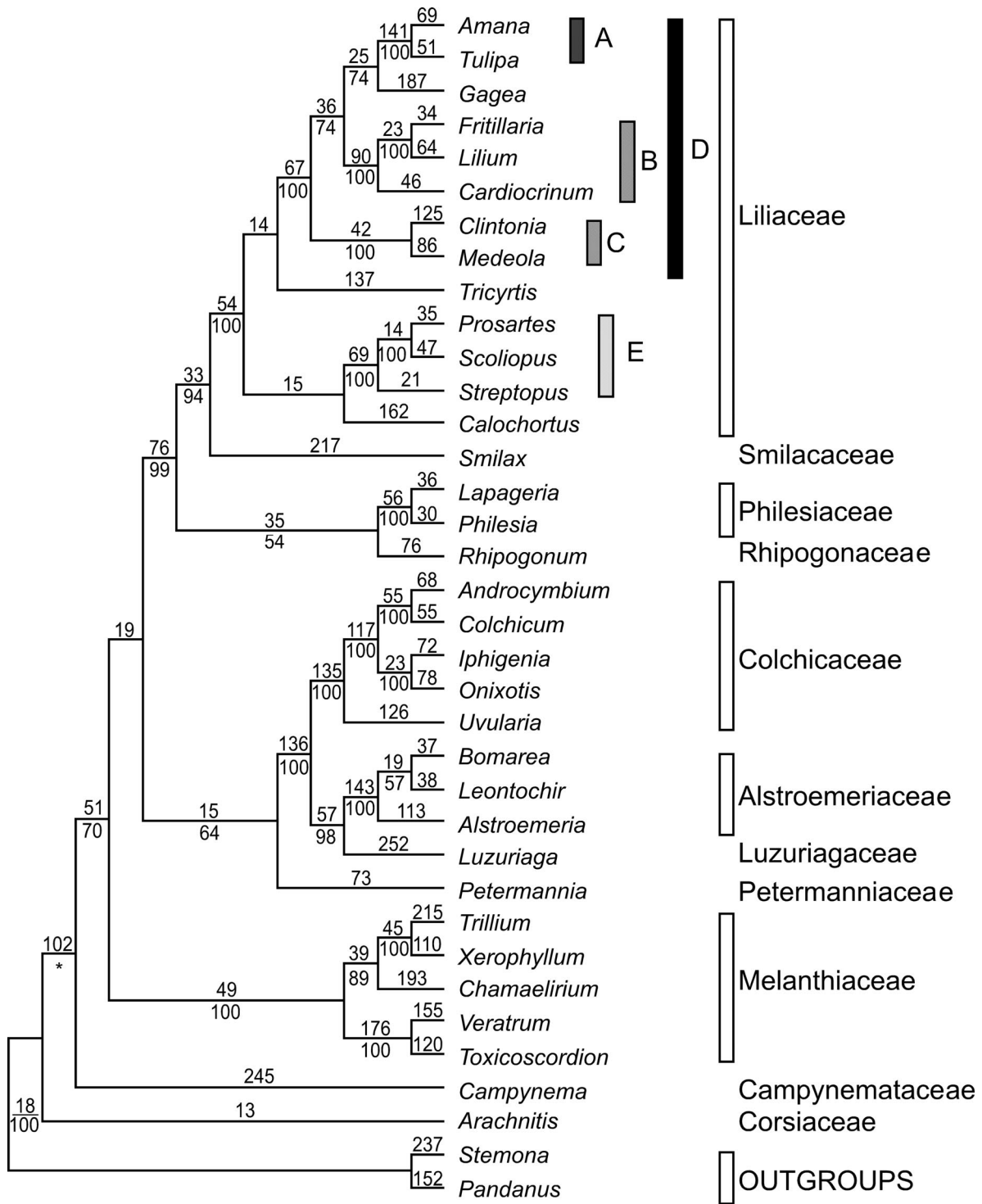


Fig. 1.—One of the two most-parsimonious trees obtained (DELTRAN optimization). Tree length = 5736 steps, consistency index = 0.60, retention index = 0.69. Branch lengths are given above the branches, bootstrap percentages below. The branch that collapses in the strict consensus is indicated with an asterisk. Bars and letters by groups within Liliaceae relate to the clades referred to in the Results section.

sis, 68% BS for the all-locus analysis). Chase et al. (2006) recommend the use of additional data and analysis to resolve this critical node in the monocots. If their topology holds in further analyses, Liliaceae sensu Dahlgren et al. (1985) are paraphyletic.

Circumscription of Liliales

The only significant change to the circumscription of the order, relative to Rudall et al. (2000) and Chase et al. (2000) is the tentative inclusion of Corsiaceae, on the basis of the

position of *Arachnitis* in the analyses of Chase et al. (2006), based on sequences for nuclear ribosomal 18S and mitochondrial *atp1*. Neyland and Hennigan (2003), using partial sequences for 26S nuclear ribosomal DNA alone, suggested that Corsiaceae may be polyphyletic. In their analysis, *Corsia* fell with Liliales, whereas *Arachnitis* fell with *Thismia* Griff. (Dioscoreales). *Arachnitis* and *Thismia* are both achlorophyllous, and plastid data for the former are lacking in the analyses of Chase et al. (2006), but we consider the result to be more robust than that of Neyland and Hennigan (2003) as it is based on data from nuclear and mitochondrial genomes and on a larger number of data points (base pairs). Rudall and Eastman (2002) examined the relationships of *Corsia* on the basis of floral anatomy and pollen morphology and found evidence that could support a relationship to Campanemataceae or *Thismia*. Thus, it appears that the placement of Corsiaceae remains problematic, and a definitive placement and assessment of its monophyly must await further data.

Petermannia falls as sister to a group of three families (Alstroemeriaceae, Colchicaceae, and Luzuriagaceae) rather than within Colchicaceae as in previous analyses. Thus, Petermanniaceae should be resurrected. This change in position is because the material labelled as *Petermannia* in earlier analyses was later shown to be misidentified *Tripladenia cunninghamii*. For further information, see Chase et al. (2006).

Family Relationships

Interrelationships among the families are the same (with the exception of Smilacaceae, see below) as that obtained by Chase et al. (2006), despite differences in taxon sampling and the loci used. Bootstrap support is also generally similar, but notable differences are the weak support for the position of Rhipogonaceae as sister to Philesiaceae [54% BS; cf. Chase et al. 2006, 100%] and the strong support for the position of Smilacaceae as sister to Liliaceae [94%; cf. Chase et al. (2006), where Smilacaceae fell as sister to (Philesiaceae + Rhipogonaceae) + Liliaceae, with only 56% BS]. The low level of support for the pattern of relationships between these families in the analysis of Chase et al. (2006) may be a reflection of the relatively low sampling density or the different loci used (in their plastid-only analysis, Smilacaceae fell as sister to Liliaceae, but again with weak BS).

Family Circumscriptions

Melanthiaceae are here recognized in a broad sense, including Trilliaceae, and this treatment is supported strongly by bootstrap analysis here and in the analyses of Zomlefer et al. (2006). Their analyses should be referred to for intra-familial relationships, as these include far more taxa in the family than do those presented here (we only used placeholders).

In Colchicaceae, *Uvularia* is strongly supported as sister to the rest of the family as sampled here, and the family is also strongly supported. Vinnersten and Reeves (2003) analyzed relationships within Colchicaceae using three plastid DNA regions and much wider taxon sampling. The relationships found here are in agreement with their analyses. They included *Burchardia* (not included in our study) in their

analyses, and this fell further outside core Colchicaceae than *Uvularia*. However, in an earlier study with less sampling and only using *rbcL*, the positions of *Uvularia* and *Burchardia* were reversed (Vinnersten and Bremer 2001). Also, *Burchardia* was included in Colchicaceae by APG (1998), APG II (2003), and Vinnersten and Reeves (2003). Due to the lability of the relative positions of *Burchardia* and *Uvularia* and in view of these recent taxonomic treatments, we recommend that *Uvularia* be included in Colchicaceae and that Uvulariaceae should not be recognized. Several genera in Colchicaceae are not monophyletic in the study of Vinnersten and Reeves (2003; see also footnote to Table 1).

Luzuriaga (Luzuriagaceae) is strongly supported as sister to Alstroemeriaceae, and these families could be combined. However, we choose not to do this for the time being in order to maintain the stability of family circumscriptions. Both Alstroemeriaceae and Luzuriagaceae have been recognized in most recent treatments (e.g., Chase et al. 2000; APG II 2003). The two families do, however, have shared characteristics, including the possession of inverted leaf blades (Dahlgren et al. 1985).

In Liliaceae, the positions of *Calochortus* and *Tricyrtis* are still not well defined. In both trees, *Calochortus* is sister to the clade containing *Prosartes*, *Scoliopus*, and *Streptopus*, but with BS <50%. *Tricyrtis* is sister to the remaining genera ("core Liliaceae" = Lilioideae and Medeoloideae), but again with BS <50%. This pattern of relationships mirrors that of Rønsted et al. (2005). In their analyses of ITS, *matK* and the *rpl16* intron, with considerably greater sampling in Liliaceae, *Tricyrtis* was sister to core Liliaceae with 91% BS. The position of *Calochortus* was, however, only weakly supported (67% BS). In the earlier study of Fay and Chase (2000), *Tricyrtis* was placed as sister to the *Prosartes/Scoliopus/Streptopus* clade and the placement of *Calochortus* was unresolved. Thus, the phylogenetic relationships of *Calochortus* and *Tricyrtis* have proved problematic. The relationships obtained here and by Rønsted et al. (2005) render Calochortaceae sensu Tamura (1998) paraphyletic. Patterson and Givnish (2003) used both Calochortaceae and Liliaceae in one article for the family containing *Calochortus*, and there is clearly a need for stability in family circumscription. For these reasons, we choose to recognize a wide circumscription of Liliaceae (as in Chase et al. 2000, and APG II 2003).

In Lilioideae, the position of *Gagea* as sister to *Tulipa* + *Amana* was only weakly supported. However, with increased taxon sampling, Rønsted et al. (2005) recovered the clade ((*Gagea* + *Lloydia*) (*Tulipa* (*Amana* + *Erythronium*))) with 97% BS. They used the tribal name Tulipeae for this clade. This varies from previous studies (see Rudall et al. [2000] and Fay and Chase [2000] in which *Tulipa*, *Amana*, and *Erythronium* were placed as sister to the remainder of core Liliaceae, including Medeoloideae, although these patterns lacked strong bootstrap support). Improving taxon sampling and increasing amounts of data have allowed us to achieve greater resolution and support within Liliaceae. Our data and those of Rønsted et al. (2005) both indicate a sister relationship of Tulipeae to Liliaceae (*Cardiocrinum*, *Fritillaria*, *Lilium*, *Notholirion*), but only with low to moderate support.

Fritillaria and *Lilium* again are shown to be closely related. In our earlier studies with fewer loci (summarized in

Fay and Chase 2000), we found two clades of *Fritillaria* and one of *Lilium* (including *Nomocharis* Franch.), but the relationship between the three clades was not well resolved. Thus, we were not able to demonstrate the monophyly of *Fritillaria*. The analysis presented here does not allow us to address this question (as we only include one species per genus), but the study conducted by Rønsted et al. (2005), in which multiple species of both genera were included, supports the hypothesis that both genera are monophyletic. *Cardiocrinum* and *Notholirion* are successive sister groups to *Fritillaria* + *Lilium* in that study, but the branch separating *Notholirion* from (*Cardiocrinum* (*Fritillaria* + *Lilium*)) is only weakly supported.

Prospects for Improvement

The main remaining problem in Liliales relates to the inclusion of Corsiaceae. Given their achlorophyllous nature and the extreme rarity of *Corsiopsis*, it may not prove possible to obtain plastid DNA data for these taxa, and it is unlikely that improving taxon sampling will be easily achieved, despite its obvious desirability. Collection of further nuclear and mitochondrial sequences for the taxa for which DNA is already available appears to be the best way forward in addressing this problem.

Elsewhere, there is scope for improving taxon sample or increasing the number of loci to address particular nodes where support is weak. The most significant of these in terms of interfamilial relationships relate to the position of Campanemataceae (and *Arachnitis*) as sister to the rest of the order and the relative positions of Melanthiaceae and the remaining two large clades that form a trichotomy in the bootstrap tree. Within Liliaceae, the relationships of *Calochortus* (and, to a lesser extent, *Tricyrtis*, *Notholirion*, and *Cardiocrinum*) still require further investigation.

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