Networks in a Large-Scale Phylogenetic Analysis: Reconstructing Evolutionary History of Asparagales (Lilianae) Based on Four Plastid Genes

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Abstract

Phylogenetic analysis aims to produce a bifurcating tree, which disregards conflicting signals and displays only those that are present in a large proportion of the data. However, any character (or tree) conflict in a dataset allows the exploration of support for various evolutionary hypotheses. Although data-display network approaches exist, biologists cannot easily and routinely use them to compute rooted phylogenetic networks on real datasets containing hundreds of taxa. Here, we constructed an original neighbour-net for a large dataset of Asparagales to highlight the aspects of the resulting network that will be important for interpreting phylogeny. The analyses were largely conducted with new data collected for the same loci as in previous studies, but from different species accessions and greater sampling in many cases than in published analyses. The network tree summarised the majority data pattern in the characters of plastid sequences before tree building, which largely confirmed the currently recognised phylogenetic relationships. Most conflicting signals are at the base of each group along the Asparagales backbone, which helps us to establish the expectancy and advance our understanding of some difficult taxa relationships and their phylogeny. The network method should play a greater role in phylogenetic analyses than it has in the past. To advance the understanding of evolutionary history of the largest order of monocots Asparagales, absolute diversification times were estimated for family-level clades using relaxed molecular clock analyses.

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Introduction

The only figure in On the Origin of Species [1] is an evolutionary tree that reflects Darwin’s vision of descent with modification from a common ancestor. Today, phylogenetic methods, or “tree-thinking” [2], form the foundation of inferences in evolutionary biology [3–5]. Bifurcating phylogenetic trees underlie our understanding of organismal evolution and are also proving instrumental in the development of a more robust classification system based on natural (evolutionary) relationships. Nevertheless, searches to determine “the tree” remain problematic, as they can often overlook conflicts in the dataset. Competing signals may arise from stochastic substitution processes, poorly fitting evolutionary models or the heuristic nature of many tree search algorithms. They may also be the result of hybridisation (including introgression), recombination, horizontal/lateral gene transfer, genome fusion, ancestral polymorphism/deep coalescence/incomplete lineage sorting and gene duplication-loss [6]. The detection of data conflicts, and the extent to which they affect analysis, becomes an important first step in phylogenetic analysis. Data-display networks may reveal reticulation patterns that are unsuspected in the data and that may have an important bearing on subsequent analyses and their interpretation. Unfortunately, this field is rather poorly developed at present [6,7], and no tools are available that biologists can easily and consistently use on real data [8].

A neighbour net [9] is a split network that visualises certain collections of splits that have been derived from a distance matrix. These splits are constructed in an iterative fashion using a criterion similar to that used in the neighbour-joining (NJ) algorithm for tree construction [6,10]. Morrison [6] reanalysed a dozen published datasets using split networks, highlighting aspects of the resulting network that could be important for interpretation of the phylogenetic tree and pointed out that the network method should play a greater role in phylogenetic analyses than it has in the past.

Asparagales is the largest order of monocots [11–16] with ca. 25,000–42,000 species (ca. 50% of monocots, or 10–15% of flowering plants), including important crop plants such as Allium, Asparagus and Vanilla, and a host of ornamentals such as irises, hyacinths and orchids [17]. The circumscription of Asparagales and the included families have undergone a series of changes in recent years. When the Angiosperm Phylogeny Group (APG) [18] was being formulated, numerous narrow circumscriptions for the
families of Asparagales largely followed those of Dahlgren et al. [19], but it was noted (APG II, 2003) that broader circumscriptions were also possible, leading to a set of sensu lato (s.l.) families being proposed with the earlier set of sensu stricto families listed in brackets. In APG III [20], the number of families in Asparagales recognised fell from 26 [19] to 14 due to the elimination of these bracketed families. Furthermore, a set of subfamilies for the expanded asparagalean families was also published to be more manageable for teaching purposes and to facilitate communication among specialists [21]. A number of studies have sampled all/most families of Asparagales sensu APG [11,14,17,18,22–28], which have generally clarified the relationships among the families within Asparagales. However, uncertainties remain in two parts of the Asparagales phylogenetic tree. First, the exact relationships of some small families (e.g. Boryaceae, Doryanthaceae, Ixioiliaceae) in lower Asparagales and Aphyllanhoideae, in higher Asparagales, remain unresolved [17,22,23]. Previous studies [17,22] found weak support for a sister relationship between Ixioiliaceae and Tecophilaeaceae, which in turn formed a polytomy or weakly supported sister group to Doryanthaceae. An analysis of morphological data, however, placed Doryanthes as sister to Iridaceae [24]. The position of Boryaceae also remains unclear relative to the rest of the families (except for the orchids) and the hypoxid clade [15,23]. The positions of all of these families require additional evidence to establish their interrelationships [15]. Fay et al. [22] demonstrated that Aphyllanhoideae (monotypic, Aphyllanhoideae) has a destabilising position within Asparagaceae s.l. Other studies found that incompatible patterns were produced when analyzing different genes [14,17]. The second problem, related to the extreme species richness, diverse morphology and complex taxonomic history of Asparagales, is that the sampling of taxa in previous studies has been limited, and many genera have not been included. Although it is clear that adding multigene sequences and sampling will produce a better hypothesis of evolutionary history, more incompatibilities could arise. Previous studies have demonstrated that bifurcating phylogenetic trees can be valuable tools for investigating the evolutionary history of Asparagales, but it is not possible to simultaneously display contradictory evolutionary signals on any such tree. Phylogenetic networks can provide a useful alternative means of analysis because they allow visualisation of competing evolutionary scenarios within a single figure [6,29]. Here, we used a phylogenetic network method, neighbour net, to reanalyze the evolutionary history of Asparagales using a new comprehensive sampling of taxa and genes. In addition, using our estimates of the time of origin, we discuss their possible evolutionary history to improve our understanding of the processes that have generated such high diversity on this branch of the tree of life.

Results

Neighbour-net Pattern of the Data

To gain a better understanding how conflicting signals were contained in the datasets, we constructed a neighbour net for the combined matrix of the four plastid genes (Figure 1), in which indeles were not considered as informative characters. The outgroup Pandanales consisting of two species (Pandanales), together with Commelinales and Liliales species, were included as they are closely related to Asparagales [26]. The centre of the neighbour net was slightly netted, implying that the data support many conflicting deep splits. Nonetheless, the clades identified appeared to be quite robust as 21 clades were generally recovered, as indicated by the colours and arc labelling in Figure 1. The neighbour net showed strong support for monophyletic Asparagales. Commelinales, Liliales and Pandanales formed a close clade as the outgroup of Asparagales. The network largely confirmed the current recognised phylogenetic relationships [14,22,28]. In addition, there were strongly supported splits (and clusters), corresponding largely to the well-supported clades in the topology of the combined tree obtained with our parsimony and Bayesian analyses (Figure 2), except Milla biflora, which netted with Orchidaceae. Furthermore, most of the difficult taxa, with conflict position or extremely low resolution from regular phylogenetic analyses, appeared in critical state on the network graph. For example, Orchidaceae competed with Boryaceae and Blandfordiaceae etc. to root of Asparagales in previously researches [12,28,30–32].

Phylogenetic Relationships

The total aligned matrix had 6,862 characters with 3,122 potentially phylogenetically informative sites for the four plastid genes: 1,472 base pairs (bp) for atpB, 1,820 bp for matK, 2,234 bp for ndhF and 1,336 bp for rbcL. In total, 163 base pairs were excluded from the combined matrix (1–17, 1449–1472, 3292–3316, 5480–5560, 6847–6862 bp), either at the beginning or end of sequences or where alignment of the ndhF sequences was ambiguous. Of the included characters, the numbers of potentially parsimony informative characters were 499 (33.9%) for atpB, 1,123 (61.7%) for matK, 1,160 (34.7%) for ndhF and 437 (32.7%) for rbcL. The matK gene was the most variable among the four genes, but gave slightly fewer parsimony informative sites than ndhF due to the longer length of the latter. The rbcL gene was length-conserved with no gaps, and atpB had only few insertions/deletions (indels), whereas matK and ndhF included a number of indels.

Parsimony analyses of the individual plastid genes gave similar topologies as expected because these genes are inherited on the same linkage group. Aphyllanthes L., has previously been discussed as a problem taxon because of its labile phylogenetic position according to the analyses by different genes [17,22]. As in previous analyses, we also performed analyses that excluded and included Aphyllanthes, which only affected position and support values in Asparagaceae s.l. Here we present the results found when Aphyllanthes was included.

The combined data Fitch analysis with equal weights (EW) produced 14,523 equally most-parsimonious trees of 24,168 steps, with a consistency index (CI), including autopomorphies) of 0.27 and a retention index (RI) of 0.73. With successive weights (SW), the number of equally most parsimonious trees was reduced to one (CI = 0.70, RI = 0.85). The SW tree is one of the trees found with Fitch weights. The Bayesian tree shows the PPs summarised from the set of recovered post-burn-in trees. The parameters of the GTR+I+G model used in this analysis are listed in Table 2. There was only one minor area of discordance between the maximum parsimony (MP) and Bayesian trees: the interrelationships among three families: Aphyllanthesae, Themidaceae and Doryanthaceae.

Due to the similarity in topology of the strict consensus parsimonious tree and the Bayesian tree, the latter having higher resolution, only the Bayesian tree found in the combined analysis is shown in Figure 2. We report three kinds of support value: parsimony bootstrap percentages with EW, SW and PP for Bayesian analysis. Pandanales was the nominated outgroup in accordance with the results of previous studies [17,22]. Within Asparagales, SW analysis had more nodes with strong support than EW, and the PP offered strong support for most nodes on the phylogenetic tree (Figure 2).
Asparagales sensu APG (1998) was monophyletic with strong support (92/100/1.0) as sister to the commelinids clade (66/93/0.9). A multiordinal clade, the commelinids monocots as a whole (Arecales, Commelinales-Zingiberales, Poales), was also strongly supported (94/100/1.0). A clade comprising Asparagales and Commelinids was grouped into a sister relationship with the Liliales clade (100/100/1.0). As in previous analyses, the order Asparagales can be divided into higher and lower asparagoid clades (sensu Chase et al. 1995a). However, this concept was recently replaced by that of core and non-core asparagoids [26,33]. The core asparagoids formed a strongly supported monophyletic group containing two well-resolved clades, Asparagaceae s.l. (72/86/1.0) and Amaryllidaceae s.l. (92/97/1.0), which was recognised in APG III (2009). The Asparagaceae s.l. included a number of subfamilies represented by two clades, which was recognised in APG III (2009). The first clade (83/97/1.0) had Lomandroideae as sister to a monophyletic group (70/53/0.99) that consisted of Asparagoideae and Nolinoideae. The second clade (63/91/1.0) consisted of four subfamilies: Agavoideae, Scilloideae, Brodiaeoideae and Aphyllanthoideae. The result also suggested that the family Amaryllidaceae s.l. had two clades: (Amaryllidoideae+Allioideae) and Agapanthoideae. The core asparagoid clade was sister (88/97/1.0) to a strongly supported (97/100/1.0) family Xanthorrhoeaceae s.l. [sensu APG III], which included three subfamily clades: Asphodeloideae, Xanthorrhoeoideae and Hemerocallidoideae. The core asparagoid and Xanthorrhoeaceae s.l. were sister (88/97/1.0) to Xeromnataceae alone. Collectively, this large clade was sister (87/97/1.0) to Iridaceae. The sister relationship between Ixioiriaceae and Tecophilaeaceae had strong support (86/96/1.0), but its position relative to Doryanthaceae remains unclear. However, a clade including Doryanthaceae, Ixioiriaceae, Tecophilaeaceae and the above-mentioned families was strongly supported (88/97/1.0). In turn, this clade was sister (60/<50/1.0) to the asteloid clade that included Boryaceae, Blandfordiaceae, Asteliaceae, Lanariaceae and Hypoxidaceae. The monophyletic Orchidaceae was the first to diverge and was sister to all other asparaguids with high support (92/100/1.0).

Divergence Time Estimation

The mean path lengths (MPL) clock tests [34] revealed significant deviations from clock-like behaviour at most nodes of the tree for Asparagales (clock tests: 265; accepted: 14; rejected: 251). Hence, we used BEAST [35], which implements a “relaxed clock” methodology that does not assume any correlation between rates (thus accounting for lineage-specific rate heterogeneity), to estimate ages and the phylogenetic tree simultaneously. At the same time, we also used PATHd8, with the mean path length method; this programme is faster for a large dataset and permits rate changes across the tree [34]. We obtained slightly younger ages in the results using PATHd8 than using BEAST.

The BEAST analysis that treated fossil priors as lognormal distributions provided an older estimated age (102–143 Ma, data not presented) for crown group of Asparagales than that using an exponential distribution (93–101 Ma), as well as larger variances around age estimates, especially at the base of the tree (also see [36]). The topology showed good agreement with previous...
analyses of these data using Bayesian methods, with a few exceptions (Agavoideae, Scilloideae, Brodiaeoideae and Aphyllanthoideae present in some one clade but in different relatively position). The age estimates for crown and stem nodes are shown in Figure 3, with a chronogram calibrated against the geological timescale. Additional sampling and age estimates for families and subfamilies of Asparagales are summarised in Table 3.

Discussion

The Network Reveals a Useful Pattern in Asparagales

The detection of data conflicts and the extent to which data conflicts will affect the data analysis becomes an important first step in a phylogenetic analysis [6]. Phylogenetic networks, such as the split graphs produced by the neighbour-net algorithm, give a broad overview of competing evolutionary scenarios within a dataset [37]. These methods have been successfully used to analyse multigene plastid datasets (e.g. ferns, [38]; Ranunculaceae, [39]), nuclear ribosomal DNA; Acer, [40]), and microbial and fungal evolution [9,41,42]. They have also been used in the context of genome sequencing surveys [43,44]. However, the use of networks as a tool for large-scale phylogenetic research has rarely been reported in the scientific literature [6].

In this study, we used the phylogenetic network method neighbour net to analyse a larger-scale sampling datasets of Asparagales. The network tree summarised the majority data pattern in plastid sequences, which with long terminal edges clusters indicated strong support for the family system of Asparagales sensu APG III that was modified to include three expanded asparagalean families proposed by Chase et al. (2009) and APG III (2009). The tree is subdivided as follows: part A, Asparagaceae and subfamilies; part B, Amaryllidaceae and Xanthorrhoeaceae and their subfamilies plus Xeronemataceae; part C, the basal nodes of Asparagales and outgroups (non-Asparagales taxa).

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Figure 2. Consensus tree from Bayesian analysis of the four combined cpDNA datasets. The 50% majority rule consensus phylogram from partitioned Bayesian analysis of a combined matrix of 284 accessions and 6699 bp from four plastid genes: atpB, matK, ndhF and rbcL. The 400,000 generations before the point when the SDF permanently fell below 0.01 (0.0016 at termination) were discarded as burn-in. Three types of support (bootstrap percentages for parsimony analyses with equal weights [EW]/successive approximations weighting [SW]/posterior probabilities for Bayesian analysis [PP]) are indicated on each branch. Major clades are named following the subfamily classification of three expanded asparagalean families proposed by Chase et al. (2009) and APG III (2009). The tree is subdivided as follows: part A, Asparagaceae and subfamilies; part B, Amaryllidaceae and Xanthorrhoeaceae and their subfamilies plus Xeronemataceae; part C, the basal nodes of Asparagales and outgroups (non-Asparagales taxa).
Figure 3. Divergence time estimates for Asparagales, based on four cpDNA genes (atpB, matK, ndhF and rbcL). The maximum clade credibility tree from the divergence times estimated with BEAST. The 95% highest posterior density (HPD) estimates for each well-supported clade are represented by bars. Numbers at nodes are fossil calibration points: ① 93 Ma, age for the most recent common ancestor (MRCA) of extant
are unstable in some previous studies. For example, Boryaceae has sometimes been placed as sister to Orchidaceae (e.g. [11]), although with weak support, and there are other topologies, including one embedding Orchidaceae in a paraphyletic Boryaceae-Hypoxidaceae clade [32]. Unexpectedly, *M. biflora* complexly netted to Orchidaceae on network analyses (Figure 1), however this taxon has been grouped within Brodiaeae (Themidaceae sensu APG II) at present parsimony and Bayesian inference (Figure 2, part A) in line with previous reports [17,22]. In case of sequencing or sampling errors, the split network is possibly more sensitive to exhibit artificial than regular phylogenetic analyses. The biased pattern of *M. biflora* suggests that resampling is necessary in order to find real situation.

The conflicting signals may be caused by homoplasy or stochastic noise rather than recombination that were not detected across the plastid genome in the core Asparagales [45]. DNA sequences from organellar genomes (e.g. mitochondria, plastids) are largely considered to be inherited uniparentally and non-recombining, with a single shared evolutionary history for the entire organellar genome [46–49]. Systematic mutational biases may also introduce conflicting phylogenetic signals within organelle sequences, especially between long-diverged taxa [50]. Although there may be reasons weak signals are introduced giving conflicting relationships, additional sequence data should allow identification of the bifurcating phylogenetic history of the organelle genome. Not unexpectedly, the continued examination of additional characters per taxon, 7 [17] and 17 plastid genes [23], and whole plastome sequences [45] gave higher resolution and bootstrap support to many clades in Asparagales.

Undoubtedly, it would be very wise to survey phylogenetic data using network methods before attempting to infer phylogenetic trees. Some attempts have begun [45], nevertheless the network methods should play a greater role in phylogenetic analyses than it has done to date. Compared with our inferred phylogenetic tree, it is worth noting that the network patterns reflect the tree bootstrap support to an extent, despite contrary opinions expressed previously [6,51].

**Phylogeny of Asparagales**

This study, with relatively dense taxon sampling and more diverse species representing more genera compared to previous phylogenetic studies, documented the stability of relationships within Asparagales. The family-level phylogenetic relationships found here were particularly congruent with other broad studies [14,22,23,26–28,45], indicating that the tree topologies in previous studies are robust with respect to the different samples used to represent genera and taxa sampled.

Relatively dense taxon sampling is generally a beneficial strategy for reducing long-branch attraction and obtaining more accurate inferences of phylogenetic relationships among and within large groups of organisms [52–55]. Long-branch attraction has been invoked for the placement of several problematic Asparagales taxa, such as Aphyllanthoideae and Ixioliriaceae, which are relatively isolated taxa with a long terminal branch. The position of *Aphyllanthes* in previous studies was labile and weakly supported [17,22,23]. In the neighbour-net tree in this study, *Aphyllanthes* had long edges that join to the base of Asparagaceae s.l., close to Lomandroideae, as has been found in other studies [17]. However, its position changed from sister to Agavoideae (Agavaceae sensu APG II) to sister to Brodiaeae (Themidaceae sensu APG II) in our MP and BI trees, respectively, but always formed a moderately to strongly supported group with Agavoideae, Scilloideae and Brodiaeae (63/91/1.0), which is also consistent with previous studies [22,23,26,28]. Based on genome data (79-plastid gene matrix), Steele et al. [45] found that *Aphyllanthes* was sister to Agavoideae with moderate support and confirmed that it links the same subfamilies mentioned above using neighbour-net analyses. Obviously, *Aphyllanthes* may be suffering from not only long branch attraction (LBA), but also too few characters to define individual nearby branches as a result of rapid radiation [45].

Ixioliriaceae was inferred as a strongly supported sister group to Tecophilaeaceae in this study, a result that had variable support in previous analyses [17,22,26,28]. Analyses of morphological data and base chromosome number support the sister relationship of these two families [56]. Doryanthaceae remain unresolved, forming either a polytomy or a weakly supported sister to the clade of Ixioliriaceae/Tecophilaeaceae and the remainder of Asparagales (except Astelioid and Orchidaceae), consistent with previous analyses [13,26,28].

Monophyly of the astelioid clade was well supported (83/91/1.0), including five small families (Boryaceae, Hypoxidaceae, Lamiastraceae, Asteliaceae and Blandfordiaceae; Figure 2, part C), consistent with most previous studies [22,23,26,29,57,58]. This clade has been demonstrated to have some shared morphological characters for all but Blandfordiaceae [57]. Little is gained by recognising the astelioid clade as a single family (Hypoxidaceae s.l.) to further reduce the number of families in Asparagales.

Our results highlight the largely robust framework for Asparagales, which is largely or completely congruent with the comparable taxonomic sampling in previous studies [14,15,17,22,23,26–28,45].

**Divergence Time Estimates**

The age estimates obtained across the major clades of Asparagales from the PATHd8 and BEAST analyses compared here overlap considerably (see Table 3). Overall PATHd8 produced slightly younger ages than BEAST. The BEAST analyses that used multiple (three) constraints with exponential distribution may be a good alternative to a lognormal distribution in the face of inadequate palaeontological information [59], which yielded a narrower 95% higher posterior density (HPD) and generally younger node ages than the latter, as noted by Bell et al. [36].

We estimated that the stem group of Asparagales dates to ca. 99–113 Ma and that the crown group dates to ca. 93–101 Ma, which agrees reasonably with Bell et al. [36], who reported a crown age range of 83–103 Ma (see Appendix S15 in their paper). However, Janssen and Bremer [31] suggested somewhat older dates of ca. 122 Ma and ca. 119 Ma, respectively. The topology within Asparagales, especially near the base, in the latter differed substantially from our results; e.g. they did not place Orchidaceae as sister to the rest of the order. Comparable results in Magallon and Castillo [60] were ca. 133.1 (stem), 125 (crown), 118.6 (stem) and 112.6 (crown) Ma for relaxed and constrained penalised likelihood dating, respectively. These molecular-based estimates suggest a Cretaceous origin of Asparagales. In this study, the estimates are obviously close to the oldest known fossil record of Asparagales (93–105 Myr old, see [61] Supplementary Methods for details).
**Table 1. Vouchers with GenBank accession number for taxa included in this study.**

| Family/Tribe Taxa | Vouchers | source type | Source (Institution) | Country | matK | rbcL | atpB | ndhF |
|-------------------|----------|-------------|----------------------|---------|------|------|------|------|
| **Asparagales**   |          |             |                      |         |      |      |      |      |
| **Elaealoeae**    |          |             |                      |         |      |      |      |      |
| Danae racemosa    | Chase 121 | DNA         | KEW DNABank          | UK      | KimJH,2010 | JX903679 | JX903260 |
| Ruscus aculeatus  | J.H. Kim s.n. 2008 Fresh |           | RBG Kew Garden       | UK      | KimJH,2010 | JX903680 | JX903261 |
| Ruscus streptophyllus | Chase 21990 DNA | KEW DNABank | UK      | KimJH,2010 | JX903681 | JX903262 |
| Semele androgyna  | Chase 997 DNA | KEW DNABank | UK      | KimJH,2010 | JX903682 | JX903263 |
| Aspidistra elatior | Z. Jang 4805 Specimen | KEW DNABank | UK      | KimJH,2010 | JX903683 | JX903264 |
| **Aspidistra**    |          |             |                      |         |      |      |      |      |
| Nolinoideae       |          |             |                      |         |      |      |      |      |
| Asparagaceae      |          |             |                      |         |      |      |      |      |
| **Convallariaceae** |          |             |                      |         |      |      |      |      |
| Convallaria majalis | D.K. Kim 04-082 Fresh | Field work | Korea    | KimJH,2010 | JX903687 | JX903268 |
| Reineckea camea    | Wu 454 DNA | KEW DNABank | UK      | KimJH,2010 | JX903688 | JX903269 |
| Speirantha gardenii | Chase 495 DNA | KEW DNABank | UK      | KimJH,2010 | JX903689 | JX903270 |
| Theropogon pallidus | Chase 2933 DNA | KEW DNABank | UK      | KimJH,2010 | JX903690 | JX903271 |
| Comospermum yedoense | Chase 833 DNA | KEW DNABank | UK      | KimJH,2010 | JX903691 | JX903272 |
| **Lilioidea**     |          |             |                      |         |      |      |      |      |
| Liriope platyphylla | D.K. Kim 07-001 Fresh | Field work | Korea    | KimJH,2010 | JX903692 | JX903273 |
| Liriope spicata    | D.K. Kim 07-002 Fresh | Field work | Korea    | KimJH,2010 | JX903693 | JX903274 |
| Ophiopogon jaburan | D.K. Kim 07-004 Fresh | Field work | Korea    | KimJH,2010 | JX903694 | JX903275 |
| Ophiopogon japonicus | D.K. Kim 07-003 Fresh | Field work | Korea    | KimJH,2010 | JX903695 | JX903276 |
| Ophiopogon sp.     | D.K. Kim 08-207 Fresh | Field work | Korea    | KimJH,2010 | JX903696 | JX903277 |
| Peliasanthes sp.   | Chase 847 DNA | KEW DNABank | UK      | JX903535 | JX903126 | JX903697 | JX903278 |
| **Reineckea**     |          |             |                      |         |      |      |      |      |
| Polyglossum chinense | D.K. Kim 04-182 Fresh | Field work | Korea    | KimDK,2012 | JX903700 | JX903279 |
| Polyglossum dilatatum | D.K. Kim 04-165 Fresh | Field work | Korea    | KimDK,2012 | JX903701 | JX903280 |
| Polyglossum stellatum | D.K. Kim 08-229 Fresh | Field work | Korea    | KimDK,2012 | JX903702 | JX903281 |
| Polygonatum desolavii | D.K. Kim 09-225 Fresh | Field work | Korea    | JX903537 | JX903127 | JX903703 | JX903282 |
| Polygonatum falcatum | D.K. Kim 09-191 Fresh | Field work | Korea    | JX903538 | JX903129 | JX903704 | JX903283 |
| Polygonatum humile | D.K. Kim 04-029 Fresh | Field work | Korea    | JX903539 | JX903130 | JX903705 | JX903284 |
| Polygonatum inflatum | D.K. Kim 04-043 Fresh | Field work | Korea    | JX903540 | JX903131 | JX903706 | JX903285 |
| Polygonatum involucratum | D.K. Kim 04-059 Fresh | Field work | Korea    | JX903541 | JX903132 | JX903707 | JX903286 |
| Polygonatum lissianthum var. coreanum | D.K. Kim 04-046 Fresh | Field work | Korea    | JX903542 | JX903133 | JX903708 | JX903287 |
| Polygonatum odoratum var. pluriflorum | D.K. Kim 04-067 Fresh | Field work | Korea    | JX903543 | JX903134 | JX903709 | JX903288 |
| Polygonatum stenophyllum | D.K. Kim 08-156 Fresh | Field work | Korea    | JX903544 | JX903135 | JX903710 | JX903289 |
| Maianthemum bicolour | D.K. Kim 04-077 Fresh | Field work | Korea    | JX903545 | JX903136 | JX903711 | JX903290 |
| Maianthemum dilatatum | D.K. Kim 04-165 Fresh | Field work | Korea    | JX903546 | JX903137 | JX903712 | JX903291 |
| Maianthemum stellatum | D.K. Kim 08-229 Fresh | Field work | Korea    | JX903547 | JX903138 | JX903713 | JX903292 |
| Dracaena aubryana | Chase 1102 DNA | KEW DNABank | UK      | KimJH,2010 | JX903714 | JX903293 |
| Dracaena deremensis | J.H. Kim 2009 s.n. Fresh | Australia Royal Botanic Garden | UK | JX903539 | *AB029848 | JX903715 | JX903294 |
| Dracaena hookeriana | D.K. Kim 09-027 Fresh | Australia Royal Botanic Garden | UK | JX903540 | *AM235113 | JX903716 | JX903295 |
| Dracaena schizantha | Chase 21514 DNA | KEW DNABank | UK      | KimJH,2010 | JX903717 | JX903296 |
| Family/Tribe Taxa                  | Vouchers       | Source type | Source (Institution) | Country | matK     | rbcL     | atpB     | ndhF     |
|-----------------------------------|----------------|-------------|----------------------|---------|----------|----------|----------|----------|
| Pleomele javanica                 | Chase 1240     | DNA         | KEW DNABank          | UK      | JX903541 | JX903130 | JX903718 | JX903299 |
| Sansevieria trifasciata           | D.K. Kim 07-005| Fresh       | Field work           | Korea   | KimJH,2010 | KimJH,2010 | JX903719 | JX903300 |
| Beaucarnea recurvata              | D.K. Kim 09-002| Fresh       | Field work           | Korea   | KimJH,2010 | KimJH,2010 | JX903723 | JX903304 |
| Calibanus hookeri                 | Chase 1006     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903724 | JX903305 |
| Daylirion wheeleri                | Chase 3469     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903725 | JX903306 |
| Nolina bigelovii                 | D.K. Kim 08-231| Fresh       | Kew Garden           | Korea   | KimJH,2010 | KimJH,2010 | JX903726 | JX903307 |
| Nolina recurvata                  | Chase 3466     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903727 | JX903308 |
| Eriospermum abyssinicum           | Chase 1006     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903720 | JX903301 |
| Eriospermum cooperi var. natalensis | Chase 2052   | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903721 | JX903302 |
| Eriospermum parvifolium           | Chase 2053     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903722 | JX903303 |
| Asparagoideae                     |                |             |                      |         |          |          |          |          |
| Asparagus cochinensis             | D.K. Kim 04-122| Fresh       | Field work           | Korea   | KimJH,2010 | KimJH,2010 | JX903789 | JX903371 |
| Asparagus densiflorus             | D.K. Kim 08-198| Fresh       | Kunming Botanic Garden | China   | KimJH,2010 | KimJH,2010 | JX903790 | JX903372 |
| Asparagus oligoclonos             | D.K. Kim 08-007| Fresh       | Field work           | Korea   | KimDK,2012 | KimDK,2012 | JX903791 | JX903373 |
| Asparagus schoberioides           | D.K. Kim 05-165| Fresh       | Field work           | Korea   | KimJH,2010 | KimJH,2010 | JX903792 | JX903374 |
| Hemiphylacus latifolius           | Chase 668      | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903793 | JX903375 |
| Lomandroideae                     |                |             |                      |         |          |          |          |          |
| Acanthocarpus preissii            | Chase 2228     | DNA         | KEW DNABank          | UK      | JX903591 | JX903182 | JX903820 | JX903403 |
| Arthropodium cirratum             | Chase 651      | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903821 | JX903404 |
| Chamaelexus serra                 | Brummitt 31374 | DNA         | KEW DNABank          | UK      | JX903593 | JX903184 | JX903823 | JX903406 |
| Cordyline cannifolia              | Chase 17936    | DNA         | KEW DNABank          | UK      | JX903594 | JX903185 | JX903824 | JX903407 |
| Cordyline pumilio                 | Chase 14228    | DNA         | KEW DNABank          | UK      | JX903595 | JX903186 | JX903825 | JX903408 |
| Laxmannia squarrosa               | Chase 2214     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903826 | JX903409 |
| Lomandra hastilis                | Brummitt       | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903827 | JX903410 |
| Lomandra longifolia               | D.K. Kim 09-038| Fresh       | Field work           | Korea   | *DQ401356 | JX903187 | JX903828 | JX903411 |
| Lomandra ordii                    | Brummitt 21345 | DNA         | KEW DNABank          | UK      | JX903596 | JX903188 | JX903829 | JX903412 |
| Sowerbaea juncea                 | Chase 454      | DNA         | KEW DNABank          | UK      | JX903597 | JX903189 | JX903830 | JX903413 |
| Thysonotus sp.                    | Chase 2218     | DNA         | KEW DNABank          | UK      | JX903598 | JX903190 | JX903831 | JX903414 |
| Trichopetalum plumosum           | Cult ADU ex    | DNA         | KEW DNABank          | UK      | JX903599 | JX903191 | JX903832 | JX903415 |
| Agavoideae                        |                |             |                      |         |          |          |          |          |
| Agave americana                   | D.K. Kim 08-193| Fresh       | Field work           | Korea   | JX903544 | JX903133 | JX903729 | JX903310 |
| Agave ghesbrechti                | Chase 3467     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903730 | JX903311 |
| Anemarrhena asphodeloides        | Kew 1156       | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903778 | JX903360 |
| Anthericum lilago                | Chase 515      | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903779 | JX903361 |
| Anthericum ramosum               | J.J. Kim       | Fresh       | Ivana Franka Boranic Garden | Ukraine  | JX903578 | JX903168 | JX903780 | JX903362 |
| Behnia reticulata                | Goldblatt 9273 | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903794 | JX903376 |
| Camassia cusickii                | Cronquist 6549 | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903801 | JX903383 |
| Chlorogalum pomeridianum         | Chase 838      | DNA         | KEW DNABank          | UK      | JX903545 | JX903134 | JX903731 | JX903312 |
| Chlorophytum orchidistrium       | Chase 2155     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903781 | JX903363 |
| Chlorophytum suffruticosum       | Chase 1043     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903782 | JX903364 |
| Chlorophytum tetraphyllum        | Chase 1044     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903783 | JX903365 |
| Echeandia sp.                    | Chase 602      | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903785 | JX903367 |
| Hagenbachia panamensis           | Correa et al.  | DNA         | KEW DNABank          | UK      | JX903579 | JX903170 | JX903786 | JX903368 |
| Herrera salisaparilha            | Chase 2154     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903795 | JX903377 |
| Family/Tribe Taxa | Vouchers | source type | Source (Institution) | Country | matK | rbcL | atpB | ndhF |
|------------------|----------|-------------|---------------------|---------|------|------|------|------|
| Herreriopsis elegans | Maurin & Rakotonasolo 90 | DNA | KEW DNABank | UK | JX903581 | JX903172 | JX903796 | JX903378 |
| Hesperocallis undulata | Cranfill & Schmid s.n. | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903797 | JX903379 |
| Hastingsia serpentinicola | Hufford 817 | DNA | KEW DNABank | UK | JX903586 | JX903177 | JX903807 | JX903389 |
| Hosta capitata | D.K. Kim 09-008 | Fresh | Field work | Korea | KimDK,2012 | KimDK,2012 | JX903732 | JX903313 |
| Hosta minor | D.K. Kim 08-086 | Fresh | Field work | Korea | KimDK,2012 | KimDK,2012 | JX903733 | JX903314 |
| Hosta plantaginea | Jin Xiow Feng s.n. | Fresh | Kunming Botanic Garden | China | KimH,2010 | KimH,2010 | JX903734 | JX903315 |
| Hosta yingeri | D.K. Kim 08-011 | Fresh | Field work | Korea | KimDK,2012 | KimDK,2012 | JX903735 | JX903316 |
| Leucocrinum montanum | Chase 795 | DNA | KEW DNABank | UK | KimJH,2010 | KimJH,2010 | JX903787 | JX903369 |
| Paradisea liliastrum | Chase 826 | DNA | KEW DNABank | UK | KimJH,2010 | KimJH,2010 | JX903736 | JX903317 |
| Paradisea minor | D.B. Yang s.n. | Specimen | KUN | China | KimH,2010 | KimH,2010 | JX903737 | JX903318 |
| Yucca filamentosa | D.K. Kim 06-077 | Fresh | Field work | Korea | KimH,2010 | KimH,2010 | JX903738 | JX903319 |
| Yucca queretaroensis | D.K. Kim 08-230 | Fresh | Field work | Korea | JX903546 | JX903135 | JX903739 | JX903320 |
| Scilloideae | | | | | | | | |
| Bellevalia pycnantha | Chase 21821 | DNA | KEW DNABank | UK | JX903582 | JX903173 | JX903798 | JX903380 |
| Bellevalia romana | D.K. Kim 08-224 | Fresh | Field work | Korea | JX903583 | JX903174 | JX903799 | JX903381 |
| Bowiera volubilis | Chase 176 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903800 | JX903382 |
| Dipcadi filifolium | Chase 1783 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903802 | JX903384 |
| Drimia alissima | Chase 1870 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903803 | JX903385 |
| Drimopsis maxima | Chase 17509 | DNA | KEW DNABank | UK | JX903584 | JX903175 | JX903804 | JX903386 |
| Eucomis humilis | Chase 1847 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903805 | JX903387 |
| Eucomis punctata | J.H. Kim 2009 s.n. | Fresh | Ivana Franka Boranic Garden | Ukraine | JX903585 | JX903176 | JX903806 | JX903388 |
| Hyacinthella nervosa | Chase 21826 | DNA | KEW DNABank | UK | JX903587 | JX903178 | JX903808 | JX903390 |
| Hyacinthoides hispanica | Chase 16564 | DNA | KEW DNABank | UK | JX903588 | JX903179 | JX903809 | JX903391 |
| Lachenalia carnosa | Chase 2261 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903810 | JX903392 |
| Ledebouria cooperi | Chase 1786 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903811 | JX903393 |
| Massonia angustifolia | Chase 5666 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903812 | JX903394 |
| Menwilla aurea | LHMS 2387 | DNA | KEW DNABank | UK | JX903589 | JX903180 | JX903813 | JX903395 |
| Muscari aucheri | Chase 21845 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903814 | JX903396 |
| Ornithogalum armeniacum | Chase 1682 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903815 | JX903397 |
| Ornithogalum caudatum | D.K. Kim 09-028 | Fresh | Field work | Korea | JX903590 | JX903181 | JX903816 | JX903398 |
| Ornithogalum shawii | Chase 1012 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903817 | JX903399 |
| Rhadamanthus convallarioides | Goldblatt, 10852 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903818 | JX903400 |
| Scilla scilloides | D.K. Kim 05-039 | Fresh | Field work | Korea | KimH,2010 | KimH,2010 | JX903819 | JX903401 |
| Urginea epigea | Chase 2055 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903819 | JX903402 |
| Brodiaeae | | | | | | | | |
| Bessera elegans | Chase 626 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903833 | JX903416 |
| Bloomeria crocea var. aura | Chase 1010 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903834 | JX903417 |
| Dandya thadhowii | Chase S.N. | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903835 | JX903418 |
| Dicholostemma multiflorum | Chase 1830 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903836 | JX903419 |
| Milla biflora | Chase 1907 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903837 | JX903420 |
| Musa maritima | Chase 779 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903838 | JX903421 |
| Triteleia peduncularis | Chase 1860 | DNA | KEW DNABank | UK | KimH,2010 | KimH,2010 | JX903839 | JX903422 |
| Afflanthoideae | | | | | | | | |
| Aphyllanthes monspeliensis | Chase 614 | DNA | KEW DNABank | UK | KimH,2010 | KimDK,2012 | JX903788 | JX903370 |
| Amaryllidaceae | | | | | | | | |
| Amaryllidoideae | | | | | | | | |
| Family/Tribe Taxa | Vouchers | source type | Source (Institution) Country | matK | rbcL | atpB | ndhF |
|------------------|----------|-------------|----------------------------|-------|------|------|------|
| Amaryllis belladona | KEW 612 DNA KEW DNABank UK | JX903555 JX903144 JX903750 JX903333 |
| Apodolirion cedarbergense | Graham Duncan DNA KEW DNABank UK | JX903556 JX903145 JX903751 JX903334 |
| Calostemma lutea | Chase 1505 DNA KEW DNABank UK | JX903557 JX903146 JX903752 JX903335 |
| Clivia nobilis | Chase 3080 DNA KEW DNABank UK | KimJH,2010 JX903147 JX903753 JX903336 |
| Crinum asiaticum var. japonicum | K.H. Tae 2004 s.n. DNA KNRRC Korea | KimJH,2010 JX903754 JX903337 |
| Cybistetes longifolia | KEW 3643 DNA KEW DNABank UK | JX903558 JX903148 JX903755 JX903338 |
| Cyrtanthus purpureus | Chase 1572 DNA KEW DNABank UK | JX903559 JX903149 JX903756 JX903339 |
| Eustephia darwinii | Chase 559 DNA KEW DNABank UK | JX903560 JX903150 JX903757 JX903340 |
| Gethyllis brittoniana | Van Jaarsveld 5618 DNA KEW DNABank UK | JX903561 JX903151 JX903758 JX903341 |
| Habranthus martinezii | Chase 1023 DNA KEW DNABank UK | JX903562 JX903152 JX903759 JX903342 |
| Haemanthus albiflos | Chase 1505 DNA KEW DNABank UK | JX903563 JX903153 JX903760 JX903343 |
| Hieronymiella var. latifolia | Chase 1901 DNA KEW DNABank UK | JX903564 JX903154 JX903761 JX903344 |
| Hippeastrum psittacinum | Chase 14823 DNA KEW DNABank UK | JX903565 JX903155 JX903762 JX903345 |
| Hymenocallis littoralis | Chase 2027 DNA KEW DNABank UK | JX903566 JX903156 JX903763 JX903346 |
| Ismene longifolia | Chase 3583 DNA KEW DNABank UK | JX903567 JX903157 JX903764 JX903347 |
| Leucojum roseum | Chase 1524 DNA KEW DNABank UK | JX903568 JX903158 JX903765 JX903348 |
| Lycoris sanguinea var. koreana | D.K. Kim 06-167 Fresh Field work Korea | KimDK,2012 KimDK,2012 JX903766 JX903349 |
| Lycoris sanguinea | D.K. Kim 06-100 Fresh Field work Korea | KimDK,2012 KimDK,2012 JX903767 JX903350 |
| Narcissus tazetta var. chinensis | D.K. Kim 06-167 Fresh Field work Korea | KimDK,2012 KimDK,2012 JX903768 JX903351 |
| Nerine alta | Chase 18199 DNA KEW DNABank UK | JX903569 JX903159 JX903769 JX903352 |
| Pancratium canariense | Chase 17733 DNA KEW DNABank UK | JX903570 JX903160 JX903770 JX903353 |
| Paramongaia weberbaueri | Chase 1594 DNA KEW DNABank UK | JX903571 JX903161 JX903771 JX903354 |
| Scadoxus cinnabarinus | Chase 549 DNA KEW DNABank UK | JX903572 JX903162 JX903772 JX903355 |
| Scadoxus puniceus | D.K. Kim 09-011 Fresh Field work Korea | JX903573 JX903163 JX903773 JX903356 |
| Stenomesson minutum | Chase 16481 DNA KEW DNABank UK | JX903574 JX903164 JX903774 JX903357 |
| Ungeria flava | Chase 3640 DNA KEW DNABank UK | JX903575 JX903165 JX903775 JX903358 |
| Vagaria parviflora | Chase 1066 DNA KEW DNABank UK | JX903576 JX903166 JX903776 JX903359 |
| Zephyranthes simpsonii | Chase 1839 DNA KEW DNABank UK | JX903577 JX903167 JX903777 JX903360 |
| Allioidae | D.K. Kim 08-002 Fresh Field work Korea | KimDK,2012 KimDK,2012 JX903740 JX903361 |
| Allium microdictyon | D.K. Kim 04-142 Fresh Field work Korea | KimDK,2012 KimDK,2012 JX903741 JX903362 |
| Allium ochotense | D.K. Kim 08-095 Fresh Field work Korea | KimDK,2012 KimDK,2012 JX903742 JX903363 |
| Allium thunbergii | D.K. Kim 08-220 Fresh Field work Korea | JX903547 JX903136 *AY147628 JX903364 |
| Ipheion uniflorum(uniflora) | Chase 449 DNA KEW DNABank UK | JX903574 JX903164 JX903774 JX903365 |
| Leucocoryne pauciflora | Chase 16462 DNA KEW DNABank UK | JX903548 JX903137 JX903743 JX903366 |
| Nothoscordum bivalve | Chase 17513 DNA KEW DNABank UK | JX903549 JX903138 JX903744 JX903367 |
| Nothoscordum texanum | Chase 1593 DNA KEW DNABank UK | JX903550 JX903139 JX903745 JX903368 |
| Tristagma nivale | Chase 2757 DNA KEW DNABank UK | JX903551 JX903140 JX903746 JX903369 |
| Tristagma uniflorum | H. Murakami 631 Specimen KYO Japan | JX903552 JX903141 JX903747 JX903370 |
| Tulbaghia simieli | Chase 17513 DNA KEW DNABank UK | JX903554 JX903143 JX903749 JX903371 |
| Agapanthoideae | Chase 627 DNA KEW DNABank UK | KimJH,2010 KimJH,2010 JX903728 JX903372 |
| Lower asparagoids | D.K. Kim 08-002 Fresh Field work Korea | JX903740 JX903373 |
| Hesperocallidioideae | D.K. Kim 04-142 Fresh Field work Korea | JX903741 JX903374 |
| Coesia contorta | Goldblatt 9406 DNA KEW DNABank UK | JX903610 JX903201 JX903858 JX903375 |
| Corynotheca micrantha | Chase 2210 DNA KEW DNABank UK | JX903611 JX903202 JX903859 JX903376 |
| Chamaescia sp. | Chase 2208 DNA KEW DNABank UK | JX903592 JX903183 JX903822 JX903405 |
| Family/Tribe Taxa            | Vouchers                  | source type | Source (Institution) | Country                  | matK        | rbcL       | atpB       | ndhF        |
|-----------------------------|---------------------------|-------------|----------------------|--------------------------|-------------|------------|------------|-------------|
| Dianella ensifolia          | Akio Naiki 5510 Specimen  | Specimen    | KUN China            | KimJH,2010               | KimJH,2010  | JX903860   | JX903444   |
| Hemerocallis dumortieri     | D.K. Kim 08-145 Fresh     | Field work  | Korea                | KimDK,2012               | JX903861    | JX903445   |
| Hemerocallis fulva          | D.K. Kim 08-152 Fresh     | Field work  | Korea                | KimDK,2012               | JX903862    | JX903446   |
| Hemerocallis hungdoidensis  | D.K. Kim 09-013 Fresh     | Field work  | Korea                | JX903612                 | *AY149364   | JX903447   |
| Hemerocallis minor          | D.K. Kim 05-091 Fresh     | Field work  | Korea                | KimJH,2010               | JX903864    | JX903448   |
| Johnsonia pubescens         | Chase 2213 DNA            | KEW DNABank  | UK                   | JX903613                 | JX903203    | JX903865   | JX903449   |
| Pasithea coerulea           | Chase 512 DNA             | KEW DNABank  | UK                   | JX903614                 | JX903204    | JX903866   | JX903450   |
| Phormium tenax              | Chase 177 DNA             | KEW DNABank  | UK                   | JX903615                 | JX903205    | JX903867   | JX903451   |
| Stawelia dimorphantha       | P.J. Rudall, s.n. DNA     | KEW DNABank  | UK                   | JX903616                | *Z77306     | JX903868   | *FJ707520  |
| Stypandra glauca            | Brummitt, George & Oliver 2123 DNA | KEW DNABank  | UK                   | JX903617                | JX903206    | JX903869   | JX903452   |
| Tricyanea elatior           | Chase 2219 DNA            | KEW DNABank  | UK                   | JX903618                | JX903207    | JX903870   | JX903453   |
| Xanthorrhoea resinosa       | Chase 192 DNA             | KEW DNABank  | UK                   | KimJH,2010               | KimJH,2010  | JX903923   | JX903504   |
| Xanthorrhoea media          | D.K. Kim 09-032 Fresh     | Field work  | Korea                | JX903650                | JX903234    | JX903922   | JX903503   |
| Aloe vera                   |                          |             |                      |                          | *AJ511390   | *AJ512309  | *AF168886  | *AY225054  |
| Asphodeline lutea           | UCI Arb. 3440 DNA         | KEW DNABank  | UK                   | JX903600                | JX903192    | JX903840   | JX903423   |
| Asphodelus aestivus         | Chase 482 DNA             | KEW DNABank  | UK                   | KimJH,2010               | KimJH,2010  | JX903841   | JX903424   |
| Astraloba fahioa            | Chase 684 DNA             | KEW DNABank  | UK                   | JX903601                | JX903193    | JX903842   | JX903425   |
| Bulbine semibracteata       | K. Dixon s.n. DNA         | KEW DNABank  | UK                   | KimJH,2010               | KimJH,2010  | JX903843   | JX903426   |
| Bulbina cauda-feliis       | UCI Arb. 359 DNA          | KEW DNABank  | UK                   | JX903602                | JX903194    | JX903844   | JX903427   |
| Eremurus chiniensis         | Qing 00317 DNA            | KEW DNABank  | UK                   | KimJH,2010               | KimJH,2010  | JX903845   | JX903428   |
| Gasteria rawlinsoi          | Chase 18179 DNA           | KEW DNABank  | UK                   | JX903603                | JX903195    | JX903846   | JX903429   |
| Haworthia coarctata         | Chase 3859 DNA            | KEW DNABank  | UK                   | JX903604                | JX903196    | JX903847   | JX903430   |
| Kniphofia sp.               | D.K. Kim 08-187 Fresh     | Field work  | Korea                | JX903605                | *Z73689     | *AJ417572  | JX903431   |
| Poellnitzia rubiflora       | KEW 6534 DNA              | KEW DNABank  | UK                   | JX903606                | JX903197    | JX903848   | JX903432   |
| Trachyandra esterhusysaeae  | Fay s.n. DNA              | KEW DNABank  | UK                   | JX903607                | JX903198    | JX903849   | JX903433   |
| Xeronema callistemon        | Chase 653 DNA             | KEW DNABank  | UK                   | KimJH,2010               | KimJH,2010  | JX903924   | JX903505   |
| Arista monticola            | Compton 11967 DNA         | KEW DNABank  | UK                   | JX903622                | JX903212    | JX903878   | JX903461   |
| Belamcanda chiniensis       | D.K. Kim 08-186 Fresh     | Field work  | Korea                | KimDK,2012               | KimDK,2012  | JX903879   | JX903462   |
| Crocus banaticus            | D.K. Kim 09-004 Fresh     | Field work  | Korea                | JX903623                | JX903213    | JX903880   | JX903463   |
| Crocus cartwright           | Chase 11726 DNA           | KEW DNABank  | UK                   | JX903624                | JX903214    | JX903881   | JX903464   |
| Dietes grandiflora          | D.K. Kim 09-021 Fresh     | Field work  | Korea                | JX903625                | JX903215    | JX903882   | JX903465   |
| Geissorhiza heterostyla      | Goldblatt & Manning 9668 DNA | KEW DNABank  | UK                   | JX903626                | JX903216    | JX903883   | JX903466   |
| Gladiolus illyricus         | Chase 9907 DNA            | KEW DNABank  | UK                   | JX903627                | KimJH,2010  | JX903884   | JX903467   |
| Hermodactylus tuberosus     | Chase I-76 DNA            | KEW DNABank  | UK                   | JX903628                | JX903217    | JX903885   | JX903468   |
| Iris confusa                | D.K. Kim 08-195 Fresh     | Field work  | Korea                | JX903629                | JX903218    | JX903886   | JX903469   |
| Iris minutaurea             | D.K. Kim 08-124 Fresh     | Field work  | Korea                | KimDK,2012               | KimDK,2012  | JX903878   | JX903470   |
| Iris odaesanensis           | S.H. Park 2008 s.n. DNA   | KEW DNABank  | UK                   | JX903881                | KimDK,2012  | JX903888   | JX903471   |
| Iris pseudoacorus           | D.K. Kim 09-055 Fresh     | Field work  | Korea                | KimDK,2012               | KimDK,2012  | JX903889   | JX903472   |
| Iris rossii                 | D.K. Kim 05-048 Fresh     | Field work  | Korea                | KimJH,2010               | KimJH,2010  | JX903890   | JX903473   |
| Iris sanguinea              | D.K. Kim 08-056 Fresh     | Field work  | Korea                | KimDK,2012               | KimDK,2012  | JX903891   | JX903474   |
| Isophysis tasmanica         | J. Bruhl, TAS DNA         | KEW DNABank  | UK                   | JX903630                | JX903219    | JX903892   | JX903475   |
| Family/Tribe Taxa | Vouchers | source type | Source (Institution) | Country | matK | rbcL | atpB | ndhF |
|-------------------|----------|-------------|----------------------|---------|------|------|------|------|
| Moraea riparia    | Goldblatt & Porter 12130 | DNA | KEW DNABank UK | JX903631 | JX903220 | JX903893 | JX903476 |
| Neomarica northiana | Solomon 6950 | DNA | KEW DNABank UK | JX903632 | JX903221 | JX903894 | JX903477 |
| Nivenia stokoei   | KEW 1223 | DNA | KEW DNABank UK | JX903633 | JX903222 | JX903895 | JX903478 |
| Pilimania templemanii | Bean s.n. | DNA | KEW DNABank UK | JX903634 | JX903223 | JX903896 | JX903479 |
| Romulea bulbocodium | Chase 21504 | DNA | KEW DNABank UK | JX903635 | JX903224 | JX903897 | JX9034780 |
| Sisyrinchium palmifolium | Chase 16458 | DNA | KEW DNABank UK | JX903636 | JX903225 | JX903898 | JX9034781 |
| Solenomelus segthii | Chase 19213 | DNA | KEW DNABank UK | JX903637 | JX903226 | JX903899 | JX9034782 |
| Thereanthus racemosus | KEW 1-224 | DNA | KEW DNABank UK | JX903638 | *AJ309663 | JX903900 | JX9034783 |
| Tigridera immaculata | Rodriguez et al, 2832 | DNA | KEW DNABank UK | JX903639 | JX903227 | JX903901 | JX9034784 |
| Trimedia martincensis | Chase 15941 | DNA | KEW DNABank UK | JX903640 | JX903228 | JX903902 | JX9034785 |
| Watsonia angusta   | Goldblatt 6904 | DNA | KEW DNABank UK | JX903641 | JX903229 | JX903903 | JX9034786 |
| Tecophilaeaceae    | Conanthera bifolia | Chase 13821 | DNA | KEW DNABank UK | JX903646 | JX903230 | JX903916 | JX903479 |
| Cyanella orchidiformis | Chase 5896 | DNA | KEW DNABank UK | JX903647 | JX903231 | JX903918 | JX903499 |
| Odontostomum hartwegii | Chase 491 | DNA | KEW DNABank UK | JX903648 | JX903232 | JX903919 | JX903500 |
| Tecophilaeaceae    | walleria gracilis | Forest & Manning 542 | DNA | KEW DNABank UK | JX903649 | JX903233 | JX903920 | JX903501 |
| Zephyra elegans    | Chase 1575 | DNA | KEW DNABank UK | JX903650 | JX903234 | JX903921 | JX903502 |
| Ixoliaceae         | Ixolirion tataricum | Chase 489 | DNA | KEW DNABank UK | JX903651 | JX903235 | JX903922 | JX903503 |
| Doryanthaceae      | Doryanthus excelsa | Chase 188 | DNA | KEW DNABank UK | JX903652 | JX903236 | JX903923 | JX903504 |
| Doryanthus palmeri | Chase 19153 | DNA | KEW DNABank UK | JX903653 | JX903237 | JX903924 | JX903505 |
| Astelioid          | Hypoxideaceae       | Curculigo capitulata | S.W. Lee 05-001 Fresh | Kunming Botanic Garden | China | Kim,H,2010 | Kim,H,2010 | JX903545 |
| Curculigo capitulata | Chase 3848 | DNA | KEW DNABank UK | JX903654 | JX903238 | JX903925 | JX903506 |
| Hypoxis hemerocallidea | Chase 3848 | DNA | KEW DNABank UK | JX903655 | JX903239 | JX903926 | JX903507 |
| Hypoxis villosa    | D.K. Kim 09-025 Fresh | Field work | Korea | JX903656 | JX903240 | JX903927 | JX903508 |
| Molineria capitulata | Chase 1292 | DNA | KEW DNABank UK | JX903657 | JX903241 | JX903928 | JX903509 |
| Pauridia longituba | D. Snijman 1440 WBG | DNA | KEW DNABank UK | JX903658 | JX903242 | JX903929 | JX903510 |
| Rhodohypoxis baurii | Chase 16460 | DNA | KEW DNABank UK | JX903659 | JX903243 | JX903930 | JX903511 |
| Rhodohypoxis milloides | Chase 479 | DNA | KEW DNABank UK | JX903660 | JX903244 | JX903931 | JX903512 |
| Spiloxene serrata  | Manning and Reeves JM&GR 2846 | DNA | KEW DNABank UK | JX903661 | JX903245 | JX903932 | JX903513 |
| Lanariaceae        | Lanaria lanata     | Goldblatt & Manning 9410 | DNA | KEW DNABank UK | JX903662 | JX903246 | JX903933 | JX903514 |
| Asteliaceae        | Astelia alpina     | Chase 1103 | DNA | KEW DNABank UK | JX903663 | JX903247 | JX903934 | JX903515 |
| Milligania stylosa | Chase 511 | DNA | KEW DNABank UK | JX903664 | JX903248 | JX903935 | JX903516 |
| Blandfordiaceae    | Blandfordia cunninghamii | R. Johnstone 2345 & A.E. Orme | DNA | KEW DNABank UK | JX903665 | JX903249 | JX903936 | JX903517 |
| Blandfordia grandiflora | A.E. Orme 583 & S. Turpin | DNA | KEW DNABank UK | JX903666 | JX903250 | JX903937 | JX903518 |
| Family/Taxa                | Vouchers       | source type | Source (Institution) | Country | matK     | rbcL     | atpB    | ndhF    |
|---------------------------|----------------|-------------|----------------------|---------|----------|----------|---------|---------|
| Blandfordia punicea       | Chase 519      | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903854 | JX903438 |
| Boryaceae                 |                |             |                      |         |           |          |         |         |
| Borya septentrionalis     | Chase 2205     | DNA         | KEW DNABank          | UK      | KimJH,2010 | KimJH,2010 | JX903855 | JX903439 |
| Orchidaceae               |                |             |                      |         |           |          |         |         |
| Apostasia wallichii       | Chase 15744    | DNA         | KEW DNABank          | UK      | KimJH,2010 | JX903642   | JX903906 | JX903489 |
| Calanthe discolor         | D.K. Kim 05-035| Fresh       | Field work           | Korea   | KimJH,2010 | JX903907   | JX903490 | JX903490 |
| Cephalanthera erecta      | D.K. Kim 08-048| Fresh       | Field work           | Korea   | KimDK,2012 | JX903908   | JX903491 | JX903491 |
| Cephalanthera falcata     | D.K. Kim 08-110| Fresh       | Field work           | Korea   | KimDK,2012 | JX903909   | JX903492 | JX903492 |
| Cephalanthera longibracteata| D.K. Kim 05-016| Fresh     | Field work           | Korea   | KimJH,2010 | JX903910   | JX903493 | JX903493 |
| Coelogynhe sp.            | T.B. Tran T-37 | Fresh       | IEBR                 | Vietnam | KimDK,2012 | JX903643   | *AF074133 | *AY147777 |
| Cymbidium goeringii       | D.K. Kim 08-028| Fresh       | Field work           | Korea   | KimDK,2012 | JX903912   | JX903494 | JX903494 |
| Cypridium calceolus       | Chase 9484     | DNA         | KEW DNABank          | UK      | KimJH,2010 | JX903913   | JX903495 | JX903495 |
| Dendrobium acinaciforme   | T.B. Tran TN-32| Fresh       | IEBR                 | Vietnam | JX903644   | *FJ216578  | JX903914 | *U20534 |
| Epipactis thunbergii      | D.K. Kim 08-030| Fresh       | Field work           | Korea   | JX903645   | JX903915   | JX903496 | JX903496 |
| Orchis rotundifolia       |                |             |                      |         |           |          |         |         |
| Commelinidae              |                |             |                      |         |           |          |         |         |
| Commelininaceae           |                |             |                      |         |           |          |         |         |
| Commelina communis        | D.K. Kim 07-006| Fresh       | Field work           | Korea   | JX903665   | JX903248   | JX903938 | JX903519 |
| Arecales                  |                |             |                      |         |           |          |         |         |
| Areaceae                  |                |             |                      |         |           |          |         |         |
| Areca triandra            | AHBLoo 301     | DNA         | KEW DNABank          | UK      | *AM114664 | JX903249   | JX903939 | *AY044535 |
| Arenga hastata            | Chase 18928    | DNA         | KEW DNABank          | UK      | JX903666   | JX903250   | JX903940 | JX903520 |
| Astrocaryum mexicanum     | Chase 21299    | DNA         | KEW DNABank          | UK      | JX903667   | JX903251   | JX903941 | JX903521 |
| Butia capitata            | Chase 21298    | DNA         | KEW DNABank          | UK      | JX903668   | JX903252   | JX903942 | JX903522 |
| Calamus castaneus         | Baker 507      | DNA         | KEW DNABank          | UK      | JX903669   | *M81810    | JX903943 | JX903523 |
| Nypa fruticans            | Chase 12603    | DNA         | KEW DNABank          | UK      | JX903670   | JX903253   | JX903944 | JX903524 |
| Phoenix dactylifera       | Barrow 77      | DNA         | KEW DNABank          | UK      | JX903671   | JX903254   | JX903945 | JX903525 |
| Ravenea sambiranensis     | Chase 18152    | DNA         | KEW DNABank          | UK      | JX903672   | JX903255   | JX903946 | *EF128297 |
| Trachycarpus martianus    | Chase 30849    | DNA         | KEW DNABank          | UK      | JX903673   | JX903256   | JX903947 | JX903526 |
| Zingiberales              |                |             |                      |         |           |          |         |         |
| Cannaceae                 |                |             |                      |         |           |          |         |         |
| Canna indica              | D.K. Kim 08-190| Fresh       | Field work           | Korea   | JX903674   | JX903257   | JX903948 | JX903527 |
| Costaceae                 |                |             |                      |         |           |          |         |         |
| Costus woodsonii          | Chase 3911     | DNA         | KEW DNABank          | UK      | JX903675   | *AF243510  | JX903949 | JX903528 |
| Zingiberaceae             |                |             |                      |         |           |          |         |         |
| Roscoea cauticoides       | Chase 19223    | DNA         | KEW DNABank          | UK      | JX903676   | JX903258   | JX903950 | JX903529 |
| Zingiber mioga            | D.K. Kim 08-069| Fresh       | Field work           | Korea   | *GU180405  | *AF243850  | JX903951 | JX903530 |
| Poales                    |                |             |                      |         |           |          |         |         |
| Juncaceae                 |                |             |                      |         |           |          |         |         |
| Juncus effusus            | D.K. Kim 09-078| Fresh       | Field work           | Korea   | JX903677   | *L12681    | *AJ235509 | *AF547015 |
| Poaceae                   |                |             |                      |         |           |          |         |         |
| Phragmites australis      |                |             |                      |         |           |          |         |         |
| Typhaceae                 |                |             |                      |         |           |          |         |         |
| Typha orientalis          | D.K. Kim 09-011| Fresh       | Field work           | Korea   | JX903678   | JX903259   | JX903952 | JX903531 |
| Liliaceae                 |                |             |                      |         |           |          |         |         |
| Colchicaceae              |                |             |                      |         |           |          |         |         |
| Disporum sessile          | D.K. Kim 04-076| Fresh       | Field work           | Korea   | JX903651   | JX903235   | JX903925 | JX903506 |
| Disporum smithianum       | D.K. Kim 04-054| Fresh       | Field work           | Korea   | JX903652   | JX903236   | JX903926 | JX903507 |
Our estimated divergence time for the families in Asparagales is much younger than previously suggested by Janssen and Bremer [31], in which most families were indicated to be older than ca. 90 Ma. Orchidaceae is the largest and one of the ecologically and morphologically most diverse families of flowering plants [62]. Our results indicated that the most recent common ancestor of extant orchids lived in the Late Cretaceous (54–82 Ma), slightly overlapping the estimated age (76–84 Ma) based on the discovery of the first unambiguous fossil of Orchidaceae and a pollinator in amber [61]. Moreover, adding two newly described orchid fossils [63], Gustafsson et al. [64] reassessed the data and reported that all extant orchids shared a most recent common ancestor in the Late Cretaceous (ca. 77 Ma), suggesting that the diversification of orchids occurred in a period of global cooling after the early Eocene climatic optimum.

Iridaceae, with over 2,030 species in 65–75 genera, is the second largest family of Asparagales [65]. Based on plastid sequences and molecular clock techniques, Goldblatt et al. [65] inferred that Iridaceae diverged from the most closely related family, Doranthaceae, ca. 82 Ma and that the crown group of the family diverged in the late Cretaceous ca. 66 Ma. The divergence of the stem group was dated to ca. 75 Ma and crown group to ca. 58 Ma. Goldblatt et al. [65] used a secondary date for the calibration point of the root node of Iridaceae, and this was suggested not to be ideal.

The split between core Asparagales and the remaining families is estimated after the K/T boundary. Furthermore, our molecular phylogenetic analyses suggest multiple rapid radiations have inferred throughout the diversification of major groups of Asparagales. For example, the largest orchid subfamilies diversification occur in a period of global cooling [64] and the possible radiation of lineages of Nolinoideae revealed from this study.

The fossil record of Asparagales is comparatively poor, with few fossils attributable to families reaching back beyond the Late Cretaceous, perhaps because of the herbaceous habit and widespread zoophilous pollination [66]. The use of more fossils with more sophisticated prior distribution affords exciting opportunities for

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### Table 1. Cont.

| Family/Tribe Taxa | Vouchers | source type | Source (Institution) | Country | matK | rbcL | atpB | ndhF |
|------------------|----------|-------------|----------------------|---------|------|------|------|------|
| Disporum uniflorum | D.K. Kim 04-089 | Fresh | Field work | Korea | JX903653 | JX903237 | JX903927 | JX903508 |
| Lilium distichum | D.K. Kim 05-046 | Fresh | Field work | Korea | JX903654 | JX903238 | JX903928 | JX903509 |
| Lilium hansonii | D.K. Kim 05-026 | Fresh | Field work | Korea | JX903655 | JX903239 | JX903929 | JX903510 |
| Lilium tsingtauense | D.K. Kim 05-176 | Fresh | Field work | Korea | JX903656 | JX903240 | JX903930 | JX903511 |
| Luzuriagaceae | Drymophila moorei | R. Coveny et al., 6377 | Fresh | Field work | Korea | JX903657 | JX903241 | JX903931 | JX903512 |
| Melanthiaceae | Chionographis japonica | D.K. Kim 04-115 | Fresh | Field work | Korea | JX903658 | JX903242 | JX903932 | JX903513 |
| Heloniopsis orientalis | D.K. Kim 06-058 | Fresh | Field work | Korea | JX903659 | JX903243 | JX903933 | JX903514 |
| Veratrum maackii var. japonicum | D.K. Kim 06-129 | Fresh | Field work | Korea | JX903660 | JX903244 | JX903934 | JX903515 |
| Smilacaceae | Smilax china | D.K. Kim 04-096 | Fresh | Field work | Korea | JX903661 | JX903245 | JX903935 | JX903516 |
| Smilax riparia var. ussurensis | D.K. Kim 04-187 | Fresh | Field work | Korea | JX903662 | JX903246 | JX903936 | JX903517 |

Orders and families circumscriptions are as in APG III (2009) and Chase et al. (2009). The vouchers of all species studied were housed in source of institution.

KimJH, 2010: KIM, J. H., D. K. KIM, F. FOREST, M. F. FAY, AND M. W. CHASE. 2010. Molecular phylogenetics of Ruscaceae sensu lato and related families (Asparagales) based on plastid and nuclear DNA sequences. Annals of Botany 106: 775-790.

KimDK, 2012: KIM, D.K., J.S.Kim, J.H.Kim. 2012. The Phylogenetic Relationships of Asparagales in Korea Based on Five Plastid DNA Regions. Journal of Plant Biology 55: 325-341.

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### Table 2. Statistics for the four genes analysed in this study.

| Characters | atpB | matK | ndhF | rbcL | Combined |
|------------|------|------|------|------|----------|
| Aligned (bp) | 1472 | 1820 | 2234 | 1336 | 6862 |
| Included (bp) | 1431 | 1819 | 2163 | 1286 | 6699 |
| Parsimony uninformative | 144 | 216 | 298 | 144 | 767 |
| Parsimony informative | 499 | 1123 | 1160 | 437 | 3122 |
| Constant | 829 | 481 | 776 | 755 | 2810 |
| Transition/Transversion | 2.58 | 1.72 | 2.57 | 3.16 | 2.18 |
| G+C (%) | 42.5 | 31.8 | 37.2 | 35.4 | 38.2 |
| Tree length | 26510 | 8275 | 7192 | 3269 | 24168 |
| CI | 0.248 | 0.295 | 0.275 | 0.258 | 0.272 |
| RI | 0.713 | 0.766 | 0.755 | 0.735 | 0.747 |
| Variant rate (%) | 33.9 | 61.7 | 34.7 | 32.7 | 45.5 |

doi:10.1371/journal.pone.0059472.t002
The taxa used for this study included 253 species of 201 genera representing all families in Asparagales [20]. In addition, 29 species representatives of Arecales, Zingiberales, Commelinales, Poales, Liliales and Pandanales were included, with two species of Pandanales as the nominated outgroup. The plant material used was either fresh or dried, collected from the field and dried, taken from specimens in herbaria, from the DNA Bank of the Royal Botanic Gardens, Kew (http://data.kew.org/dnabank/DnaBankForm.html) or the Medicinal Plant Resources Bank of the Korea National Research Resource Centre (KNRRC) at Gachon University (for details, see Table 1). All necessary permissions and approvals for the described plant and specimen sampling were obtained from the respective curators, i.e. RBG Kew Gardens (Dr. M. W. Chase), Kunming Botanic Garden (MOU), Ivana Franka Botanic Garden (MOU), Australia Royal Botanic Garden (MOU), KEW DNA Bank. Voucher specimens of the taxa were prepared; source, voucher information and database accession numbers are listed in Table 1.

**DNA Extraction and Polymerase Chain Reaction Sequencing**

Total genomic DNA was extracted from 0.5–1.0 g fresh or silica gel-dried leaves using the 2× CTAB buffer method [67]. 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. The ATPB gene was amplified using the primers and protocols of White et al. [60], Nickrent and Soltis [69] and Soltis and Soltis [70]. The matK gene was amplified with the primers and protocols of Johnson and Soltis [71] and Hiu et al. [25]; ndhF was amplified with the primers reported by Terry et al. [72] and Olmstead et al. [73]; and rbcL was amplified with the primers and protocols of Olmstead et al. [74], Shinwari et al. [75] and Fay and Chase [76]. 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 MgCl2), 2.5 mM dNTPs, and 5 pmol μL−1 forward and reverse primers using a Perkin-Elmer 9700 (Applied Biosystems, ABI, Beverly, MA, USA). Dimethyl sulphoxide (DMSO; 2%) was added to reduce the secondary structure in the polymerase chain reaction (PCR). PCR conditions consisted of an initial denaturation at 94°C for 2 min, followed by 30–35 cycles at 94°C for 1 min, 50°C–55°C for 1 min and 72°C for 3 min, followed by a final 7-min extension 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 an ABI Prism BigDye Reaction Kit (ver. 3.1) in accordance with the manufacturer’s protocols. Products were run on an ABI 3700 Genetic Analyser according to the manufacturer’s protocols. Sequence editing and assembly of contigs were carried out using the Sequence Navigator and AutoAssembler software (ABI).

**Sequence Alignment**

All sequences were aligned initially in MUSCLE [77] and MacClade (ver. 4.0) [78] and then adjusted manually following the guidelines of Kelchner [79]. Manual alignment of rbcL and ATPB was accomplished easily because no insertions/deletions occurred for rbcL and they were rare for ATPB. In contrast, matK and ndhF were subject to length variation. These two genes were aligned and further edited manually by deleting small sections in which the homology of characters across taxa could not be determined with confidence. In total, the combined alignment was 6,699 characters in length (Table 2). The aligned matrix has been submitted as Appendix S1.

**Neighbour Net**

Neighbour nets have the attractive property of always being represented in the plane through a circular ordering of the taxa. Although closely related to split decomposition [80], for larger datasets, the neighbour-net method often provides better resolution than split decomposition due to the criterion used to calculate support for relationships among taxa [9]. To construct neighbour nets, the default settings in SplitsTree4 [81] were used, applying uncorrected P distances with gaps and ambiguous sites coded as missing data. Bootstrap support for internal splits, which define clusters, was calculated with 1,000 replicates.

**Parsimony Analysis**

PAUP* ver. 4.10b for Macintosh [82] was used for parsimony analysis. Tree searches were conducted using the Fitch (equal weight, EW) [83] criterion with 1,000 random sequence additions and tree bisection/reconnection (TBR) branch swapping, but permitting only five trees to be held at each step to reduce the time spent searching suboptimal “islands” of trees. All shortest trees collected in the 1,000 replicates were swapped on to completion without a tree limit. DELTRAN character optimisation was used to illustrate branch length throughout. To evaluate internal support, 1,000 bootstrap replicates were conducted with equal weights (EW) and successive approximation weights (SW; [84]), and TBR branch swapping with five trees held at each step and simple taxon addition [85]. The following descriptions for categories of bootstrap percentages were used: weak, ≤ 74; moderate, 75–88; well supported, 85–100 [14].

**Bayesian Analysis**

Further phylogenetic analyses were performed using BI as implemented in MrBayes ver. 3.12 [86]. PAUP* ver. 4.10b and MrModeltest ver. 2.2 [87] were used to determine the best model of DNA substitution for each partition by evaluating all models against defaults of the programme. The GTR+G model (a general time-reversible model with a proportion of invariable sites and a gamma-shaped distribution of rates across sites) was chosen as the best-fit substitution model in all four partitions. Consequently, the combined data matrix was assigned a model of six substitution types (α = 6) with a proportion of invariable sites. Four simultaneous Markov chain Monte Carlo (MCMC) chains were run for 1 × 10⁷ generations and sampled every 1,000 generations, and the first 25% sampled trees were excluded as burn-in. Post-burn-in samples of trees were used to construct a 50% majority rule consensus cladogram in PAUP* ver. 4.10b. The proportions of bifurcations found in this consensus tree are given as posterior clade probabilities (PPs). Bayesian analysis was performed twice to ensure convergence of the results.

**Molecular Dating and Fossil Calibration**

We used the combined dataset to estimate the age of origin of Asparagales using the programmes PATHd8 [34] and BEAST v1.7.4 [35,88]. The phylogenetic trees were constructed using MP with PAUP*4.0. The branch lengths on this tree were estimated using DELTRAN optimisation. We used the mean path length method of the PATHd8 programme. The MLE clock tests were used to test the molecular clock. The PATHd8 programme requires at least one reference point to be fixed. We used the oldest monocot fossil estimate of 120 Ma [89] as the fixed crown age of the root to calibrate the clock. BEAST v1.7.4 was also used to estimate the divergence times using multiple calibration points and a relaxed molecular clock approach. The BEAUti interface was used to create input files for BEAST with the tree priors set as follows: 1) age for the most recent common ancestor (MRCA) of extant Asparagales: exponential distribution with a mean of 2.0 and an offset 93 Ma that equalled the minimum age of the fossil (see discussion in [61], labelled # in Figure 3); 2) age for the MRCA of Zingiberales: exponential distribution with a mean of 2.0 and an offset 83.5 Ma which equalled the minimum age of the fossil (see [36,90], labelled # in Figure 3); 3) age for the root of the tree (The upper age constraint of 120 Ma for the calibrations above corresponds to the oldest known monocot fossil [89]; normal prior distribution with mean 106.5 Ma and standard deviation of 5.5 (giving a 95% CI ranging from 93–120 Ma, labelled # in Figure 3).

The general time-reversible (GTR+G) nucleotide-substitution model was used for the molecular clock model and Yule Process was chosen as speciation process for data set. Several short BEAST runs were first performed to examine the performance of the MCMC. After optimal operator adjustment, as suggested by the output diagnostics, three final BEAST runs each containing 10,000,000 generations were performed, and a tree was saved every 1,000 generations. All resulting trees were then combined with LogCombiner v1.7.4 [35], with a burn-in of ca. 45%. Log files were analysed with Tracer v1.5 [91], to assess convergence and confirm that the combined effective sample sizes for all parameters were enough. A maximum credibility tree was then
produced using TrecAnnotator v1.7.4 [35,88]. These were visualised using FigTree v.1.3.1 with means and 95% HPDs of age estimates. An XML file for analyses has been submitted as Appendix S2.

Supporting Information

Appendix S1 The aligned data matrix in this study (Nexus).

Appendix S2 The XML file used for divergence time estimates in BEAST analysis.

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Author Contributions

Conceived and designed the experiments: JWK MWC.Performed the experiments: DKK JWK. Analyzed the data: SC DKK. Contributed reagents/materials/analysis tools: JHK DKK MWC. Wrote the paper: SC JHK DKK MWC.
