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Molecular phylogenetics of Ruscaceae *sensu lato* and related families (Asparagales) based on plastid and nuclear DNA sequences

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• *Background* Previous phylogenetics studies of Asparagales, although extensive and generally well supported, have left several sets of taxa unclearly placed and have not addressed all relationships within certain clades thoroughly (some clades were relatively sparsely sampled). One of the most important of these is sampling within and placement of Nolinoideae (Ruscaceae *s.l.*) of Asparagaceae *sensu* Angiosperm Phylogeny Group (APG) III, which subfamily includes taxa previously referred to Convallariaceae, Dracaenaaceae, Eriospermaceae, Nolinaceae and Ruscaceae.
• *Methods* A phylogenetic analysis of a combined data set for 126 taxa of Ruscaceae *s.l.* and related groups in Asparagales based on three nuclear and plastid DNA coding genes, 18S rDNA (1796 bp), *rbcL* (1338 bp) and *matK* (1668 bp), representing a total of approx. 4·8 kb is presented. Parsimony and Bayesian inference analyses were conducted to elucidate relationships of Ruscaceae *s.l.* and related groups, and parsimony bootstrap analysis was performed to assess support of clades.
• *Key Results* The combination of the three genes results in the most highly resolved and strongly supported topology yet obtained for Asparagales including Ruscaceae *s.l.* Asparagales relationships are nearly congruent with previous combined gene analyses, which were reflected in the APG III classification. Parsimony and Bayesian analyses yield identical relationships except for some slight variation among the core asparagoid families, which nevertheless form a strongly supported group in both types of analyses. In core asparagoids, five major clades are identified: (1) Alliaceae *s.l.* (*sensu* APG III, Amarylidaceae–Agapanthaceae–Alliaceae); (2) Asparagaceae–Laxmanniaceae–Ruscaceae *s.l.*; (3) Themidaceae; (4) Hyacinthaceae; (5) Anemarrhenaceae–Behniaceae–Herreriaceae–Agavaceae (clades 2–5 collectively Asparagaceae *s.l. sensu* APG III). The position of *Aphyllanthes* is labile, but it is sister to Themidaceae in the combined maximum-parsimony tree and sister to Anemarrhenaceae in the Bayesian analysis. The highly supported clade of Xanthorrhoeaceae *s.l.* (*sensu* APG III, including Asphodelaceae and Hemerocallidaceae) is sister to the core asparagoids. Ruscaceae *s.l.* are a well-supported group. Asparagaceae *s.s.* are sister to Ruscaceae *s.l.*, even though the clade of the two families is weakly supported; Laxmanniaceae are strongly supported as sister to Ruscaceae *s.l.* and Asparagaceae. Ruscaceae *s.l.* include six principal clades that often reflect previously named groups: (1) tribe Polygonateae (excluding *Disporopsis*); (2) tribe Ophiopogoneae; (3) tribe Convallarieae (excluding *Theropogon*); (4) Ruscaceae *s.s.* + Dracaenaceae + Theropogon + Disporopsis + Comospermum; (5) Nolinaceae, (6) Eriospermum.
• *Conclusions* The analyses here were largely conducted with new data collected for the same loci as in previous studies, but in this case from different species/DNA accessions and greater sampling in many cases than in previously published analyses; nonetheless, the results largely mirror those of previously conducted studies. This demonstrates the robustness of these results and answers questions often raised about reproducibility of DNA results, given the often sparse sampling of taxa in some studies, particularly the earliest ones. The results also provide a clear set of patterns on which to base a new classification of the subfamilies of Asparagaceae *s.l.*, particularly Ruscaceae *s.l.* (= Nolinoideae of Asparagaceae *s.l.*), and examine other putatively important characters of Asparagales.

Key words: *Aphyllanthes*, Asparagaceae, Convallariaceae, Dracaenaceae, *Eriospermum*, monocot phylogenetics, Nolinaceae, Nolinoideae.

INTRODUCTION

Asparagales are the largest order among the five orders of Lilianae (= Liliiflorae) *sensu* Dahlgren *et al.* (1985), who followed the concepts of Huber (1969). There are up to 29 families [APG (Angiosperm Phylogeny Group), 1998] in the order, which has been considered monophyletic on the basis of their phytomelan-containing seed coat and several other characteristics (Huber, 1969; Rudall *et al.*, 2000; Chase *et al.*, 2006). Chase *et al.* (1995*a*) performed the first extensively sampled phylogenetic analysis to examine their circumscription. This analysis led to the recircumscription of Asparagales to include Orchidaceae (including the former Apostasiaceae and Cypripediaceae) and Iridaceae (including the former Geosiridaceae), both families formerly Liliales/Orchidales, and to exclude Dasypogonaceae *s.l.*, Hanguanaceae,

Luzuriagaceae and Philesiaceae. The boundary between Asparagales and Liliales can be difficult to define with morphological data alone because several characters are shared by some lilioids and asparagoids, especially net-veined taxa (Conran, 1989; Rudall *et al.*, 2000). The combined molecular–morphology analysis (Chase *et al.*, 1995*b*) indicated that although the lilioid monocots were monophyletic, several asparagoid families were paraphyletic or polyphyletic (Chase *et al.*, 1995*a*, 2006). Within Asparagales there was a paraphyletic grade (predominantly characterized by simultaneous microsporogenesis and inferior ovaries) and a 'core asparagoid' clade, uniformly characterized by successive microsporogenesis and mostly superior ovaries (Rudall *et al.*, 1997; Furness and Rudall, 1999). The combined plastid DNA (including *rbcL*, *atpB*, *trnL* intron, and *trnL-F* intergenic spacer) analyses by Fay *et al.* (2000) and additional DNA sequences by Pires *et al.* (2006) further resolved phylogenetic relationships within Asparagales. To accord with the molecular and morphological studies (Chase *et al.*, 1995*b*, *b*; Fay and Chase, 1996; Rudall *et al.*, 1997, 2000; Fay *et al.*, 2000), many families in Asparagales have been recircumscribed (APG, 1998; APG II, 2003), and several new families have been erected (Chase *et al.*, 1996, 1997; Conran *et al.*, 1997; Fay and Chase, 1996; Rudall and Chase, 1996).

Ruscaceae *sensu lato* are a recently recognized family in the broad sense (Chase *et al.*, 1995*a*; Rudall *et al.*, 2000; APG, 1998); they include Ruscaceae *s.s.*, Convallariaceae, Nolinaceae, Dracaenaceae, Eriospermaceae and *Comospermum* (the last of highly speculative placement in Dahlgren *et al.*, 1985). Ruscaceae *s.l.* can be distinguished from other higher asparagoid groups by usually possessing berries or other indehiscent fruit types and absence of phytomelan in the seed coat. One might suppose that indehiscent fruits and absence of phytomelan could be correlated characters, but in *Asparagus* (Asparagaceae *s.s.*) berries and phytomelan co-occur. The combined analysis of *rbcL* and morphology (Chase *et al.*, 1995*b*; Rudall *et al.*, 1997) indicated that several genera that had been included in Convallariaceae were members of other families or were embedded within a larger clade; this larger clade was recognized as the newly circumscribed broad-sense Convallariaceae (APG, 1998; Fay *et al.*, 2000). Rudall *et al.* (2000) suggested Ruscaceae Sprengel (1826) had priority over Convallariaceae Horaninow (1834), and they are now generally referred to as Ruscaceae *s.l.* (Jang and Pfosser, 2002; APG II, 2003), which could also be included in a much-expanded circumscription of Asparagaceae. The latter was presented as an alternative classification in APG II. In APG III (2009), the broadly circumscribed families (including Asparagaceae *s.l.*) were accepted as the only circumscription in accord with APG, in which case this clade would be referred to as subfamily Nolinoideae.

Ruscaceae *s.s.*, comprising three genera (*Ruscus*, *Danae* and *Semele*), are distributed in the Mediterranean–Macronesian area; they have woody stems, scale-like leaves, berries, and a basic chromosome number of $x = 20$. Dahlgren *et al.* (1985) and Takhtajan (1997) regarded Ruscaceae *s.s.* as the most closely related group to Asparagaceae *s.s.*, but there has been no clear evidence on relationships of these families. The two families have several similarities including phylloclades (but even for this character there are questions about homology; Arber, 1924; Cooney-Sovetts and Sattler, 1986), baccate fruits and similar karyotypes (Sato, 1942; Tamura, 1995), and they have differences in the position of inflorescences and seed coat (Conran and Tamura, 1998). Rudall *et al.* (1998) recognized that the karyotype of Ruscaceae ($x = 20$) is more similar to Convallariaceae (usually $x = 19$, rarely 18, 20) than to that of Asparagaceae *s.s.* (mostly $x = 10$). Serological analyses and lack of phytomelan in the seed coat indicated a closer relationship between Ruscaceae *s.s.* and Convallariaceae than either to Asparagaceae *s.s.* (Chupov and Cutjavina, 1980).

Convallariaceae are rhizomatous perennial herbs distributed in the Northern Hemisphere; they are abundant in eastern and southeastern Asia and comprise four tribes: Polygonateae, Ophiopogoneae, Convallarieae and Aspidistreae (Dahlgren *et al.*, 1985; Tamura, 1995). They share calcium oxalate crystals and two ovules (rarely or over) per locule, but it is not so easy to identify distinguishing morphological characters for the tribes in Convallariaceae, and Dahlgren *et al.* (1985) used plesiomorphic characters for the taxonomic key, including baccate fruits, non-phytomelaniferous seeds and nuclear endosperm formation. Polygonateae share a sympodial rhizome, an elongated aerial stem and berries, and the position and shape of inflorescences (axillary in *Polygonatum* and *Disporopsis*, terminal in *Smilacina* and *Maianthemum*, and axillary and terminal in *Heteropolygonatum*) are variable in the tribe. Ophiopogoneae have a sympodial rhizome, fruits that rupture at an early stage, seeds with sarcotesta, and basic chromosome number $x = 18$; this tribe comprises three genera (*Liriope*, *Ophiopogon* and *Peliosanthes*) distributed in eastern and southeastern Asia. Convallarieae and Aspidistreae have a monopodial rhizome and a short stem, usually berries (except drupes in *Tricalistra*), and basic chromosome number usually of $x = 19$, rarely 20 (*Theropogon*) or 18 (some *Aspidistra*). Conran and Tamura (1998) merged Aspidistreae with Convallarieae. The plastid *trnK* sequence analysis of Yamashita and Tamura (2000) supported the treatment of Conran and Tamura (1998).

Nolinaceae are arborescent, anomalously woody plants with terminal rosette leaves and indehiscent nutlets, and they comprise four genera, *Nolina*, *Dasylirion*, *Calibanus* and *Beaucarnea*, found in warm, dry regions of North America. Nolinaceae were often previously included in a broadly defined family Liliaceae near *Dracaena*, and they had been treated in the tribe Dracaeneae (Bentham and Hooker, 1883) or Nolineae (Krause, 1930). Hutchinson (1934) included Nolinaceae, Yuccoideae and Dracaenae in Agavaceae because of their anomalous woody growth (via a secondary thickening meristem) and fibrous leaves, but this treatment was not supported by other morphological characters (flowers, fruits and seeds) and karyology (Sharma and Chaudhuri, 1964). Nolinaceae were excluded from Agavaceae and arranged near Dracaenaceae in Dahlgren *et al.* (1985).

Dracaenaceae include perennial plants with a more or less woody trunk, but many do not have a trunk; they comprise two genera, *Dracaena* and *Sansevieria* (perhaps best combined into one genus), which occur in subtropical to tropical regions of the Old World. Dracaenaceae are distinguished from Nolinaceae in having berries, no oils in guard cells and mucilage-filled cells with crystal raphides in vegetative parts.

Eriospermaceae are perennial herbs with various types of tubers and free perianth parts. They comprise a single genus (*Eriospermum*) distributed in southern parts of Africa. This family shows seasonal developmental differences between leaves and inflorescences. Because they have extraordinary characters such as leaf appendages, epidermal hairs on the seeds and embryological attributes but have successive microsporogenesis and thin testa, Dahlgren *et al.* (1995) suggested that treatment as a family separate from related groups was probably best. The taxonomic position of Eriospermaceae has been controversial whether included (Rudall *et al.*, 2000) or not (Jang and Pfosser, 2002) in Ruscaceae *s.l.*

Ruscaceae *s.l.* have no distinguishable synapomorphic characters from the other higher asparagoids except the absence of phytomelan in the seed coat, but analysis of the combined molecular and morphology matrix (Chase *et al.*, 1995*b*) indicated that Ruscaceae *s.l.* was a well-supported clade that was largely unresolved relative to the related families and genera. This grouping of Ruscaceae *s.l.* (= Convallariaceae *s.l.*) was supported by plastid DNA restriction-site analyses of some taxa, although Bogler and Simpson (1995) lacked some of the core taxa such as Ruscaceae *s.s.* and *Comospermum*. Several molecular studies supported monophyly of Ruscaceae *s.l.* (Rudall *et al.*, 1997, 2000). Yamashita and Tamura (2000) sequenced the plastid *trnK* region (including the *matK* exon) for 39 Convallariaceae species and related families, which indicated that there were six major clades; Convallariaceae *s.s.* were paraphyletic in this analysis. They compared the *trnK* tree with the *rbcL* tree and looked at basic chromosome numbers, but they occasionally had unresolved relationships due to a lack of informative characters and sampling of potential sister groups; they nonetheless found evidence to support the tribal limits in Convallariaceae of Conran and Tamura (1998). Jang and Pfosser (2002) performed a phylogenetic analysis based on *rbcL* and *trnL-F* intron/spacer sequences, but there were no improved assessments of relationships because of poor sampling of taxa in Ruscaceae *s.l.*

Asparagaceae *s.s.* have been usually considered sister to Ruscaceae *s.l.* due to their cytological and morphological similarities (Tamura, 1995). *Aphyllanthes* (Aphyllanthaceae) was also indicated as a possible sister group to Ruscaceae (Conran, 1998; Yamashita and Tamura, 2000), but Fay *et al.* (2000) made a cautious accessment of *Aphyllanthes*, a taxonomically isolated Mediterranean genus, because of its labile phylogenetic position. Laxmanniaceae were sister to Ruscaceae *s.l.* plus Asparagaceae (Rudall *et al.*, 1997; Fay *et al.*, 2000; Bogler *et al.*, 2006; Givnish *et al.*, 2006; Graham *et al.*, 2006; Pires *et al.*, 2006). APG II (2003) and APG III (2009) suggested a broader circumscription of Asparagaceae based largely on results of analysis for four plastid DNA regions (Fay *et al.*, 2000); Ruscaceae *s.l.* was treated as an optional circumscription along with Agavaceae *s.l.* (including Anemarrhenaceae, Anthericaceae, Behniaceae, Herreriaceae and Hesperocallidaceae) and related families such as Aphyllanthaceae, Hyacinthaceae, Laxmanniaceae and Themidaceae.

A molecular phylogenetic study was conducted to re-evaluate delimitation of Ruscaceae *s.l.* of Rudall *et al.* (2000) and related families (APG, 1998; APG II, 2003; APG III, 2009; Chase *et al.*, 2006), especially to assess their possible sister groups in Asparagales and evaluate phylogenetic relationships with the related families in the core asparagoids. The aim was to investigate relationships in Asparagales by sequencing three genes, 18S nuclear ribosomal DNA and plastid *rbcL* and *matK*, for 121 taxa of Asparagales. These genes were chosen because of their use in recent studies of familial and higher-level phylogenetics (Chase *et al.*, 1995*a*, 2006; Soltis *et al.*, 1997, 2000; Fay *et al.*, 2000; Yamashita and Tamura, 2000; Hilu *et al.*, 2003; Devey *et al.*, 2006). The impact of these data on the classification of Ruscaceae *s.l.* and related families was also evaluated. New sequences from mostly new accessions of the sampled taxa were produced for this study; this was done to avoid possibile misidentification of taxa in the earlier published studies or sequences with errors due to the prelatively primitive techniques used to produce *rbcL* and 18S rDNA sequences in the early period of DNA sequencing.

MATERIALS AND METHODS

Plant materials

The taxa used for this study included all genera (except *Heteropolygonatum*) in Ruscaceae *s.l. sensu* Rudall *et al.* (2000) and representatives of all families of Asparagales (APG). The plant material used was either fresh, collected from the field and dried, taken from specimens in the herbarium, or was a DNA sample borrowed from the Royal Botanic Gardens, Kew, DNA Bank (http://data.kew.org/dnabank/DnaBankForm.html). Voucher specimens of the taxa were prepared; source, voucher information and database accession numbers are listed in the Appendix. Provenance and distributions were also prepared from voucher specimens and the World Checklist of Selected Plant Families (http://apps.kew.org/wcsp/home.do). For one taxon (*Bulbine* sp.), sequences from different species (*B. succulenta* and *B. frutescens*) in GenBank were used, and several sequences (six for 18S rDNA, nine for *rbcL* and ten for *matK*) were from GenBank and previous papers (Chase *et al.*, 2006). Otherwise, new sequences were prepared.

DNA extraction, PCR, sequencing and alignment

Total genomic DNA was extracted from 0·5–1·0 g of fresh or silica gel-dried leaves using the 2× CTAB buffer method (Doyle and Doyle, 1987). Lipids were removed with SEVAG solution (24 : 1 chloroform : isoamyl alcohol), and DNA was precipitated with isopropanol at −20 °C. Total extracted DNA was dissolved in 1× TE buffer and stored at −70 °C, and the concentration of DNA was determined with GeneQuant pro (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, USA) before use.

The 18S rDNA gene was amplified using the primers and protocols of White *et al.* (1990), Nickrent and Soltis (1995), and Soltis and Soltis (1998); *matK* was amplified with primers and protocols of Johnson and Soltis (1995) and Hilu *et al.* (2003), and the *rbcL* gene was amplified with primers and protocols of Omstead *et al.* (1992), Shinwari

et al. (1994) and Fay and Chase (1996). Amplifications were carried out in 50-μL reactions, containing 2 units Taq DNA polymerase, 5 μL 10× reaction buffer (100 mM Tris–HCl, 500 mM KCl, 15 mM $MgCl_2$), 2·5 mM dNTPs, 5 pmol μL^{-1} forward and reverse primers, using Perkin-Elmer 9700 machine (Applied Biosystems, Inc., Beverly, MA, USA). DMSO (2 %) was added to reduce the secondary structure in PCR. PCR conditions were premelt of 94 °C for 2 min, followed by 30–35 cycles of denaturation at 94 °C for 1 min, annealing at 50–55 °C for 1 min, extension at 72 °C for 3 min, followed by a final extension of 7 min at 72 °C.

All PCR products were purified using ExoSAP-IT (USB Corporation, Cleveland, OH, USA) according to the manufacturer's protocols. Dideoxy cycle sequencing was performed using the chain-termination method and the ABI prism big dye reaction kit (ver. 3·1) following the manufacturer's protocols. Products were run on an ABI 3700 genetic analyser or MegaBace1000 (Amersham Pharmacia Biotech, Inc.) using the manufacturers' protocols. Sequence editing and assembly of contigs were carried out using Sequence Navigator and AutoAssembler software (ABI).

All sequences were aligned initially in ClustalX (ver. 1·83; Thompson *et al.*, 1997) and MacClade (ver. 4·0; Maddison and Maddison, 2000) and then manually adjusted following the guidelines of Kelchner (2000). Alignment of sequences for these coding genes was easily performed because there were no insertions/deletions (indels) among the sequences of Ruscaceae *s.l.*, but there were indels in the sequences of other Asparagales and outgroups: three in 18S rDNA and nine in *matK*; the aligned matrix is available from kimjh@dju.ac.kr or m.chase@kew.org. The three indels in 18S rDNA correspond to positions 496–501, 666–672 and 1363–1369 on the reference sequence of *Glycine max* (L.) Merr. (Soltis *et al.*, 1997, 2000; Soltis and Soltis, 1998).

Parsimony analysis

Two separate sets of analyses were carried out. The first (analysis A) comprised the plastid sequences of 121 taxa representing all 29 families of Asparagales, and the second (analysis B) comprised the combined 18S rDNA and plastid DNA sequences for the same taxa. Orchidaceae were designated as the outgroup for both analyses based on previous results (Chase *et al.*, 1995*a*, 2000*b*; Fay *et al.*, 2000). PAUP* (ver. 4·10b; Swofford, 2007) was used for parsimony analysis and followed the widely used parsimony analysis with successive approximations weighting and bootstrapping (Fay *et al.*, 2000; Clarkson *et al.*, 2004; the bootstrap did not use the relative weights). In analyses A and B, tree searches were performed under the Fitch (equal weight, EW; Fitch, 1971) criterion with 1000 random sequence additions and tree–bisection–reconnection (TBR) branch swapping, permitting ten trees to be held at each step (Multrees on) to reduce time searching suboptimal 'islands' of trees (Chase *et al.*, 2006). All shortest trees collected in the 1000 replicates were swapped on to completion without a tree limit. Successive approximation weighting (SW; Farris, 1989) was carried out to select the most stable trees (Carpenter, 1988) according to the rescaled consistency index, using the maximum value (best fit) criterion and a base weight of 1·0, followed by 100 replicates of heuristic search with random sequence additions and subtree pruning-regrafting (SPR) swapping. All shortest trees from these 100 replicates were then swapped to completion, after which another round of weighting was implemented. This process was repeated until the same tree length was obtained twice in succession. DELTRAN character optimization was used to illustrate branch length throughout. To evaluate internal support, 1000 bootstrap replicates were carried out with equal weights, TBR branch swapping with five trees held at each step and simple taxon addition (Felsenstein, 1985). The following descriptions for categories of bootstrap support were used: weak, 50–74; moderate; 75–84; well supported, 85–100 % (Chase *et al.*, 2000*a*).

Bayesian analysis

Further phylogenetic analyses were performed using Bayesian inference as implemented in MrBayes (ver. 3·12; Ronquist *et al.*, 2005). MrModeltest (ver. 2·2; Nylander, 2005) was used to determine the best model of DNA substitution for each partition, evaluating all models against defaults of the program. The GTR + I + G model (a general time reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites) was chosen for the three genes as the best-fitting among the 24 models compared. Thus, all three genes were assigned a model of six substitution types ($n = 6$) with a proportion of invariable sites. Four simultaneous Markov Chain Monte Carlo (MCMC) chains were run for 5×10^6 generations and sampled every 100 generations, and the first 1×10^5 trees were excluded ('burn-in'). Post-burn-in samples of trees drawn from the posterior probability distribution were summarized, and this tree is illustrated (see Fig. 3). Bayesian analysis was performed three times to ensure convergence of results.

RESULTS

A summary of characteristics of the DNA data is presented in Table 1. The aligned number of characters was 4802, but 71 positions for 18S rDNA were excluded from phylogenetic analyses as in previous studies due to ambiguous alignments in these short sections of the matrix (Soltis *et al.*, 1997, 2000; Soltis and Soltis, 1998). The total number of included bases was 4731 of which 1851 were variable (39·1 %) and 1301 (27·5 %) were potentially parsimony informative. The number of positions in the matrix included 1338 for *rbcL*, 1668 for *matK* and 1725 for 18S rDNA. The *matK* gene was the most variable among the three genes and gave the greatest number of parsimony informative sites; 18S rDNA showed the lowest variation. The number of parsimony-informative characters was 327 (25·1 %) for *rbcL*, 784 (60·3 %) for *matK* and 190 (14·6 %) for 18S rDNA.

Parsimony analysis based on plastid DNA (analysis A)

The final alignment of the combined (*rbcL* and *matK*) plastid DNA matrix comprised 3006 positions, of which 1534 were variable (51·0 %) and 1111 (37·0 %) were potentially parsimony informative. Fitch analysis (EW; Table 1) produced

TABLE 1. *Statistics for the three genes analysed in this study*

Characters	*rbcL* (I)	*matK* (II)	18S rDNA (III)	Plastid data (I + II)	Combined (I + II + III)
Aligned	1338	1668	1796	3006	4802
Included	1338	1668	1725	3006	4731
Parsimony informative	327	784	190	1111	1301
Variable	462	1072	317	1534	1851
Constant	876	596	1408	1472	2880
Transition/transversion	877/456 (1·87)	1868/1189 (1·52)	507/179 (2·52)		
G + C (%)	43·45	31·37	50·43		
Tree length (EW/SW)	(1572/407·660)	(3744/1156·147)	(902/290·889)	(5435/1537·590)	(6442/1811·619)
CI (EW/SW)	(0·40/0·71)	(0·44/0·70)	(0·46/0·80)	(0·42/0·70)	(0·42/0·71)
RI (EW/SW)	(0·69/0·84)	(0·74/0·86)	(0·65/0·82)	(0·72/0·85)	(0·70/0·84)

EW, Equally weighted; SW, successive weighted; CI, consistence index; RI, retention index.

5760 equally most-parsimonious trees [length = 5435 steps; CI (consistency index, including autapomorphies) = 0·42; RI (retention index) = 0·72]. Successive weighting (SW) identified one shortest tree as optimal with an SW score of 1537·59 (5435 Fitch length; CI = 0·70, RI = 0·85). The SW tree is therefore one of the trees found with equal weights; it is shown with its Fitch branch lengths (DELTRAN optimization) in Fig. 1. Groups (nodes) not found in the consensus tree of Fitch analysis are marked with triangles. Bootstrap percentages (BP) consistent with the strict consensus tree are shown below each branch; groups with BP < 50 are not indicated.

In this study, only the core asparagoids are presented for the plastid DNA tree (Fig. 1) since it showed a topology similar to that of the combined DNA tree except for relationships among Ruscaceae *s.l.* and related families. The core asparagoids formed a strongly supported group (BP 100), and the other asparagoids were paraphyletic (not shown). The core asparagoids fell into two clades, one moderately (BP 84) and the other well supported (BP 90). The first consisted of four families including Agavaceae *s.l. sensu* APG I (BP 96), Hyacinthaceae (BP 100) and Themidaceae (BP 100), as well as Aphyllanthaceae. The second consisted of Ruscaceae *s.l.*, Asparagaceae, Laxmanniaceae, Alliaceae, Agapanthaceae and Amaryllidaceae.

Within the second group, Ruscaceae *s.l.* were well supported (BP 90; Fig. 1). Asparagaceae *s.s.* were strongly supported (BP 100) and sister to Ruscaceae *s.l.*, but the two families together were weakly supported (BP 50); Laxmanniaceae were strongly supported (BP 96) as a member of the clade with Ruscaceae *s.l.* and Asparagaceae *s.s.* Alliaceae *s.l. sensu* APG (1998) including Alliaceae *s.s.*, Agapanthaceae and Amaryllidaceae form a moderately supported clade (BP 75) as the sister of the rest (Fig. 1).

The tree topology of Ruscaceae *s.l.* in this study did not accord or was only partly congruent with previous studies (Rudall *et al.*, 1997; Yamashita and Tamura, 2000; Jang and Pfosser, 2002). Ruscaceae *s.l.* were strongly supported (BP 90), and within this clade fell Ruscaceae *s.s.*, Dracaenaceae, Convallariaceae, Nolinaceae and Eriospermaceae (Fig. 1). The combined Ruscaceae *s.s.* and Dracaenaceae clade was moderately supported (BP 75), and they were interdigitated within clades of Convallariaceae. Within Convallariaceae, Aspidistreae (BP 96; including *Campylandra*, *Rohdea*, *Tupistra* and *Aspidistra*) and Ophiopogoneae (BP 98; including *Liriope*, *Ophiopogon* and *Peliosanthes*) were strongly supported. Convallarieae were not monophyletic, and Polygonateae were only weakly supported as monophyletic (BP 64) and excluded *Disporopsis* (BP 100). Eriospermaceae (BP 100) were sister to highly supported Nolinaceae (BP 100).

Parsimony analysis based on combined DNA (analysis B)

The number of positions included in the combined analysis (18S rDNA, *rbcL* and *matK*) was 4731. The number of bases contributed by each individual gene was 1338 for *rbcL*, 1668 for *matK* and 1725 for 18S rDNA. The number of variable sites was 1851 (39·1 %), and 1301 (27·5 %) were potentially parsimony informative. Fitch analysis (EW), including 121 asparagoid monocots (Table 1), produced 5721 equally most-parsimonious trees of 6442 steps with CI (including autapomorphies) = 0·42 and RI = 0·70. Successive weighting (SW) identified one shortest tree as optimal with an SW score of 1811·62 (6442 Fitch length; CI = 0·71, RI = 0·85). The SW tree was one of the Fitch trees, and it is shown with its Fitch branch lengths (DELTRAN optimization) in Fig. 2. Groups not found in the strict consensus tree of the Fitch analysis are marked with triangles. Bootstrap percentages (BP; equal weights) consistent with the strict consensus tree are shown below each branch, but groups with BP < 50 are not indicated (Fig. 2).

The topology of the combined DNA tree for Asparagales largely followed the previous analyses in the broad sense of the core asparagoids concept (Chase *et al.*, 1995*a*; Fay *et al.*, 2000; Pires *et al.*, 2006). The core asparagoids formed a strongly supported group (BP 100) with the rest of the families of Asparagales forming a grade relative to the core group (Fig. 2). The core asparagoids fell into two big clades, one with strong support (BP 86; group B) and the other with weak support (BP 56; group A). The former consisted of four families including Agavaceae *s.l. sensu* APG II (BP 94), Hyacinthaceae (BP 100), Themidaceae (BP 100) and Aphyllanthaceae. The other consisted of Ruscaceae *s.l.*, Asparagaceae *s.s.*, Laxmanniaceae and Alliaceae *s.l.*

Within group A in the core asparagoids, Ruscaceae *s.l.* were well-supported (BP 88; Fig. 2). Asparagaceae *s.s.* were strongly supported (BP 100) as sister to Ruscaceae *s.l.*, even though the clade of the two was weakly supported (BP 50), and Laxmanniaceae appeared as sister (BP 96) to Ruscaceae *s.l.* and Asparagaceae. Alliaceae *s.l. sensu* APG (1998) were weakly supported (BP 73) as sister to group A (Fig. 2).

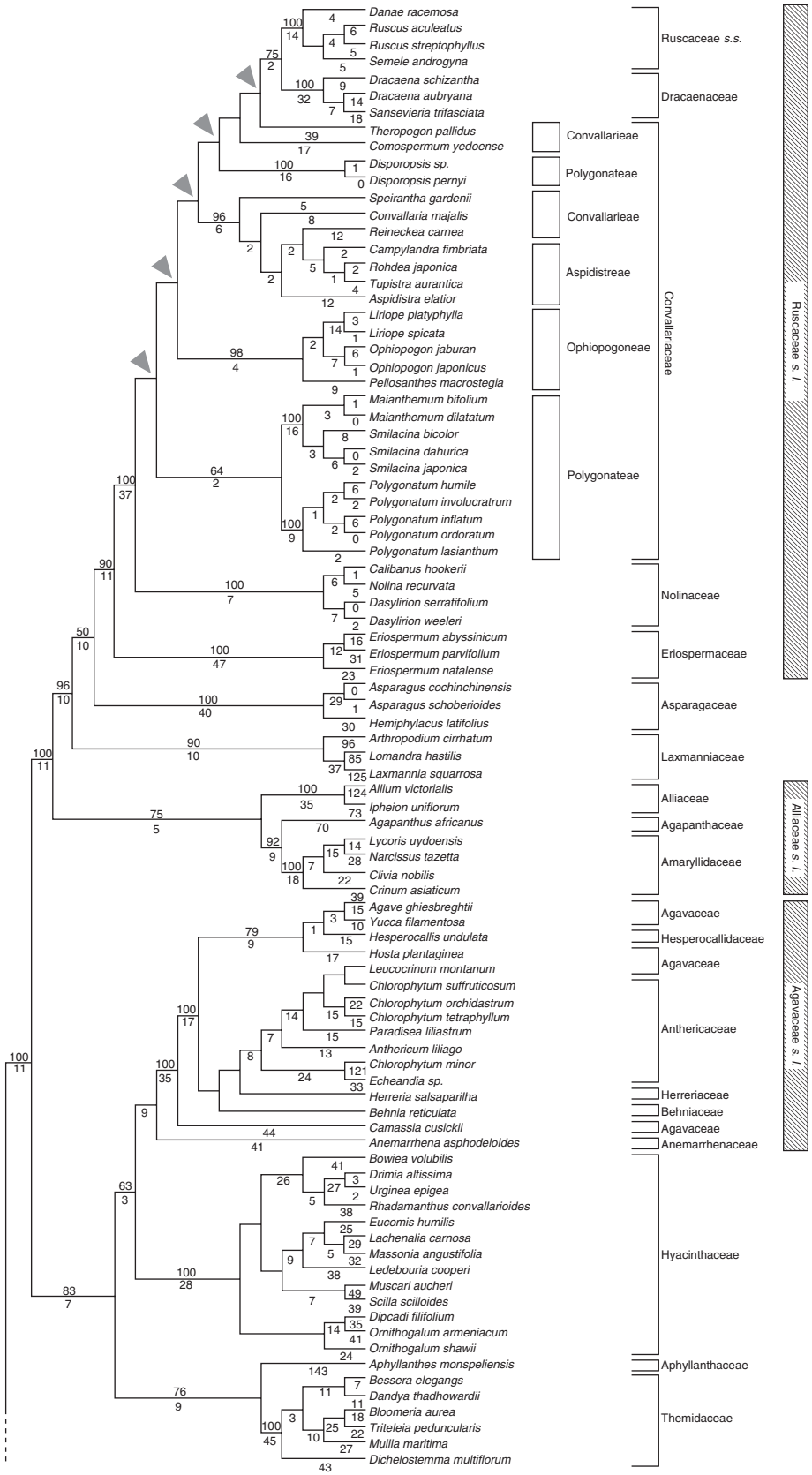

FIG. 1. The single shortest tree from successive weighting of plastid *rbcL* and *matK* (analysis A) for Ruscaceae *s.l.* and related groups of Asparagales. Numbers of substitutions are indicated below each branch (DELTRAN optimization), and bootstrap percentages >50 % are given above each branch. Triangles indicate branches not present in the strict consensus tree of 5760 equally MP trees by Fitch analysis (equal weight). Tree length is 5435 steps with CI = 0·70 and RI = 0·85. The dashed line in the lower left-hand corner marks the point where the non-core asparagoids are attached to this part of the tree (non-core taxa are not shown; this part of the tree is identical to that show in Fig. 2).

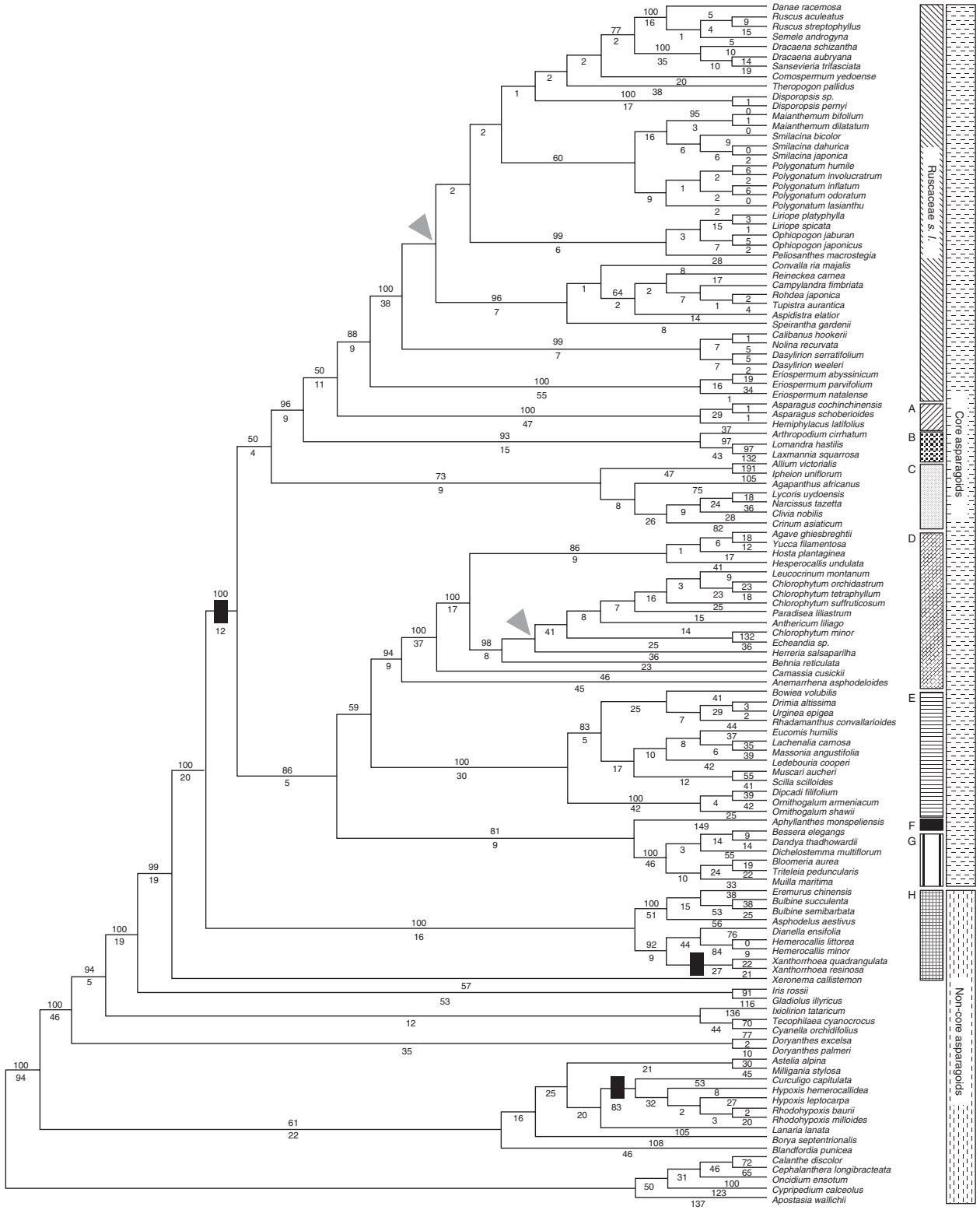

FIG. 2. One MP tree from combined DNA data (analysis B) for 121 taxa of Asparagales. Numbers of substitutions are indicated below each branch, and bootstrap percentages >50 % are given above each branch. Triangles indicate groups not present in the strict consensus tree of the Fitch analysis. Bars show points at which changes in microsporogenesis have taken place. Tree length is 6442 steps with CI = 0·71 and RI = 0·85. A, Asparagaceae; B, Laxmanniaceae; C, Alliaceae *s.l.*; D, Agavaceae *s.l.*; E, Hyacinthaceae; F, Aphyllanthaceae; G, Themidaceae; H, Xanthorrhoeaceae *s.l.*

The tree topology of Ruscaceae *s.l.* from the combined analysis did not accord with or was only partly congruent with previous plastid analyses (Rudall *et al.*, 1997; Yamashita and Tamura, 2000; Jang and Pfosser, 2002). Ruscaceae *s.l.* were grouped together in one strongly supported clade (BP 88; Fig. 2), and Ruscaceae *s.s.*, Dracaenaceae, Nolinaceae and Eriospermaceae received strong bootstrap support (BP > 99 %) even though Convallariaceae were polyphyletic (Fig. 2). For the tribes of non-monophyletic Convallariaceae, Aspidistreae (BP 64) and Ophiopogoneae (BP 99) were monophyletic, but Convallarieae were not monophyletic. Polygonateae were monophyletic but weakly supported (BP 60). Nolinaceae were sister to the rest of Ruscaceae *s.l.* minus *Eriospermum* (BP 99). Strongly supported Eriospermaceae (BP 100) were sister to the rest of Ruscaceae *s.l.*

Outside the core asparagoids, the tree topology from analysis of the combined DNA data was congruent with those from previous analyses (Fay *et al.*, 2000; Pires *et al.*, 2006). The major differences between combined and plastid results were mostly not in topology but rather in levels of support. The core asparagoids were sister to Xanthorrhoeaceae *s.l. sensu* APG III (2009) with strong support (BP 100). Xeronemataceae were sister to the large clade (core asparagoids and Xanthorrhoeaceae *s.l.*; BP 99), which was strongly supported. Iridaceae were strongly supported (BP 100) as sister to the above clade. The relationships among the next asparagoid families [(Ixioliriaceae + Tecophilaceae) Doryanthaceae] were strongly supported (BP < 94), and the sister to the above clade (BP 100) consisted of <Branfordiaceae {Boryaceae [Asteliaceae (Hypoxidaceae + Lanariaceae)]}>. The final clade in Asparagales was Orchidaceae, which was designated in this study as outgroup to the rest of the order (BP 100), following results of broader monocot analyses that demonstrated Orchidaceae to be sister to the rest of Asparagales (Chase *et al.*, 2006).

Bayesian analysis of combined matrix (Analysis C)

The Bayesian tree (Fig. 3) shows the posterior probabilities summarized from the set of recovered post-burn-in trees; parameters of the GTR + I + G model used in this analysis are listed in Table 2. Although one node in the core asparagoids had low posterior probability (PP), 0·62, the majority of nodes in the tree are supported by PPs >0·95. Bayesian analysis produced a similar overall topology to that of the maximum parsimony analysis (Fig. 3), but it showed a few differences in the core asparagoids. The core asparagoids were strongly supported (1·00 PP; Fig. 3). Within the core asparagoids, a big clade consisting of Ruscaceae *s.l.* (PP 0·99), Asparagaceae (PP 1·00) and Laxmanniaceae (PP 1·00) was highly supported (PP 1·00). Among the taxa of Ruscaceae *s.l.*, Dracaenaceae (PP 1·00), Ruscaceae *s.s.* (PP 1·00) and Eriospermaceae (PP 1·00) were strongly supported, but the former four tribes of Convallariaceae were not monophyletic except for Ophiopogoneae (PP 1·00). Agavaceae *s.l.* (*sensu* APG III) including Anemarrhenaceae, Anthericaceae, Behniaceae and Herreriaceae were weakly supported (PP 0·86). A combined clade with Agavaceae *s.l.* and Aphyllanthaceae showed low PP (PP 0·37), and the node with Themidaceae (PP 1·00) and Hyacinthaceae (PP 1·00) was highly supported (PP 0·92). Amaryllidaceae *s.l.* (*sensu* APG III) consisting of Alliaceae (PP 1·00), Amaryllidaceae *s.s.* (PP 1·00) and Agapanthaceae were strongly supported (PP 1·00).

The spine of the tree among the non-core asparagoids was nearly congruent to that of the maximum-parsimony (MP) tree with high PP (1·00; Fig. 3). All nodes were strongly supported (PP > 0·89) with only one exceptional branch (PP 0·30), that linking Doryanthaceae (PP 1·00), Ixiolirionaceae and Tecophilaceae (PP 1·00). Also Xanthorrhoaceae *s.l.* (PP 1·00) were sister to the core asparagoids (Fig. 3); Xeronemataceae were sister to Xanthorrhoaceae *s.l.* plus core asparagoids.

DISCUSSION

The tree topology in Asparagales from analysis of three genes is nearly congruent with those of previous analyses, although this study used Orchidaceae as the only outgroup (Chase *et al.*, 1995*a*, *b*; Rudall *et al.*, 2000; Fay *et al.*, 2000; Pires *et al.*, 2006). The overall results produced here, with different accesissions of species and a different set of taxa, indicate that the tree topologies from the previous studies are robust with respect to the samples used to represent genera and the taxa sampled. The core asparagoid clade was strongly supported, and the tree topology of the asparagoids characterized by simultaneous microsporogenesis and inferior ovaries, is congruent with the previous analyses and has strong support (Figs 2 and 3; Chase *et al.*, 1995*a*; Fay *et al*, 2000; Pires *et al.*, 2006). The family composition of the core asparagoids is the same as that in APG (1998) and characterized by a reversal to successive microsporogenesis, although there are a few parallel occurrences in Xanthorrhoeaceae and Hypoxidaceae (Rudall *et al.*, 1997). In this study, the core asparagoids was split into two subclades: (1) Ruscaceae *s.l.* + Asparagaceae *s.s.* + Laxmanniaceae + Alliaceae *s.l. sensu* APG II; and (2) Agavaceae *s.l. sensu* APG II + Hesperocallidaceae + Hyacinthaceae + Themidaceae with Aphyllanthaceae. These two major clades differ from the two identified in the study by Pires *et al.* (2006), upon which the APG III set of families was based (see below for more discussion). The present study also supports Xanthorrhoeaceae *s.l. sensu* APG III as sister to all core asparagoids.

The most variable gene was *matK*, and 18S rDNA exhibited the lowest level of variation. The variable positions in the two plastid DNA genes changed twice as fast as those in 18S rDNA. The topologies exhibited similar patterns in the asparagoids for each analysis from three genes separately (not shown) as in the previous combined analyses (Rudall *et al.*, 2000; Fay *et al.*, 2000).

Phylogenetics of Ruscaceae s.l. *and related families*

Ruscaceae *s.l.* are a recently recognized family (APG, 1998; Rudall *et al*, 2000), which can be distinguished by the absence of phytomelan in the seed coat and indehiscent or berry-like fruits (Rudall *et al.*, 2000). Ruscaceae *s.l.* represent a well-supported clade in DNA alone (Fay *et al.*, 2000; Pires *et al.*, 2006) and combined DNA–morphological analyses

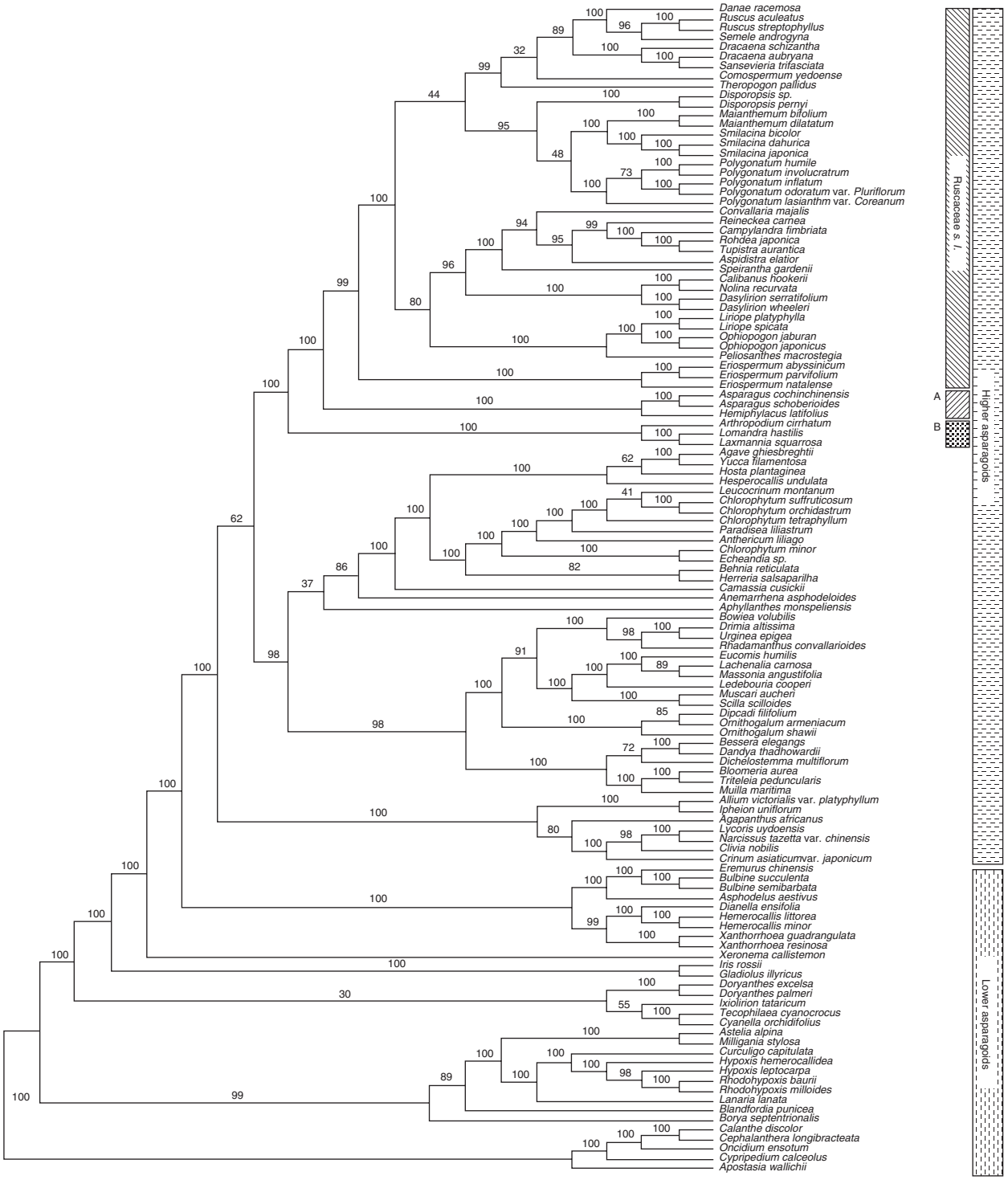

FIG. 3. Bayesian tree from combined DNA analysis (analysis C) for 121 taxa of Asparagales. The numbers above branches are posterior probabilities from 5×10^6 generations with the GTR + I + G model. A, Asparagaceae; B, Laxmanniaceae.

(Rudall *et al.*, 2000). This study strongly supports monophyly of Ruscaceae *s.l.* (BP 90 from plastid DNA alone, and BP 88 from the combined data). Asparagaceae *s.s.* were monophyletic (BP 100, plastid; BP 100, combined data), and the sister group to Ruscaceae *s.l.* (BP 90, plastid; BP 93, combined) was Laxmanniaceae, as in previous analyses (Fay *et al.*, 2000; Pires *et al.*, 2006). The clade with Ruscaceae *s.l.*, Asparagaceae and Laxmanniaceae was sister to Amaryllidaceae *s.l.* (APG III, 2009), including Alliaceae, Amaryllidaceae *s.s.* and Agapanthaceae. This set of relationships, particularly with respect to the position of Amaryllidaceae *s.l.*, was a little different from previous

TABLE 2. *Parameters of models for each gene as estimated by MrModeltest 2·1*

Parameters*	*rbc*L	*mat*K	18S rDNA
r(G ↔ T)	1	1	1
r(C ↔ T)	4·0874	2·9017	11·9400
r(C ↔ G)	1·1394	0·9554	0·4848
r(A ↔ T)	0·4645	0·2741	2·1859
r(A ↔ G)	2·6483	3·2593	1·9161
r(A ↔ C)	0·8335	1·7435	0·9482
freqA	0·2854	0·3060	0·2573
freqC	0·1900	0·1488	0·2134
freqG	0·2278	0·1425	0·2724
freqT	0·2968	0·4062	0·2569
Shape	0·7818	1·1016	0·5748
Pinvar	0·5254	0·0879	0·6916

* *r*(N ↔ N), Substitution rates for each nucleotide pair; freqA, freqC, freqG, freqT, empirical base frequency; Shape, gamma distribution shape parameter; Pinvar, proportion of invariable sites.

results. However, the relationships identified here were only moderately supported and contradicted by Pires *et al.* (2006), who found Asparagaceae *s.l.* (*sensu* APG II) to be sister to Amaryllidaceae *s.l.*; the core asparagoids were thus composed of three clades in the strict consensus tree (not shown) of the equally weighted analysis of the plastid DNA data. Yamashita and Tamura (2000) suggested that the outgroups for Convallariaceae were *Eriospermum*, *Aphyllanthes* and former Anthericaceae genera in their *trnK* region analyses, but the present study shows that *Aphyllanthes* and Anthericaceae have a more remote relationship to that family than Asparagaceae *s.s.*

Aphyllanthes has been a problem taxon in core asparagoid phylogenetics. In this study *Aphyllanthes* was found to be sister to Themidaceae (BP 100) in both MP analyses, and this combined clade (77/81 BP) of *Aphyllanthes* and Themidaceae was the sister to Hyacinthaceae (100/100 BP) and Agavaceae *s.l.* (94/94 BP). Also, in the present MP analyses that excluded *Aphyllanthes* (results not shown) there was no change in tree topology and a small increase in internal support, but in the Bayesian tree (Fig. 3) *Aphyllanthes* was sister to Anemarrhenaceae in Agavaceae *s.l.*, although this result was weakly supported (37 PP). Further detailed studies are required to establish the phylogenetic relationships of *Aphyllanthes*. If Asparagaceae *s.l.* is recognized as in APG III (2009), then at least the problem becomes one of within-family phylogenetics.

Phylogenetics within Ruscaceae s.l.

Although Asparagales were established with phytomelaneous seeds as the synapomorphic character by Huber (1969), Ruscaceae *s.l.*, which have non-phytomelaneous seeds, were controversially included within the core asparagoids that exhibit successive microsporogenesis. Most taxa in Ruscaceae *s.l.* have several additional synapomorphies, such as articulate pedicels, septal nectaries and berries. Chase *et al.* (1995*a*) first mentioned the expanded range of taxa in Ruscaceae *s.l.* including Convallariaceae *s.s.*, Ruscaceae *s.s.*, Nolinaceae, Dracaenaceae, Eriospermaceae and *Comospermum*, and this group of taxa was treated as Convallariaceae *s.l.* in some papers (Rudall *et al.*, 1997; APG, 1998; Fay *et al.*, 2000), but Ruscaceae has priority (Rudall *et al.*, 2000).

This study confirmed the monophyly of Ruscaceae *s.l.* with strong support (BP 90, Fig. 1), including Eriospermaceae (BP 100, Figs 1 and 2; Rudall *et al.*, 2000). Based on the combined three-gene analyses, Ruscaceae *s.l.* consist of six subclades: (1) Polygonateae (excluding *Disporopsis*), (2) Ophiopogoneae, (3) Convallarieae (excluding *Theropogon*), (4) Ruscaceae *s.s.* + Dracaenaceae + *Theropogon* + *Disporopsis* + *Comospermum*, (5) Nolinaceae and (6) *Eriospermum*. This result corresponds with that of Rudall *et al.* (2000): (1) *Eriospermum*, (2) *Comospermum*, (3) nolinoids (Nolinaceae, Ophiopogoneae except *Peliosanthes*), (4) dracaenoids (Dracaenaceae), (5) Polygonateae, (6) Convallarieae with ruscoids (Ruscaceae *s.s.*) and *Peliosanthes*. Yamashita *et al.* (2000) also found six groups: (1) Polygonateae, (2) Ophiopogoneae, (3) Convallarieae, (4) Nolinaceae, (5) Ruscaceae (with Dracaenaceae) and (6) *Comospermum.* Only *Eriospermum* and Polygonateae were consistent in the results from all three sets of analyses.

Within the Ruscaceae *s.l.* clade, Eriospermaceae (BP 100/PP 1·00), Nolinaceae (BP 100/PP 1·00), Ruscaceae *s.s.* (BP 100/PP 1·00) and Dracaenaceae (BP 100/PP 1·00) were well supported. However, Convallariaceae are paraphyletic (Figs 1 and 2) as in previous studies (Rudall *et al.*, 2000; Yamashita and Tamura, 2000; Jang and Pfosser, 2002). If Convallariaceae is to be recognized, it should be recircumscribed; this result has been well supported by the results of molecular and combined molecular and morphological data (Chase *et al.*, 1995*b*; Rudall *et al.*, 1997; Fay *et al.*, 2000; Rudall *et al.*, 2000; Yamashita *et al.*, 2000; Tamura and Yamashita, 2004).

Relationships of Ophiopogoneae. In this study, Ophiopogoneae (BP 98/PP 100) were the only monophyletic tribe among the four previously recognized in Convallariaceae. Ophiopogoneae share hypodermal fibres and well-developed fruits with a thin, papery pericarp and fleshy seeds (Conran and Tamura, 1998). The leaf epidemal cells are ridged and sculptured with the subsidary cells surrounding the guard cells in *Liliope* and *Ophiopogon*, and flowers are perigynous in *Ophiopogon* and *Peliosanthes* (Cutler, 1992).

Polygonateae. Monophyly of Polygonateae has been supported in previous studies (Rudall *et al.*, 2000; Yamashita *et al.*, 2000; Tamura and Yamashita, 2004). Polygonateae share sympodial rhizomes, elongate stems and broad leaves relative to those of Ophiopogoneae. Their chromosome numbers and karyotypes are diverse: *Polygonatum*, $x = 9–15$; *Heteropolygonatum*, $x = 16$; *Maianthemum* (including *Smilacina*, $x = 18$); *Disporopsis*, $x = 20$. It was reported recently that variation in chromosome numbers of Polygonateae was derived from an ancestral basic one ($x = 19$) in Ruscaceae *s.l.* (Yamashita and Tamura, 2004). Polygonateae including *Disporopsis* was strongly supported as monophyletic in Bayesian tree (PP 0·95; Fig. 3).

Smilacina was treated within *Maianthemum* by LaFrankie (1985*a*, *b*; 1986), and many studies have agreed with combining these two genera (Conran and Tamura, 1998; Yamashita

et al, 2000; Rudall *et al.*, 1997; 2000; Shinwari, 2000). The two genera exhibit several distinguishing characters. For example, *Smilacina* has trimerous flowers, multiple (>6) leaves, and adventitious roots from both nodes and internodes of the rhizome, whereas *Maianthemum* has dimerous flowers, 2–5 leaves and adventitious roots only from the internodes of the rhizome. Kim and Lee (2007) also proposed to merge the two genera based on analyses of the *trnK* data (including *matK*). We also agree with the previous studies that proposed their merger (LaFrankie, 1985*a*; Conran and Tamura, 1998; Yamashita *et al*, 2000; Rudall *et al.*, 1997, 2000; Shinwari, 2000), but more intensive studies including distributional diversity and more samples are needed to elucidate this relationship more clearly.

Convallarieae clade. Dahlgren *et al.* (1985) divided Convallariaceae into four tribes, Polygonateae, Ophiopogoneae, Convallarieae and Aspidistreae, but they did not suggest any obvious characteristics to delimit Convallarieae relative to Aspidistreae. After Dahlgren *et al.* (1985), most of studies treated Convallariaceae as composed of three tribes and merged Aspidistreae with Convallarieae (Conran and Tamura, 1998; Yamashita *et al.* 2000; Rudall *et al*, 2000), which was supported here. *Theropogon* differs from Convallarieae in anatomical features (Utech, 1979), basic chromosome number and floral morphology. Rudall *et al.* (2000) mentioned close relationships of Convallarieae, Ruscaceae *s.s.* and *Peliosanthes*, but Ruscaceae *s.s.* and *Peliosanthes* are different in their basic chromosome numbers ($x = 20$ and $x = 18$, respectively) and septal nectaries from Convallarieae. Also, *Peliosanthes* is included in Ophiopogoneae, which have some special fruit features and perigynous flowers. Convallarieae/Aspidistreae have several synapomorphies such as basic chromosome numbers ($x = 19$), monopodial rhizomes and shoots and non-septal nectaries (Dahlgren *et al.*, 1985; Tamura, 1995). In the Convallariae/Aspidistreae clade, *Campylandra*, *Rohdea*, *Tupistra*, *Aspidistra*, *Convallaria* and *Speirantha* formed a group (BP 96/PP 0·96), but the genera are not monophyletic.

Ruscaceae s.s. + *Dracaenaceae* + Theropogon + Comospermum *clade.* The close relationships of Ruscaceae *s.s.*, Dracaenaceae and *Comospermum* have been found in previous studies (Tamura, 1995; Rudall *et al.*, 1997), which all have tenuinucellate parietal cells and the same basic chromosome number ($x = 20$). The basic chromosome numbers of *Theropogon* ($x = 19$) differs from Convallarieae and Polygonateae, and it has septal nectaries, otherwise found only in Convallarieae. Additional molecular and morphological studies should be pursued to resolve the phylogenetic problems and controversies concerning relationships of *Theropogon*.

Nolinaceae clade. It has been previously reported that Nolinaceae have a close relationship with Dracaenaceae. They were often treated in tribe Dracaeneae (Bentham and Hooker, 1883) or Nolineae (Krause, 1930). Recently several studies suggested that they are close to Dracaenaceae and Convallariaceae (Bogler and Simpson, 1995, 1996), particularly Ophiopogoneae, even though there are no obvious morphological characters to support this (Rudall *et al.*, 2000). Nolinaceae are sister to Convallariaceae–Ruscaceae *s.s.*–Dracaenaceae (BP 100) in the MP tree but sister to Convallariae/Aspidistreae alone with BA (PP 0·96).

Eriospermum clade. Eriospermum, endemic to southern Africa, is strongly supported (BP 100/PP 1·00) as sister to Ruscaceae *s.l.* (BP 100/PP 0·99; Figs 2 and 3). In previous studies, *Eriospermum* with *Aphyllanthes* were close to Ruscaceae *s.l.* (Rudall *et al.*, 1997; Fay *et al.*, 2000) or proposed to be included in Convallariaceae (Yamashita and Tamura, 2000). However, Jang and Pfosser (2002) suggested *Aphyllanthes* should go with Anthericaceae and *Eriospermum* should be included in Ruscaceae *s.l.*; *Eriospermum* and Ruscaceae *s.l.* share many characters such as seeds without phytomelan, articulate peduncles and septal nectaries, but *Eriospermum* differs in its seed trichomes, special leaf appendages, large ovules and oily perisperm (Dahlgren *et al.*, 1985; Lu, 1985). The phylogenetic position of *Eriospermum* seems secure; it shares many of the traits of Ruscaceae *s.l.* Little is gained by recognizing it as a family on its own.

Conclusions

This study with different taxon sampling and different species representing genera than in previous phylogenetic studies documents the stability of relationships within Asparagales. Moreover, a better-supported topology for relationships within Ruscaceae (Nolinoideae of Asparagaceae *sensu* APG III, 2009) than in any previous study is provided here, and it is documented that there are still subjects for more detailed future studies of genera and tribes in this clade. The higher-level relationships (interfamilial) found in this study are not totally in agreement with other broad studies (e.g. Pires *et al.*, 2006), in particular the parsimony analysis in this study does not find support for the broader circumscription of Asparagaceae *sensu* APG III. Amaryllidaceae *s.l.* are supported, but in this study Asparagaceae *s.l.* are paraphyletic to Amaryllidaceae *s.l.* However, this set of relationships is not strongly supported. In contrast, the Bayesian analysis found that Asparagaceae *s.l.* were sister to Amaryllidaceae *s.l.* but with PP < 95. All other aspects of the higher-level relationships within Asparagales are similar to those found previously. We intend to collect more data to evaluate this disagreement in greater detail and also to investigate relationships in Ruscaceae further by increasing both taxa and numbers of loci.

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LITERATURE CITED

APG (Angiosperm Phylogeny Group). 1998. An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden* **18**: 531–553.

APG II. 2003. An update of the Angiosperm Phylogeny Group Classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* **141**: 399–436.

APG III. 2009. An update of the Angiosperm Phylogeny Group Classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161**: 105–121.

Arber A. 1924. *Danae, Ruscus* and *Semele*: a morphological study. *Annals of Botany* **38**: 229–260.

Bentham G, Hooker JD. 1883. Liliaceae. *Genera Plantarum*. London: Reeve.

Bogler DJ, Simpson BB. 1995. A chloroplast DNA study of the Agavaceae. *Systematic Botany* **20**: 191–205.

Bogler DJ, Simpson BB. 1996. Phylogeny of Agavaceae based on ITS rDNA sequence variation. *American Journal of Botany* **83**: 1225–1235.

Bogler DJ, Pires JC, Francisco-Ortega J. 2006. Phylogeny of Agavaceae based on *ndhF, rbcL* and ITS sequences: implications of molecular data for classification. In: Columbus JT, Friar EA, Hamilton CW, Porter JM, Prince LM, Simpson MG eds. *Monocots: comparative biology and evolution*. Claremont, CA: Rancho Santa Ana Botanic Garden, 313–328.

Carpenter JM. 1988. Choosing among multiple equally parsimonious cladograms. *Cladistics* **4**: 291–296.

Chase MW, Duvall MR, Hills HG, *et al*. 1995*a*. Molecular phylogenetic of Lilianae. In: Rudall PJ, Cribb PJ, Cutler DF, Humphries CJ eds. *Monocotyledons: systematics and evolution*. Richmond: Royal Botanic Gardens, Kew, 109–137.

Chase MW, Stevenson DW, Wilkin P, Rudall PJ. 1995*b*. Monocots systematics: a combined analysis. In: Rudall PJ, Cribb PJ, Cutler DF, Humphries CJ eds. *Monocotyledons: systematics and evolution*. Richmond: Royal Botanic Gardens, Kew, 685–730.

Chase MW, Rudall PJ, Conran JG. 1996. New circumscription and a new family of asparagoid lilies: genera formerly included in Anthericaceae. *Kew Bulletin* **51**: 667–680.

Chase MW, Rudall PJ, Conran JG. 1997. Validation of the family name Boryaceae. *Kew Bulletin* **52**: 416.

Chase MW, de Bruijn AY, Cox AV, *et al*. 2000*a*. Phylogenetics of Asphodelaceae (Asparagales): an analysis of plastid *rbcL* and *trnL-F* DNA sequences. *Annals of Botany* **86**: 935–951.

Chase MW, Solitis DS, Soltis PS, *et al*. 2000*b*. Higher-level systematics of the monocotyledons: an assessment of current knowledge and a new classification. In: Wilson KL, Morrison DA eds. *Monocots, systematics and evolution*. Melbourne: CSIRO, 3–16.

Chase MW, Fay MF, Devey DS, *et al*. 2006. Multigene analyses of monocot relationships: a summary. In: JT Columbus, Friar EA, Hamilton CW, Porter JM, Prince LM, Simpson MG eds. *Monocots: comparative biology and evolution*. Claremont, CA: Rancho Santa Ana Botanic Garden, 63–75.

Chupov VS, Cutjavina NG. 1980. Phylogeny of some groups of Liliales based on the data of serological analysis. In: Zhilin SG ed. *Systematics and evolution of higher plants*. Leningrad: Nauka, 101–110.

Clarkson JJ, Knapp S, Garcia VF, Olmstead RG, Leitch AR, Chase MW. 2004. Phylogenetic relationships in *Nicotiana* (Solanaceae) inferred from multiple plastid DNA regions. *Molecular Phylogenetics and Evolution* **33**: 75–90.

Conran JG. 1989. Cladisitic analyses of some net-veined Liliiflorae. *Plant Systematics and Evolution* **168**: 123–141.

Conran JG. 1998. Aphyllanthaceae. In: Kubitzki K ed. *The families and genera of vascular plants*, Vol. 3. Berlin: Springer-Verlag.

Conran JG, Tamura MN. 1998. Convallariaceae. In: Kubitzki K ed. *The families and genera of vascular plants*, Vol. 3. Berlin: Springer-Verlag.

Conran JG, Chase MW, Rudall PJ. 1997. Two new monocot families: Anemarrhenaceae and Behniaceae (Lilianae: Asparagales). *Kew Bulletin* **52**: 995–999.

Cooney-Sovetts C, Sattler R. 1986. Phylloclade development in Asparagaceae: an example of homoeosis. *Botanical Journal of the Linnean Society* **94**: 327–371.

Cutler DF. 1992. Vegetative anatomy of Ophiopogoneae (Convallariaceae). *Botanical Journal of the Linnean Society* **110**: 385–419.

Dahlgren RMT, Clifford HT, Yeo PF. 1985. *The families of the monocotyledons*. Berlin: Springer-Verlag.

Devey DS, Leitch I, Rudall PJ, Pires JC, Pillon Y, Chase MW. 2006. Systematics of Xanthorrhoeaceae *sensu lato*, with an emphasis on *Bulbine*. In: JT Columbus, Friar EA, Hamilton CW, Porter JM, Prince LM, Simpson MG eds. *Monocots: comparative biology and evolution.* Claremont, CA: Rancho Santa Ana Botanic Garden, 345–351.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissues. *Phytochemical Bulletin* **19**: 11–15.

Farris JS. 1989. The retention index and the rescaled consistency index. *Cladistics* **5**: 417–419.

Fay MF, Chase MW. 1996. Resurrection of Themidaceae for *Brodiaea* alliance and recircumscription of Alliaceae, Amaryllidaceae and Agapanthoideae. *Taxon* **45**: 441–451.

Fay MF, Rudall PJ, Sullivan S, *et al*. 2000. Phylogenetic studies of Asparagales based on four plastid DNA regions. In: Wilson KL, Morrison DA eds. *Monocots: systematics and evolution*. Melbourne: CSIRO, 360–371.

Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783–791.

Fitch WM. 1971. Toward defining the course of evolution: minimum change for a specific tree topology. *Systematic Zoology* **20**: 406–416.

Furness CA, Rudall PJ. 1999. Microsporogenesis in monocotyledons. *Annals of Botany* **84**: 475–499.

Givnish TJ, Pires JC, Graham SW, *et al*. 2006. Phylogenetic relationships of monocots based on the highly informative plastid gene *ndhF*: evidence for widespread concerted convergence. In: Columbus JT, Friar EA, Hamilton CW, Porter JM, Prince LM, Simpson MG eds. *Monocots: comparative biology and evolution.* Claremont, CA: Rancho Santa Ana Botanic Garden, 28–51.

Graham SW, Zgurski JM, McPherson MC, *et al*. 2006. Robust inference of monocot deep phylogeny using an expanded multigene plastid data set. In: Columbus JT, Friar EA, Hamilton CW, Porter JM, Prince LM, Simpson MG eds. *Monocots: comparative biology and evolution*. Claremont, CA: Rancho Santa Ana Botanic Garden, 3–21.

Hilu K, Borsch DT, Muller K, *et al*. 2003. Angiosperm phylogeny based on *matK* sequence information. *American Journal of Botany* **90**: 1758–1776.

Huber H. 1969. Die Samenmerkale und Verwandtschaftsverhältnisse der Liliifloren. *Mitteilungen der Botanischen Staatssammlung München* **8**: 219–538.

Hutchinson J. 1934. *The families of flowering plants*. Vol. 2. *Monocotyledons*. Oxford: Clarendon Press.

Jang CG, Pfosser M. 2002. Phylogenetics of Ruscaceae *sensu lato* based on plastid *rbcL* and *trnL-F* DNA sequences. *Stapfia* **80**: 333–348.

Johnson LA, Soltis DE. 1995. Phylogenetic inference in Saxifragaceae *sensu lato* and *Gilla* (Polemoniaceae) using *matK* sequences. *Annals of the Missouri Botanical Garden* **82**: 149–175.

Kelchner SA. 2000. The evolution of non-coding chloroplast DNA and its application in plant systematics. *Annals of the Missouri Botanical Garden* **87**: 482–498.

Kim S-C, Lee NS. 2007. Generic delimitation and biogeography of *Maianthemum* and *Smilacina* (Ruscaceae *sensu lato*): preliminary results based on partial 3′ *matK* gene and *trnK* 3’ intron sequences of cpDNA. *Plant Systematics and Evolution* **265**: 1–12.

Krause K. 1930. Liliaceae. In: Engler A, Prantl K eds. *Die Natürlichen Pflanzanfamilien*, Vol. 15a. Leipzig: Engelmann, 227–386.

LaFrankie JV. 1985*a*. *Morphological and systematic studies in the genus Smilacina (Liliaceae)*. Ph.D. Dissertation, Harvard University, Cambridge, MA.

LaFrankie JV. 1985*b*. A note on seedling morphology and establishment growth in the genus *Smilacina* (Liliaceae). *Bulletin of the Torrey Botanical Club* **112**: 313–317.

LaFrankie JV. 1986. Transfer of species of *Smilacina* to *Maianthemum* (Liliaceae). *Taxon* **35**: 584–589.

Lu AM. 1985. Embryology and possible relationships of *Eriospermum. Nordic Journal of Botany* **5**: 229–240.

Maddison WP, Maddison DR. 2000. *MacClade: analysis of phylogeny and character evolution. ver. 4·0*. Sunderland, MA: Sinauer Associates.

Nickrent DL, Soltis DE. 1995. A comparison of angiosperm phylogenies from nuclear 18S rDNA and *rbcL* sequences. *Annals of the Missouri Botanical Garden* **82**: 208–234.

Nylander JAA. 2005. MrModeltest ver. 2·2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala.

Olmstead RG, Michaels HJ, Scott KM, Palmer JD. 1992. Monophyly of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbcL. Annals of the Missouri Botanical Garden* **79**: 249–265.

Pires JC, Maureira IJ, Givnish TJ, *et al*. 2006. Phylogeny, genome size, and chromosome evolution of Asparagales. In: Columbus JT, Friar EA, Hamilton CW, Porter JM, Prince LM, Simpson MG eds. *Monocots:*

comparative biology and evolution. Claremont, CA: Rancho Santa Ana Botanic Garden, 287–304.

Ronquist F, Huelsenbeck JP, van der Mark P. 2005. *MrBayes 3·1 Manual*. http://mrbayes.csit.fsu.edu/manual.php.

Rudall PJ, Campbell G. 1999. Flower and pollen structure of Ruscaceae in relation to Aspidistreae and other Convallariaceae. *Flora* **194**: 201–214.

Rudall PJ, Chase MW. 1996. Systematics of Xanthorrhoeaceae *sensu lato*: evidence for polyphyly. *Telopea* **6**: 629–647.

Rudall PJ, Furness CA, Chase MW, Fay MF. 1997. Microsporogenesis and pollen sulcus type in Asparagales (Lilianae). *Canadian Journal of Botany* **75**: 408–430.

Rudall PJ, Engelman EM, Hanson L, Chase MW. 1998. Systematics of *Hemiphylacus, Anemarrhena* and *Asparagus*. *Plant Systematics and Evolution* **211**: 181–199.

Rudall PJ, Conran JG, Chase MW. 2000. Systematics of Ruscaceae/Convallariaceae: a combined morphological and molecular investigation. *Botanical Journal of the Linnean Society* **134**: 73–92.

Sato D. 1942. Karyotype alteration and phylogeny in Liliaceae and allied families. *Japanese Journal of Botany* **12**: 57–161.

Sharma AK, Chaudhuri U. 1964. Cytological studies as an aid in assessing the status of *Sansevieria*, *Ophiopogon* and *Curculigo*. *Nucleus* **7**: 43–58.

Shinwari ZK. 2000. Chloroplast DNA variation in Polygonatae *sensu lato* (Liliaceae). *Pakistan Journal of Botany* **32**: 7–14.

Shinwari ZK, Kato H, Terauchi R, Kawano S. 1994. Phylogenetic relationships among genera in the Liliaceae-Asparagoideae-Polygonatae *s.l.* inferred from *rbcL* gene sequence data. *Plant Systematics and Evolution* **192**: 263–277.

Soltis DE, Soltis PS, Nickrent DL, *et al*. 1997. Angiosperm phylogeny inferred from 18S ribosomal DNA sequences. *Annals of the Missouri Botanical Garden* **84**: 1–49.

Soltis DE, Soltis PS, Chase MW, *et al*. 2000. Angiosperm phylogeny inferred from 18S rDNA, *rbcL*, and *atpB* sequences. *Botanical Journal of the Linnean Society* **133**: 381–461.

Soltis PS, Soltis DE. 1998. Molecular evolution of 18S rDNA in angiosperms: implications for character weighting in phylogenetic analysis. In: Soltis DE, Soltis PS, Doyle JJ eds. *Molecular systematics of plants. II. DNA sequencing*. Boston, MA: Kluwer, 188–210.

Swofford DL. 2007. *PAUP*: phylogenetic analysis using parsimony, ver. 4·10b*. Sunderland, MA: Sinauer Associates.

Takhtajan AL. 1997. *Diversity and classification of flowering plants*. New York, NY: Columbia University Press.

Tamura MN. 1995. A karyological review of the orders Asparagales and Liliales (Monocotyledonae). *Feddes Report* **106**: 83–111.

Tamura MN, Yamashita J. 2004. Molecular phylogeny of monocotyledons inferred from combined analysis of plastid *matK* and *rbcL* gene sequences. *Journal of Plant Research* **117**: 109–120.

Thomson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**: 4876–4882.

Utech FH. 1979. Floral vascular anatomy of the Himalayan *Theropogon pallidus* Maxim. (Liliaceae-Convallarieae). *Annals of Carnegie Museum* **48**: 25–41.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ eds. *A guide to methods and applications*. New York, NY: Academic Press, 315–322.

World Checklist of Selected Plant Families. 2008. Published by the Board of Trustees of the Royal Botanic Gardens, Kew, on the Internet (http://www.kew.org/wcsp/), accessed in January 2008.

Yamashita J, Tamura MN. 2000. Molecular phylogeny of the Convallariaceae (Asparagales). In: Wilson KL, Morrison DA eds. *Monocots: systematics and evolution*. Melbourne: CSIRO, 387–400.

Yamashita J, Tamura MN. 2004. Phylogenetic analyses and chromosome evolution in Convallarieae (Ruscaceae *sensu lato*), with some taxonomic treatments. *Journal of Plant Research* **117**: 363–370.

APPENDIX

Voucher data and GenBank accession numbers for Ruscaceae *s.l.* and related groups in the order Asparagales. Order and family circumscriptions are as in APG (1988) with slight modification (Chase *et al.*, 2000). Names with asterisks are the family circumscriptions of Dahlgren *et al.* (1985).

Family/Tribe	Taxon	Source/voucher	Origin/distribution	18S	*matK*	*rbcL*
Agapanthaceae	*Agapanthus africanus*	*Chase 627* (K)	SW Cape, S Africa	HM640715	HM640599	HM640485
Agavaceae	*Agave ghiesbreghtii*	*Chase 3467* (K)	Mexico, N and C America	HM640709	HM640592	HM640478
	Yucca filamentosa	*DK Kim 06-077* (TUT)	E USA, N America	HM640713	HM640596	HM640482
	Leucocrinum montanum	*Chase 795* (K)	S USA. N America	HM640712	HM640595	HM640481
	Hosta plantaginea	*JX Feng s.n.* (HZU)	Hangzhou, China, E Asia	HM640711	HM640594	HM640480
Alliaceae	*Allium victorialis* var. *platyphyllum*	*DK Kim 04-142* (TUT)	Korea, E Asia	HM640714	HM640597	HM640483
	Ipheion uniflorum	*Murakami 631* (KYO)	N Argentina, S America	–	HM640598	HM640484
Amaryllidaceae	*Lycoris uydoensis*	*DK Kim 05-102* (TUT)	Korea, E Asia	HM640716	HM640600	HM640486
	Narcissus tazetta var. *chinensis*	*DK Kim 06–167* (TUT)	W Mediterranean	HM640717	HM640601	HM640487
	Crinum asiaticum var. *japonicum*	*GH Tae s.n.* (TUT)	Korea, E Asia	HM640718	HM640602	HM640488
	Clivia nobilis	*Chase 3080* (K)	E Cape, S Africa	AF206889	HM640603	Chase *et al.*, 2006
Anemarrhenaceae*	*Anemarrhena asphodeloides*	*TCMK 312* (K)	Korea, NE Asia	HM640719	HM640604	HM640489
Anthericaceae*	*Anthericum liliago*	*Chase 515* (K)	N, C and S Europe	HM640720	HM640605	HM640490
	Chlorophytum minor	*BY Ding s.n.* (KUN)	Zambia, Africa	HM640721	HM640606	HM640491
	Chlorophytum suffruticosum	*Chase 1043* (K)	E Africa	HM640723	HM640608	HM640493

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APPENDIX *Continued*

Family/Tribe	Taxon	Source/voucher	Origin/distribution	18S	*matK*	*rbcL*
	Chlorophytum orchidastrum	*Chase 2155* (K)	W and C Africa	HM640722	HM640607	HM640492
	Chlorophytum tetraphyllum	*Chase 1044* (K)	Ethiopia, N Africa	HM640724	HM640609	L05031
	Comospermum yedoense	*Chase 833* (K)	Japan, E Asia	HM640725	HM640610	HM640494
	Echeandia sp.	*Chase 602* (K)	S and C America	HM640727	HM640612	HM640495
	Paradisea liliastrum	*Chase 826* (K)	Pyrenees, Alps, S Europe	HM640728	HM640613	HM640496
Aphyllanthaceae	*Aphyllanthes monspeliensis*	*Chase 614* (K)	W and C Mediterranean	HM640729	HM640614	Z77259
Asparagaceae	*Asparagus cochinchinensis*	*DK Kim 04–122* (TUT)	Korea, E and SE Asia	HM640730	HM640615	HM640497
	Asparagus schoberioides	*DK Kim 04–165* (TUT)	Korea, NE Asia	HM640731	HM640616	HM640498
	Hemiphylacus latifolius	*Chase 668* (K)	Mexico, N America	HM640732	HM640617	HM640499
Behniaceae	*Behnia reticulata*	*Goldbladtt 9273* (MO)	S and E Africa	HM640733	HM640618	HM640500
Convallariaceae*						
Convallarieae	*Convallaria majalis*	*DK Kim 04–082* (TUT)	Korea, NE Asia, Europe	HM640672	HM640557	HM640443
	Reineckea carnea	*DK Kim 05–008* (TUT)	Korea, E Asia	HM640673	HM640558	HM640444
	Speirantha gardenii	*Chase 495* (K)	SE China	HM640674	HM640559	HM640445
	Theropogon pallidus	*Chase 2933* (K)	SW China, Himalaya	HM640675	HM640560	HM640446
Aspidistreae	*Aspidistra elatior*	*DK Kim 05-013* (TUT)	Korea, E Asia	HM640676	HM640561	HM640447
	Campylandra fimbriata	*Liu Yang 484* (KUN)	Himalaya, NW China	HM640677	HM640562	HM640448
	Rohdea japonica	*DK Kim 05-005* (TUT)	Korea, E Asia	HM640678	HM640563	HM640449
	Tupistra aurantiaca	*Chase 1100* (K)	Yunnan, SW China, E Asia	HM640679	HM640564	HM640450
Polygonateae	*Disporopsis pernyi*	*Chase 493* (K)	S China, E Asia	HM640681	HM640566	HM640452
	Disporopsis sp.	*DK Kim 05-136* (TUT)	Sichuan, China, E Asia	HM640680	HM640565	HM640451
	Maianthemum bifolium	*DK Kim 04-182* (TUT)	Korea, temperate Eurasia	HM640682	HM640567	HM640453
	Maianthemum dilatatum	*DK Kim 04-165* (TUT)	Korea, NE Asia	HM640683	HM640568	HM640454
	Polygonatum humile	*DK Kim 04-029* (TUT)	Korea, C and E Asia	HM640684	HM640569	HM640455
	Polygonatum inflatum	*DK Kim 04-043* (TUT)	Korea, NE Asia	HM640685	HM640570	HM640456
	Polygonatum involucratum	*DK Kim 04-059* (TUT)	Korea, NE Asia	HM640686	HM640571	HM640457
	Polygonatum lasianthum var. *coreanum*	*DK Kim 04-046* (TUT)	Korea, NE Asia	HM640687	HM640572	HM640458
	Polygonatum odoratum var. *pluriflorum*	*DK Kim 04-067* (TUT)	Korea, NE Asia	HM640688	HM640573	HM640459
	Smilacina bicolor	*DK Kim 04-077* (TUT)	Korea, E Asia	HM640689	HM640574	HM640460
	Smilacina dahurica	*DK Kim 04-082* (TUT)	Korea, NE Asia	HM640690	HM640575	HM640461
	Smilacina japonica	*DK Kim 04-039* (TUT)	Korea, NE Asia	HM640691	HM640576	HM640462
Ophiopogoneae	*Liriope platyphylla*	*DK Kim 07-001* (TUT)	Korea, E Asia	HM640692	HM640577	HM640463
	Liriope spicata	*DK Kim 07-002* (TUT)	Japan, E Asia	HM640693	HM640578	HM640464
	Ophiopogon jaburan	*DK Kim 07-004* (TUT)	Korea, E Asia	HM640694	HM640579	HM640465
	Ophiopogon japonicus	*DK Kim 07-003* (TUT)	Korea, E Asia	HM640695	HM640580	HM640466
	Peliosanthes macrostegia	*G Murata 44832* (KYO)	S China, E Asia	HM640696	HM640581	HM640467
Dracaenaceae*	*Dracaena schizantha*	*Chase 21514* (K)	Ethiopia, NE Africa	HM640698	HM640582	HM640469
	Dracaena aubryana	*Chase 1102* (K)	Uganda, WC Africa	HM640699	HM640583	HM640470
	Sansevieria trifasciata	*DK Kim 07-05* (TUT)	Nigeria, WC Africa	HM640700	HM640584	HM640471
Eriospermaceae*	*Eriospermum abyssinicum*	*Chase 2051* (K)	S Africa	HM640706	HM640589	HM640475
	Eriospermum natalense	*Chase 2052* (K)	S Africa	HM640707	HM640590	HM640476
	Eriospermum parvifolium	*Chase 2053* (K)	W Cape, S Africa	HM640708	HM640591	HM640477
Herreriaceae*	*Herreria salsaparilha*	*Chase 2154* (K)	Brazil, S America	HM640734	HM640619	HM640501
Hesperocallidaceae	*Hesperocallis undulata*	*Cranfill & Schmid s.n.* (JEPS)	SW USA, N America	HM640735	HM640620	HM640502
Hyacinthaceae*	*Bowiea volubilis*	*Chase 176* (K)	Uganda, C and S Africa	HM640736	HM640621	HM640503
	Camassia cusickii	*Cronquist 6549* (RSA)	C USA, N America	HM640710	HM640593	HM640479
	Dipcardi filifolium	*Chase 1783* (K)	C Asia, Africa, India	HM640737	HM640622	HM640504
	Drimia altissima	*Chase 1870* (K)	C and S Africa	HM640738	HM640623	HM640505
	Eucomis humilis	*Chase 1847* (K)	Lesotho, S Africa	HM640739	HM640624	HM640506
	Lachenalia carnosa	*Chase 2261* (K)	W Cape, S Africa	HM640740	HM640625	HM640507
	Ledebouria cooperi	*Chase 1786* (K)	S Africa	HM640741	HM640626	HM640508
	Massonia angustifolia	*Chase 5666* (K)	Cape, S Africa	HM640742	HM640627	HM640509
	Muscari aucheri	*Chase 21845* (K)	Turkey, Med. to Caucasus	HM640743	HM640628	HM640510
	Ornithogalum armeniacum	*Chase 1682* (K)	Turkey to Macedonia	HM640744	HM640629	HM640511
	Ornithogalum shawii	*Chase 1012* (K)	S Africa	HM640745	HM640630	HM640512
	Rhadamanthus convallarioides	*Goldblatt 10852* (A)	Cape, S Africa	HM640746	HM640631	HM640513
	Scilla scilloides	*DK Kim 05-039* (TUT)	Korea, E Asia	HM640747	HM640632	HM640514

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APPENDIX *Continued*

Family/Tribe	Taxon	Source/voucher	Origin/distribution	18S	*matK*	*rbcL*
	Urginea epigea	*Chase 2055* (K)	S Africa	HM640748	HM640633	HM640515
Laxmanniaceae	*Arthropodium cirrhatum*	*Chase 651* (NCU)	New Zealand, Australia	HM640749	HM640634	HM640516
	Laxmannia squarrosa	*Chase 2214* (K)	W and S Australia	HM640751	HM640636	HM640518
	Lomandra hastilis	*Brummitt et al. 21239* (K)	W and SW Australia	HM640750	HM640635	HM640517
Nolinaceae*	*Calibanus hookeri*	*Chase 1006* (K)	Mexico, N America	HM640702	HM640585	HM640472
	Dasylirion serratifolium	*Abisai et al., s.n.* (RSA)	Mexico, N America	HM640704	HM640587	AB029847
	Dasylirion wheeleri	*Chase 3469* (K)	Texas, S USA, N America	HM640705	HM640588	HM640474
	Nolina recurvata	*Chase 3466* (K)	Mexico, N America	HM640703	HM640586	HM640473
Ruscaceae*	*Danae racemosa*	*Chase 121* (K)	Turkey, Syria, Iran, Caucasus	HM640668	HM640553	HM640439
	Ruscus aculeatus	*Bohuslavek 1348* (RSA)	W and C Europe, Medit.	HM640669	HM640554	HM640440
	Ruscus streptophyllus	*Chase 21990* (K)	Madeira	HM640670	HM640555	HM640441
	Semele androgyna	*Chase 997* (K)	Canary Is., Madeira	HM640671	HM640556	HM640442
Themidaceae	*Bessera elegans*	*Chase 626* (K)	Mexico, N America	HM640752	HM640637	HM640519
	Bloomeria aurea	*Chase 1010* (K)	SW USA, N America	HM640753	HM640638	HM640520
	Dandya thadhowardii	*Chase s.n.* (K)	Mexico, N America	HM640754	HM640639	HM640521
	Dichelostemma multiflorum	*Chase 1830* (K)	SW USA, N America	HM640755	HM640640	HM640522
	Muilla maritime	*Chase 779* (K)	SW USA to Mexico, N America	HM640757	HM640642	HM640524
	Triteleia peduncularis	*Chase 1860* (K)	California, W USA, N America	HM640758	HM640643	HM640525
Asphodelaceae	*Eremurus chinensis*	*Qing 00317* (KUN)	Tibet to S Gansu, W China	HM640759	HM640644	HM640526
	Asphodelus aestivus	*Chase 482* (K)	Portugal, Spain, SW Europe	HM640760	HM640645	HM640527
	Bulbine semibarbata	*K Dixon, s.n.* (KPBG)	S and E Australia	HM640761	HM640646	HM640528
	Bulbine succulenta	*Chase 5518* (K)	Cape, S Africa	AF206876		Z73684
	Bulbine frutescens	*Chase 9215* (K)	S Africa		AJ511414	
Asteliaceae	*Astelia alpina*	*Chase 1103* (K)	NSW to Tasmania, S Australia	HM640762	HM640648	HM640530
	Milligania stylosa	*Chase 511* (K)	Tasmania, S Australia	HM640763	HM640649	HM640531
Brandfordiaceae	*Brandfordia punicea*	*MRK Rambert 787* (K)	Tasmania, S Australia	HM640764	HM640650	HM640532
Boryaceae	*Borya septentrionalis*	*Chase 2205* (K)	Perth, W Australia	HM640765	HM640651	HM640533
Doryanthaceae	*Doryanthes excelsa*	*Chase 188* (K)	NSW, SE Australia	HM640766	HM640652	HM640534
	Doryanthes palmeri	*Chase 19153* (K)	Queensland, SE Australia	HM640767	HM640653	HM640535
Hemerocallidaceae	*Dianella ensifolia*	*Nakai 5510* (KYO)	Taiwan, SE and Tropical Asia	HM640768	HM640654	HM640536
	Hemerocallis minor	*DK Kim 05-091* (TUT)	Korea, NE Asia	HM640769	HM640655	HM640537
	Hemerocallis littorea	*Chase 3833* (K)	Korea, Japan, E Asia	Chase *et al.*, 2006	AJ581422	AY149364
Hypoxidaceae	*Curculigo capitulata*	*SW Lee 05-001* (TUT)	Yunnan, S Asia to N Australia	HM640770	HM640656	HM640538
	Rhodohypoxis milloides	*Chase 479* (K)	E Cape, S Africa	AF207008	AY368377	Z77280
	Rhodohypoxis baurii	*Chase 16460* (K)	Cape, S Africa	HM640772	HM640658	HM640540
	Hypoxis leptocarpa	*Chase 108* (NCU)	Duke, SE USA, N America	AF135209	AY368375	Z73702
	Hypoxis hemerocallidea	*Chase 1045* (K)	Tropical and S Africa	HM640771	HM640657	HM640539
Iridaceae	*Iris rossii*	*DK Kim 05-048* (TUT)	Korea, NE Asia	HM640773	HM640659	HM640541
	Gladiolus illyricus	*Chase 9907* (K)	Portugal, SW Europe	HM640774		HM640542
	Gladiolus papilio	*Goldblatt & Manning 9841* (MO)	S Africa		AJ579956	
Ixioliriaceae	*Ixiolirion tataricum*	*Chase 489B* (K)	E Turkey to Kashmir, W Asia	HM640775	HM640660	HM640543
Lanariaceae	*Lanaria lanata*	*Goldblatt 9410* (MO)	Cape, S Africa	Chase *et al.*, 2006	AY368376	Z77313
Orchidaceae	*Calanthe discolor*	*DK Kim 05-035* (TUT)	Korea, E Asia	HM640776	HM640665	HM640548
	Cephalanthera longibracteata	*DK Kim 05-016* (TUT)	Korea, NE Asia	HM640777	HM640666	HM640549
	Cypripedium calceolus	*Chase 9484* (K)	Estonia, Europe to Asia	HM640778	HM640667	HM640550
	Oncidium ensatum	*Chase 9671* (K)	Tropical C and S America	HM640779	AY368423	HM640551
	Apostasia wallichii	*Chase 15744* (K)	Sri Lanka, S Asia to N Australia	HM640780	AY557212	HM640552
Tecophilaceae	*Tecophilaea cyanocrocus*	*Chase 447* (K)	Chile, S America	HM640781	HM640661	HM640544

Continued

APPENDIX *Continued*

Family/Tribe	Taxon	Source/voucher	Origin/distribution	18S	*matK*	*rbcL*
	Cyanella orchidiformis	*Chase 5896* (K)	Cape, S Africa	HM640782	HM640662	HM640545
Xanthorrhoeaceae	*Xanthorrhoea resinosa*	*Chase 192* (NCU)	NSW, Australia	HM640783	HM640663	HM640546
	Xanthorrhoea quadrangulata	*Hahn 6978* (WIS)	S Australia	U42064		
		Qiu 97039 (NID)			DQ401345	
		Unvouchered				Z73710
Xeronemataceae	*Xeronema callistemon*	*Chase 653* (K)	Poor Night Is., New Zealand	HM640784	HM640664	HM640547