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Quality and quantity of data recovered from massively parallel sequencing: Examples in Asparagales and Poaceae[1]

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- *Premise of the study:* Genome survey sequences (GSS) from massively parallel sequencing have potential to provide large, cost-effective data sets for phylogenetic inference, replace single gene or spacer regions as DNA barcodes, and provide a plethora of data for other comparative molecular evolution studies. Here we report on the application of this method to estimating the molecular phylogeny of core Asparagales, investigating plastid gene losses, assembling complete plastid genomes, and determining the type and quality of assembled genomic data attainable from Illumina 80–120-bp reads.
- *Methods:* We sequenced total genomic DNA from samples in two lineages of monocotyledonous plants, Poaceae and Asparagales, on the Illumina platform in a multiplex arrangement. We compared reference-based assemblies to de novo contigs, evaluated consistency of assemblies resulting from use of various references sequences, and assessed our methods to obtain sequence assemblies in nonmodel taxa.
- *Key results:* Our method returned reliable, robust organellar and nrDNA sequences in a variety of plant lineages. High quality assemblies are not dependent on genome size, amount of plastid present in the total genomic DNA template, or relatedness of available reference sequences for assembly. Phylogenetic results revealed familial and subfamilial relationships within Asparagales with high bootstrap support, although placement of the monotypic genus *Aphyllanthes* was placed with moderate confidence.
- *Conclusions:* The well-supported molecular phylogeny provides evidence for delineation of subfamilies within core Asparagales. With advances in technology and bioinformatics tools, the use of massively parallel sequencing will continue to become easier and more affordable for phylogenomic and molecular evolutionary biology investigations.

Key words: angiosperms; *Aphyllanthes*; chloroplast; DNA barcoding; mitochondria; next-generation sequencing; nuclear ribosomal DNA; phylogenetics; plastid.

Advances in DNA sequencing technologies have enhanced evolutionary biology investigations, but the typically nontargeted approach to next-generation sequencing and the volume of data produced by these technologies have hindered use of next-generation sequence data in large-scale molecular systematic studies (Steele and Pires, 2011; Harrison and Kidner, 2011). Questions have emerged regarding data obtainable and the robustness of assembled sequences (e.g., Nock et al., 2011). In this study we reveal answers to these questions through phylogenomic analyses of two monocot plant lineages, Asparagales and Poaceae.

Massively parallel sequencing (MPS, often called next-generation sequencing) technologies have revolutionized comparative research across the life sciences by allowing genomic sequencing of many more organisms than was previously possible. MPS methods can provide orders-of-magnitude more data than traditional Sanger sequencing at a reduced cost per nucleotide (see recent reviews by Ansorge, 2009; Lister et al., 2009; Pettersson et al., 2009; Metzker, 2010). While this technology is allowing unprecedented access to raw sequence data, storing, managing, and processing such data remains daunting. MPS is being used in a wide range of applications from whole nuclear genome sequencing of humans for health purposes (e.g., James D. Watson, Wheeler et al., 2008) to functional genomics (Morozova and Marra, 2008) to assessment of genetic biodiversity across multispecies communities (Steele and Pires, 2011).

Genome survey sequencing (GSS) presents an enticing alternative to complete genome sequencing and assembly. GSS utilizes nontargeted MPS of total genomic DNA to shallowly sequence the entire genomic compliment with low coverage and redundancy. While GSS projects do not aim to assemble complete nuclear genome sequences, genomic elements present in high copy number, including organellar (plastid and mitochondrial) and nuclear ribosomal genes (nrDNA), are easily assembled. The terms plastome and mitogenome have been described in various contexts, referring to only the genic (coding) portions of the genome or the entire genomic compliment. Here we use plastome and mitogenome to refer to the complete

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genome in each respective organelle, including introns and intergenic regions. We describe how GSS data can be used to assemble these genomes through a combination of de novo and reference-based assembly approaches.

Standards for complete genome sequencing require high coverage to ensure assembly and prevent sequencing errors. In fact, many questions in comparative genomics are impossible to answer with sparse coverage (Green, 2007). However, low coverage GSS has yielded impressive results when comparisons with closely related reference species are sought. For example, overlaying 0.66× coverage of the pig genome to a human–mouse alignment revealed comparisons between 38% of the coding fraction of the genome (Wernersson et al., 2005). Similar coverage (0.1×) in scuttle fly allowed almost complete reconstruction of the mitogenome as well as information about repetitive elements and some functional genes (Rasmussen and Noor, 2009). When syntenically aligned to a well-assembled and annotated reference genome, sparse sequences of related taxa can provide such robust information to infer levels of recombination, introgression, and chromosomal restructuring (Kulathinal et al., 2009).

The studies cited used either conventional Sanger sequencing or 454 MPS data. While these methods provide relatively long sequence reads (~800 and ~400 bp, respectively), they are costly and/or labor intensive. Illumina (formerly Solexa) sequencing is an alternative MPS technology that provides shorter sequence reads (for this study, ~80–120 bp) at a much lower cost per taxon. Nock et al. (2011) sequenced total genomic DNA on one Illumina lane (36-bp reads) per taxon for five grass species. When compared to a previously sequenced rice plastome reference, they were able to assemble complete plastomes for the target species with 100–750× median coverage. Their success contrasts with prior expectations that plastomes could only be assembled from sequencing of DNAs enriched for plastids (i.e., chloroplast isolations, Atherton et al., 2010).

Plastomes are targeted for MPS projects because of their phylogenetic utility (Shaw et al., 2005, 2007) and high frequency relative to low-copy nuclear genes in total genomic DNA extractions. The majority of the nearly 200 plastomes available on GenBank are generally conserved in genome size, gene content, and gene order. Most have a quadripartite structure with a large single-copy (LSC) region and a small single-copy (SSC) region with two copies of a large inverted repeat (IR) region separating them. For seed plants, these plastomes generally range in size from 108 to 165 kb, containing 110–130 genes (reviewed by Raubeson and Jansen, 2005; Bock, 2007). For nearly a decade, targeted sequencing of full plastomes has been employed to address recalcitrant problems in plant systematics (e.g., Goremykin et al., 2003; Pombert et al., 2005; Leebens-Mack et al., 2005, Turmel et al., 2006; Jansen et al., 2007; Moore et al., 2007; Givnish et al., 2010). The earliest of these studies (Goremykin et al., 2003) sparked well-placed concern about the influence of long-branch attraction in studies with sparse taxon sampling and thousands of characters (e.g., Soltis and Soltis, 2004; Stefanović et al., 2004; Leebens-Mack et al., 2005). More recent studies strove to increase taxon sampling and seriously consider the impact of long-branch attraction (Givnish et al., 2010). Further, plastome-based phylogenetic analyses are now being applied to understanding relationships at lower taxonomic levels (Goremykin et al., 2003; Soltis and Soltis, 2004; Stefanović et al., 2004; Leebens-Mack et al., 2005; Pombert et al., 2005; Turmel et al., 2006; Jansen et al., 2007; Moore et al., 2007; Parks et al., 2009; Givnish et al., 2010). Whereas all of these studies relied largely on targeted plastome sequencing, GSS offers new opportunities for cost-effective sequencing, assembly, and analysis of many more individuals for robust phylogenetic inference across a wide range of taxonomic levels.

Obtaining sequences from nuclear and organellar genomes from Illumina GSS has been proposed for a broad range of systematic applications (Steele and Pires, 2011), including use as a DNA barcode for species identification (Hollingsworth, 2011). Compared to the plastome, little is known about the evolution of plant mitogenomes, partly due to larger size of this organellar genome (Alverson et al., 2010), high rates of evolution (Adams et al., 2002), and fewer targeted sequencing efforts. Additional information about plant mitogenomes could prove useful for comparative studies. High-copy nrDNA loci should also be easy to assemble, and these sequences can provide independent confirmation of species identification or phylogenetic signal.

Despite the apparent advantages to assembling plastomes, mitogenomes, and nrDNA from GSS, several outstanding questions hinder implementation of these methods in a wider breadth of taxa. First, most genome sequencing projects to date, including GSS, have targeted taxa with relatively small genome sizes. Larger genomes have higher repetitive element compliments that not only obscure genic content in genomes, but also confound efforts to reliably assemble large genomic contigs, or contiguous sections of assembled short reads (Rabinowicz and Bennetzen, 2006). It is unclear how genome size, which can vary dramatically among plant lineages (Bennett and Leitch, 2011), affects assembly quality for both nuclear genes and organellar genomes (Nock et al., 2011). Second, little work has investigated how phylogenetic distance of reference taxa affects assembly quality of the target genome (Nock et al., 2011), particularly in nonmodel taxa.

Asparagales is an economically and horticulturally important order of plants in the monocot clade of angiosperms that includes onions, asparagus, agave, orchids, and irises. The core Asparagales comprises a well-supported monophyly of asparagoid plants. Recently, the number of families in the core Asparagales has been reduced from 14 to three, and clades within these families have been given subfamily status (APG III, 2009; Chase et al., 2009). Relationships among these families and subfamilies have, for the most part, been resolved but with low bootstrap support for some clades (Fig. 1). In particular, the monotypic genus *Aphyllanthes* (Aphyllanthoideae) was placed sister to both Scilloideae (Fay et al., 2000) and Lomandroideae (Pires et al., 2006). The position of *Aphyllanthes* was labile in trees published by Kim et al. (2010): sister to Brodiaeoideae in their combined maximum-parsimony tree (bootstrap value = 81) and sister to Agavoideae in their Bayesian analysis (posterior probability = 0.37). Pires et al. (2006) found that inclusion of *Aphyllanthes* in phylogenetic analyses of Asparagales greatly changed relationships outside Aphyllanthoideae. To date, only one complete plastome in Asparagales has been placed in GenBank (*Phalaenopsis aphrodite* subsp. *formosana,* Orchidaceae; Chang et al., 2006), and most of the protein-coding genes have been published for 14 additional species within the Asparagales (Leebens-Mack et al., 2005; Jansen et al., 2007; Givnish et al., 2010). For the present study, we analyzed 50 Asparagales plastome sequences assembled from GSS data and used these assemblies to evaluate relationships among major clades within the order.

More generally, we investigated the use of GSS to (1) estimate phylogenetic relationships in core Asparagales from all plastid and selected mitochondrial and nrDNA genes, (2)

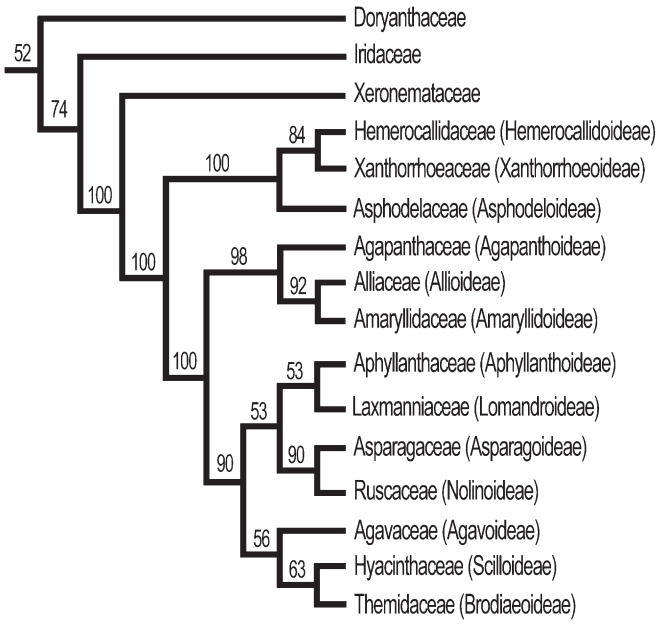

Fig. 1. Partial cladogram (reproduced from Pires et al., 2006) of Asparagales relationships inferred from plastid (*atpB*, *matK*, *ndhF*, *rbcL*, *trnL* intron, and *trnL-F* intergenic spacer) and mitochondrial (*atp1*) sequence data. Numbers above branches indicate MP bootstrap values based on 1000 replicates. Taxonomy follows APG II (2003) with APG III (2009) and Chase et al. (2009) names in parentheses.

assemble complete plastomes for selected taxa, and (3) evaluate suitability and robustness of these methods for broad-scale phylogenetic studies. Taxonomic sampling included a wide range of taxa from Asparagales, and limited sampling of grass (Poaceae) species, for which there are published sequences available for comparison. We implemented both reference-based and de novo sequence assembly methods to test reliability of our assembled sequences. We found that our easily implemented, low-cost approach to sequencing total genomic DNA can return reliable, robust organellar and nuclear ribosomal sequences in a variety of plant lineages. We used these results to test whether robust sequences are dependent on genome size, amount of plastid present in the total genomic DNA template, or availability of closely related reference sequences.

MATERIALS AND METHODS

***Taxon sampling*—**Sampling included taxa (quantities in parentheses) from several genera in each subfamily of core Asparagales: Amaryllidaceae-Agapanthoideae (1), Allioideae (4), Amaryllidoideae (4); Asparagaceae-Agavoideae (6), Aphyllanthoideae (1), Asparagoideae (3), Brodiaeoideae (6), Lomandroideae (3), Nolinoideae (8), and Scilloideae (5); Xanthorrhoeaceae-Asphodeloideae (4), Hemerocallidoideae (1), Xanthorrhoeoideae (1); and Xeronemataceae (1). Outgroups were chosen based on previous phylogenies of Asparagales (Pires et al., 2006) and included Iridaceae (1) and Doryanthaceae (1). The 50 samples, including source/collection information and taxonomic assignment, are listed in Appendices 1 and 2. We obtained genome size estimates for our Asparagales taxa via flow cytometry at the Benaroya Research Institute at Virginia Mason in Seattle, Washington using a protocol modified from Arumuganathan and Earle (1991) (Appendix S1, see Supplemental Data with the online version of this article). When fresh leaf material from the exact accession was not available, we averaged genome sizes from individuals of the same species or used values reported from the RBG Kew Angiosperm DNA C-values database (Bennett and Leitch, 2010).

We sequenced limited taxa from an additional monocot lineage to evaluate the robustness of assembled sequences. Poaceae is comprised of many agriculturally and ecologically important herbaceous species, for which complete genome sequences have been published or are in progress for many taxa. Three taxa (*Oryza sativa* subsp. *japonica* cv. Nipponbare, *Sorghum bicolor* cv. BTx623, and *Zea mays* subsp. *mays* cv. B73, hereafter *Oryza*, *Sorghum*, and *Zea* B73) have substantial genomic information, including complete cytotype-specific plastomes, available through GenBank. These taxa were sequenced because the wealth of available genomic information allows them to serve as controls for the efficacy of our sequence and assembly methods, especially in the presence of structural variation (i.e., plastomes in Poales, Doyle et al., 1992; Bortiri et al., 2008; Guisinger et al., 2010).

***Illumina sequencing*—**Methods for Illumina sequencing are explained briefly here with details in online Appendix S1. We extracted total genomic DNA from ca. 20 mg silica dried or an equivalent amount of fresh leaf tissue using a DNeasy Plant Mini Kit (Qiagen, Germantown, Maryland, USA). For Asparagales taxa, we performed RT-PCR to obtain a Ct (cycle threshold) value, or number of cycles required to reach the florescence threshold (indicating a signal stronger than background fluorescence). In our case, smaller Ct values indicated more plastome present in total genomic DNA. All taxa except *Asparagus asparagoides* had a Ct value less than 21.0. Two samples (*Camassia* and *Hosta*), were sequenced from chloroplast isolations.

For Illumina library preparation, we performed end repair on 2–5 μg of sheared genomic DNA prior to ligating barcoding adapters for multiplexing, using NEB Prep kit E600L (New England Biolabs, Ipswich, Massachusetts, USA). Oligonucleotides used as adapters are shown in online Appendix S1. Adapter preparation included combining equal volumes of each adapter at 100 μmol/L in a tube, floating them in a beaker of water (preheated to boiling) resting on a bench-top for 30 min, and then snap cooling them on ice. We size-selected samples for ~300 bp and enriched these fragments using PCR in 50 μL volumes containing 3 μL of ligation product, 20 μL of ddH2O, 25 μL master mix (from NEB kit), and 1 μL each of a 25 μmol/L solution of each forward and reverse primer (forward 5′-AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CTT TCC CTA CAC GAC GCT CTT CCG ATC* T-3′; reverse 5′-CAA GCA GAA GAC GGC ATA CGA GAT CGG TCT CGG CAT TCC TGC TGA ACC GCT CTT CCG ATC*-3′; both HPLC purified). Thermal cycle routine was as follows: 98°C for 30 s, followed by 15 cycles of 98°C for 10 s, 65°C for 30 s, and 72°C for 30 s, with a final extension step of 72°C for 5 min. Enriched products were run against a size standard on a 2% low-melt agarose gel at 120 V for 1 h. Using x-tracta disposable gel extraction tools (USA Scientific, Ocala, Florida, USA), the complete band was extracted from the gel, and samples were purified with a Gel Extraction kit (Qiagen). We sent the final product to the University of Missouri DNA Core for quantitation, fragment size verification, and sequencing on an Illumina GAIIx Genome Analyzer using the Illumina Cluster Generation Kit v2-GA II, Cycle Sequencing Kit v3, and image analysis using Illumina RTA 1.4.15.0. All samples ran on one sixth of an Illumina lane with single-end 80- or 120-bp reads.

***Sanger sequencing*—**We performed Sanger sequencing of select plastome regions (*clpP*, *ndhF*, *rpL32*, *rps16*, and *rps19*) to confirm gene loss for selected taxa. Amplifications were performed using PCR in 25 μL volumes containing 15.75 μL of ddH_2O, 7.5 μL of FailSafe buffer—PreMix C (EPICENTRE Biotechnologies, Madison, Wisconsin, USA), 0.25 μL of a 20 μmol/L solution of each forward and reverse primer, 0.25 μL of *Taq* polymerase (Invitrogen, Carlsbad, California, USA), and 1 μL of unquantified DNA template. Forward and reverse primers are as follows: *clpP*: 5′-GGC TCC GTT CAG AAA ATA CC-3′, 5′-GCT GAC ATA AAA RYA TCC C-3′; *ndhF*: 5′-CAC AAA GAG AGA GCG TAC C-3′, 5′-CT TTT ATG GCA GCT AAA TAT CC-3′; *rpl32*: 5′-GGC AGT TCC AAA AAA GCG-3′, 5′-CCA AGA TTT TAT TAC TTG TTT GTC G-3′; *rps16*: 5′-GGA ATG TTA TGG TAA AAC WTC G-3′, 5′-CCT WAA AAT ATC ATG AAC AGT TCC-3′; *rps19*: 5′-CGA GAT TTA TTA TCG TTT CTC GC-3′, 5′-CCT TTT GTA GCT AAT CAT TTA TCC-3′. PCR reaction conditions were as follows: one round of amplification consisting of denaturation at 96°C for 3 min, 53°C for 45 s, and extension at 72°C for 1 min; followed by 35 cycles of 94°C for 35 s, annealing temperature for 45 s, and 72°C for 1 min; with a final extension step of 72°C for 12 min. Amplifications were visualized on 1% agarose gels with ethidium bromide and a size standard to estimate fragment sizes and DNA concentration. PCR amplicons were cleaned with QIAquick PCR Purification kit (Qiagen). Sequencing was conducted at the DNA Core Facility at the University of Missouri-Columbia, using ABI Big Dye chemistry.

Sequence assembly, annotation, and analysis—We parsed raw reads from sequencing of a single Illumina lane into six bins (one for each taxon in the lane) and removed barcoding adaptor tags using a custom perl script (Appendix S1). The same scripts also deleted sequences containing more than five ambiguous states (represented in raw sequence data as "N"). We employed a reference-based assembly strategy to mine GSS for desired sequences using YASRA (Yet Another Short Read Assembler, http://www.bx.psu.edu/miller_lab/), a reference-based assembly algorithm designed for assembly of short reads into organellar genomes (Ratan, 2009). We used high quality sequences from closely related taxa as references downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank) to assemble target sequences using the medium threshold parameter (~85% similarity) in YASRA.

Asparagales sequence assembly, annotation, and analysis—The ultimate goal of plastome assembly is to obtain a single contig representing all portions of the plastid genome, including the IR, LSC, and SSC. Toward that end, we used an iterative process to extend the flanking regions of contigs to join them together into a single sequence for Asparagales. We input the initial result from YASRA containing multiple contigs into the program Geneious Pro 5.0.4 (Drummond et al., 2011) to align overlapping regions. The resulting sequence was fed back into YASRA as the reference sequence and run against the entire compliment of Illumina reads from that sample. This process was repeated as many times as was necessary to obtain a complete plastome, which was annotated in Geneious. The last step was to input the complete plastid sequence into YASRA as the reference to obtain accurate summary statistics for that taxon. The percentage plastome reported here is the percentage of reads saved and integrated into the assembly from the full complement of Illumina reads, while plastome coverage indicates the average depth of coverage (i.e., 50× coverage of 120 000-bp template).

We used two methods to confirm reference-based plastome assembly results. First, forward and reverse Sanger sequences of all PCR products verifying missing genes were assembled, edited, and aligned with assemblies in Geneious. Second, we assembled complete plastomes of three Asparagales taxa—*Asparagus officinalis* (Asparagoideae), *Sansevieria trifasciata* (Nolinoideae), and *Trichopetalum plumosum* (Lomandroideae)—with the de novo assembler in the program NextGene (SoftGenetics, State College, Pennsylvania, USA). NextGene was run in an iterative process with CAP3 (Huang and Madan, 1999) for additional assembly of NextGene output contigs (Appendix S1). We assembled whole plastid genomes for these species by verifying reference-based assemblies with assembled de novo contigs. Assembling complete plastomes for all taxa was outside the scope of this study because we only used genic regions for phylogenetics.

The lability of size and structure in plant mitogenomes makes assembly difficult, especially given the paucity of available reference sequences. Furthermore, reference-based assemblies for entire mitogenomes in monocots are computationally intensive and generate hundreds or even thousands of contigs (K. L. Hertweck, unpublished data), making them suboptimal for large-scale phylogenetic studies. We selected the five most frequently published mitochondrial genes for Asparagales in GenBank (*atp4*, *atp6*, *cox3*, *nad9*, and *rps13*) and performed a single iteration of reference-based assembly in YASRA on all taxa. The same procedure was repeated for each of three coding nrDNA regions.

Phylogenetic analyses—All sequences used for phylogenetic analyses were uploaded to GenBank (accession numbers in Appendix 1). Plastid genes and mitochondrial genes were concatenated separately to represent one marker each, and then aligned in Geneious using the MAFFT (Katoh et al., 2002) plug-in. Concatenated chloroplast genes, concatenated mitochondrial genes, and concatenated nrDNA genes were analyzed independently, and then all were combined into one data set for analysis to evaluate total evidence (TreeBase accession 12173).

Maximum parsimony (MP) analyses were performed with the program PAUP* 4.0b10 (Swofford, 2003) on the plastid marker and combined data set. For each data set, heuristic searches were conducted using 100 random addition replicates with tree-bisection-reconnection (TBR) branch swapping. Additionally, searches used characters equally weighted, gaps treated as missing, and the MulTrees option. Support for internal nodes was assessed using bootstrap analysis (Felsenstein, 1985) of 500 replicates with 1 random addition per replicate.

Maximum likelihood (ML) analyses were performed using the program Garli 0.951 (Zwickl, 2006), accessed through the online CIPRES Science Gateway (http://www.phylo.org) with the default model of evolution (GTR+I+G). The ML analyses used the automated stopping criterion, terminating a search when the likelihood score remained constant for 20 000 consecutive generations. Likelihood scores of the optimal tree generated by Garli were calculated using PAUP*, which better optimizes branch lengths (Zwickl, 2006). ML bootstrap analyses were performed in Garli on 500 replicates using an automated stopping criterion set at 20 000 generations.

Resolution of the phylogenetic placement of *Aphyllanthes* within the core Asparagales has been a long-standing point of contention (Fay et al., 2000; Pires et al., 2006; Kim et al., 2010). The difficulty in placing Aphyllanthes may be due to long-branch artifacts (Felsenstein, 1978; Hendy and Penny, 1989) or real variation in single gene histories due to recombination following lineage sorting, hybrid speciation, or interspecific gene flow. Recombination among plastid genes is not generally expected, but we assessed the possibility of variation in phylogenetic signal within the plastid genome sequences using the network analyses implemented in the program Splitstree v.4.0 (Huson, 1998). A Neighbor-Net (Bryant and Moulton, 2004) analysis was performed to visualize incompatible splits in networks calculated on the plastome data matrix. In addition, recombination among regions within the plastome was tested using the Phi test (Bruen et al., 2006) implemented in Splitstree.

Poaceae sequence assembly, annotation, and analysis—For grasses, we assembled plastomes using the published sequence for each taxon, which should be highly similar to the assembly. We reported values from the first complete YASRA assembly for Poaceae and indicate the total number of contigs generated per assembly as a measure of the difficulty of assembling that target genome. Fewer and longer contigs are preferable for ease of assembly and annotation. We also tested the effect of phylogenetic distance of the reference from the target taxon on assembly quality by reassembling each of the grass genomes with 11 reference sequences (*Oryza*, *Triticum*, *Agrostis*, *Bambusa*, *Zea*, *Sorghum*, *Typha*, *Phoenix*, *Dioscorea*, *Amborella*, *Cycas*), ranging from closely related grasses to a distantly related cycad (GenBank accession numbers for reference sequences are listed in Appendix S2, see online Supplemental Data). The final step of YASRA reports the percentage sequence identity (similarity) between the reference and target sequences, which provides a crude estimate of phylogenetic distance. We also assembled plastomes from *Oryza*, *Sorghum*, and *Zea* B73 de novo contigs to evaluate their quality.

We evaluated how relative size of the target and reference plastomes affects plastome assembly in Poaceae using the genome length ratio (GLR), the ratio of the size (length in bp) of the target taxon to the reference taxon. We interpret this ratio as follows: GLR = 1 indicates target and reference plastomes are nearly equal in length, GLR > 1 indicates the target taxon plastome is larger than the reference and GLR < 1 indicates the target plastome is smaller than the reference.

RESULTS

Genome size in Asparagales—Genome sizes are represented as pg/2C, or mass of DNA in a diploid (somatic) cell. In Asparagales, these values ranged from 1.3 pg/2C in *Aphyllanthes* to 50.9 pg/2C in *Amaryllis*; the average genome size for the 43 taxa for which data were available was 16.9 pg/2C (SD = ±13.8).

Ct values in Asparagales—Our samples had a Ct value of 21.0 or below with the exception *Asparagus asparagoides* (Ct = 24.1), as we were unable to obtain a DNA sample with a Ct value within the desirable range. The lowest Ct value for our samples was 14.2 in *Trichopetalum*, and the average Ct value was 17.5 (SD = ±1.8).

Plastome assembly in Asparagales—For two samples (*Camassia* and *Hosta*), sequencing was conducted from chloroplast isolations. Whole plastome sequences, including 79 protein-coding genes were recovered for each of the 50 samples. Several taxa were missing genes or contained pseudogenes (Appendix 2). For samples sequenced from total genomic DNA, the total number of reads ranged from 1.28 million (*Agapanthus*) to 6.86 million (*Brodiaea*). The percentage of Illumina reads assembling into plastomes ranged from 0.5 to 13.3%

(*Scadoxus* and *Cordyline*, respectively), while average plastome depth of sequence ranged from 12.5 to 482.8× (*Eucharis* and *Cordyline*).

Assembly of whole, contiguous plastomes verified by de novo assemblies did not alter the reference-based assemblies for *Sansevaria trifasciata* (Nolinoideae) and *Trichopetalum plumosum* (Lomandroideae). However, this verification revealed two small (ca. 15 bp each) mis-assemblies in noncoding regions of *Asparagus officinalis* (Asparagoideae) which were subsequently corrected. All three plastid genomes had the same genome structure and gene arrangement as that of the typical land plant genome of *Nicotiana tabacum* (Wakasugi et al., 2001) with the exception of *trnH* and *rps19* shifting from the large single-copy region into the inverted repeat. For the circular sequence map of genes for *A. officinalis*, the complete genome was 154 664 bp in length (LSC = 83 821 bp, SSC = 17 901 bp, and IRa/b = 26 471 bp each; Fig. 2).

We tested the effects of genome size and Ct value on plastome assembly using only the 48 taxa sequenced from total genomic DNA. Plastome coverage generally increased as percentage plastome increased (Fig. 3A), but we tested both genome size and Ct value against each variable for confirmation. Ct value was unrelated to genome size (Fig. 3B). Removing an outlier (*Asparagus asparagoides*, with a Ct value higher than our desired threshold) had little impact on the relationship. As genome size increased, both percentage plastome and plastome coverage decreased, although relationships were weak (Fig. 3C, D). Finally, there was no correlation between Ct value and either percentage plastome or plastome coverage (Fig. 3E, F).

***Mitochondrial gene assembly in Asparagales*—**We attempted to assemble five mitochondrial genes for each taxon (online Appendix S3): *atp4* (complete coding region ~588 bp in length), *atp6* (819 bp), *cox3* (798 bp), *nad9* (573 bp), and *rps13* (351 bp). No mitochondrial genes were recovered from the two samples sequenced from chloroplast isolations (*Hosta* and *Camassia*) or from *Agapanthus* and *Oziroe*. We were able to assemble multiple genes for each of the other taxa. Average depth of sequence coverage was much lower than plastome assemblies and varied by gene; assemblies contained less than 0.01% of total reads. Additionally, the entire coding region for each gene was not recovered for every sample, and ranged as follows: 157–604 bp (*atp4*), 213–820 bp (*atp6*), 190–810 bp (*cox3*), 172–583 bp (*nad9*), and 161–351 bp (*rps13*).

Unlike our plastid genomes, our mitochondrial gene YASRA-based assemblies had low average sequencing depth. Therefore, we looked at genes from a subset of the taxa that had low overall numbers of reads obtained from sequencing and found that we were unable to align reads across the entirety of some of the genes. For this reason, those genes are unsuitable for phylogenetic analysis.

However, samples with greater total reads assembled mitochondrial genes with extensive depth (see Appendix S3). Therefore, our work illustrates that, given the degree of sequencing provided by some of our samples, our method is suitable for assembling mitochondrial genes for phylogenetic construction.

***Nuclear ribosomal DNA assembly in Asparagales*—**We attempted to assemble three nrDNA genes for each sample (Appendix S3): 18S (1790 bp), 5.8S (115 bp), 26S (estimated to be

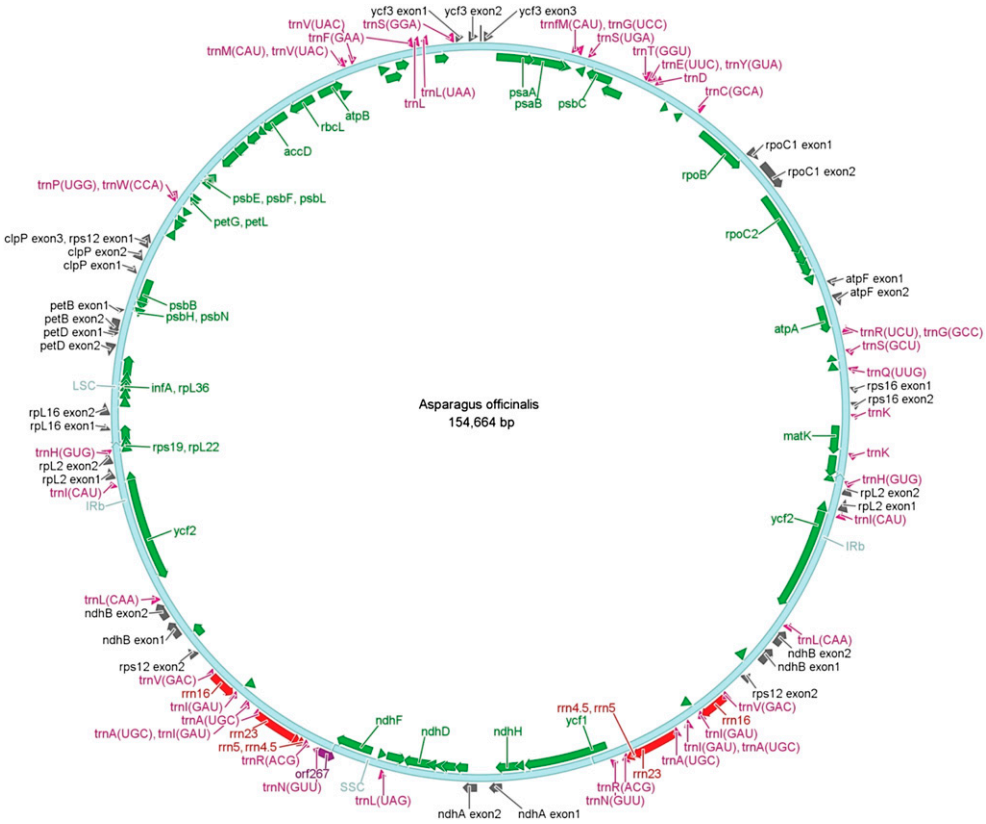

Fig. 2. Complete plastid genome of *Asparagus officinalis*, sequenced with Illumina technology from total DNA extractions. Total length = 154 664 bp.

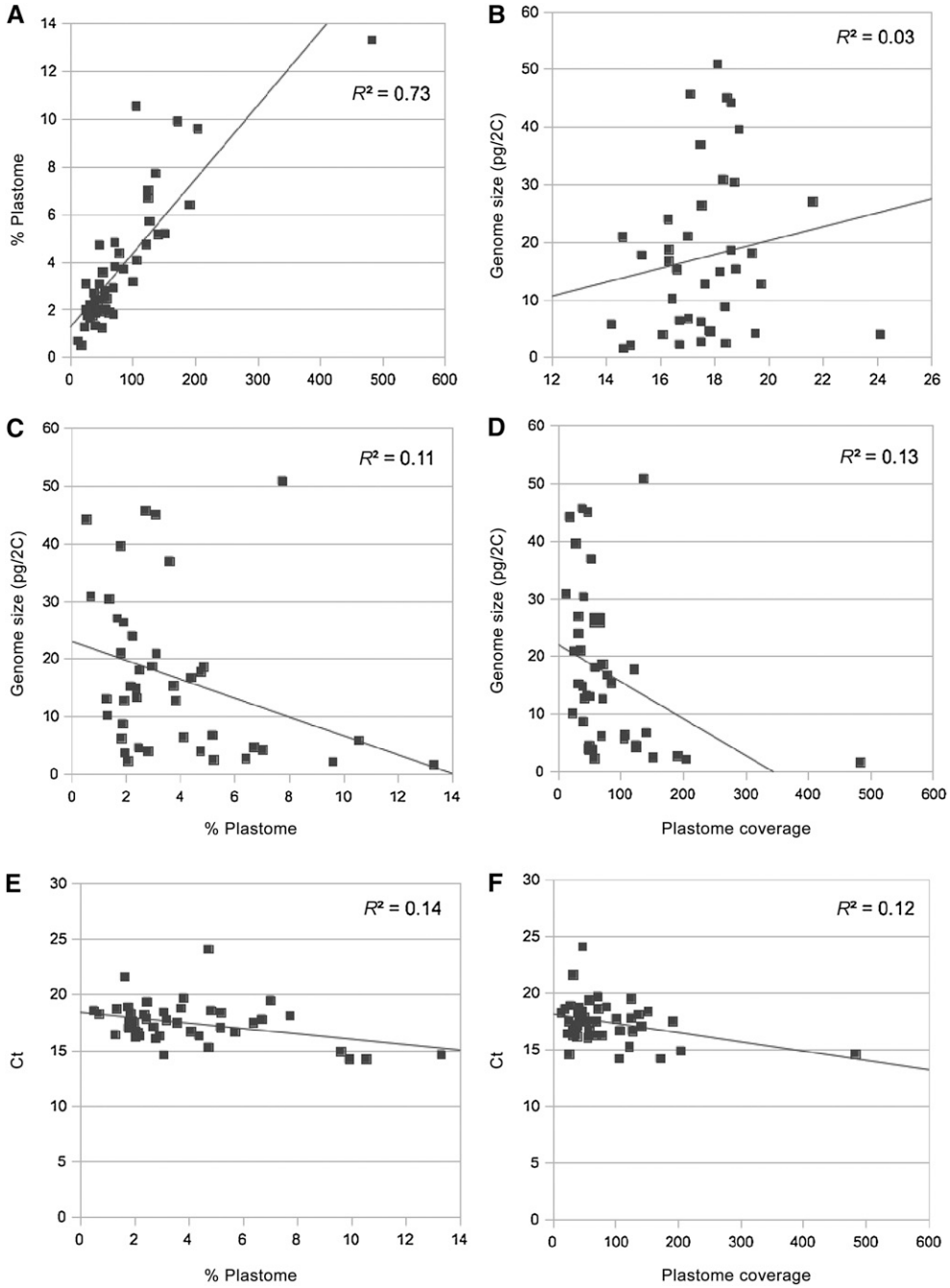

Fig. 3. Effect of Ct value and genome size on plastome assembly in Asparagales. (A) Average depth of coverage in plastome assembly and percentage of Illumina reads from the plastome; removal of (*Cordyline australis*) does not change relationship ($R^2 = 0.72$). (B) Ct value and genome size; removal of outlier (*Asparagus asparagoides*) does not change strength of relationship ($R^2 = 0.09$). (C) Percentage of Illumina reads from the plastome and genome size; removal of outlier (*Amaryllis belladonna*) slightly strengthens the relationship ($R^2 = 0.32$). (D) Average depth of coverage in plastome assembly and genome size; removal of outliers (*Cordyline australis* and *Amaryllis belladonna*) slightly strengthens the relationship ($R^2 = 0.4$). (E) Percentage of Illumina reads from the plastome and Ct value; removal of outlier (*Asparagus asparagoides*) strengthens the relationship ($R^2 = 0.25$). (F) Average depth of coverage in plastome assembly and Ct value; removal of outlier (*Cordyline australis*) decreases the strength of the relationship ($R^2 = 0.08$).

3360 bp). No nrDNA genes were recovered from the two samples sequenced from chloroplast isolations. For the other 48 samples, 18S was recovered in all samples, 5.8S in 45 samples, and 26S in 47 samples. Average read coverage was generally higher than that for plastomes, and the percentage of reads assembled into nrDNA genes was intermediate between plastome and mitochondrial gene results (<0.01–0.06%). One exception was in *Trichopetalum* for which percentages were 0.14% (18S and 5.8S) and 0.26% (26S). For those samples in which 5.8S was found, the entire coding region was recovered; however, as with some of the mitochondrial genes, the entire coding region for 18S and 26S was not recovered for all samples. Recovered

lengths varied as follows: 1085–1792 bp (18S) and 356–531 bp (26S). For none of the samples was the entire coding region for 26S recovered.

Phylogenetic analysis in Asparagales—*Plastid data*—Plastid genes for phylogenetic analysis were relatively consistent in length and presence throughout the data set (Appendix S3). Average pairwise identity for all samples ranges from 85.3% similarity in *ycf1* to 99.3% in *ndhB* with an average of 95.8%. Percentage of identical sites ranges from 31.6% in *ycf1* to 91.7% in *ndhB*. Two genes for Trichopetalum (*ycf1* and *accD*) had less than 55% sequence identity. Seventy-nine protein-coding genes were concatenated into one plastid marker for an aligned matrix length of 73 585 bp (Table 1). MP and ML analyses yielded topologically identical trees (Fig. 4). All ingroup families and subfamilies were strongly supported as monophyletic. All intergeneric relationships were also strongly supported, except the sister relationship between *Smilacina* and *Calibanus* + *Dasylirion*, with a MP/ML bootstrap value (BSV) of 53/74. The placement of *Aphyllanthes* was moderately well supported (BSV = 60/71). Well-supported subfamily sister relationships included Brodiaeoideae sister to Scilloideae, Brodiaeoideae + Scilloideae sister to Agavoideae + Aphyllanthoideae, Nolinoideae sister to Asparagoideae, Nolinoideae + Asparagoideae sister to Lomandroideae, Amaryllidoideae sister to Allioideae, Amaryllidoideae + Allioideae sister to Agapanthoideae, and Hemerocallidoideae + Xanthorrhoeoideae sister to Asphodeloideae.

Among-site variation in phylogenetic signal across the plastome alignment was assessed by visualizing incomparable splits in a neighbor-net analysis (Bryant and Moulton, 2004) run in Splitstree 4.0 (Huson, 1998). As seen in the ML tree (Fig. 4), relationships among the Aphyllanthoideae, Agavoideae, and Brodiaeoideae + Scilloideae clades within the Asparagaceae were ambiguous (Appendix S3). Whereas alternative networks are apparent in the neighbor-net output, the links to *Aphyllanthes* were no more variable than those to any of the subfamily clades in the Asparagaceae. Moreover, the Phi test of Bruen et al. (2006) failed to detect evidence for recombination across the plastid genome ($p = 0.79$). Taken together, phylogenetic and network analyses of the plastome date suggests that the difficulty in placing *Aphyllanthes* within the Asparagaceae phylogeny is due to a rapid radiation of lineages leading to the Aphyllanthoideae, Agavoideae, and Brodiaeoideae + Scilloideae clades and a long branch leading to *Aphyllanthes* (Fig. 4; Appendix S3).

Mitochondrial data—Average pairwise identity for all five genes ranges from 96.7% similarity in *atp4* to 98.5% in *nad9* with an average of 97.8% (Appendix S3). Percentage of identical sites ranges from 67.6% in *atp4* to 86.9% in *cox3*. Concatenation of the five genes yielded an alignment 3256 bp in length for the 46 samples from which at least one gene was obtained (Table 1). The ML mitochondrial tree (Appendix S3) was poorly resolved, presented differing topology from the plastome tree, and yielded poor (below 50) bootstrap support. Those relationships with BSVs greater than 50 agree with the plastid tree, with two exceptions. The mitochondrial tree supported the sister relationship of *Amaryllis* + *Scadoxus* (BSV = 81) to *Eucharis* (BSV = 92), whereas the plastid tree supported *Amaryllis* + *Eucharis* (BSV = 100/100) sister to *Scadoxus* (BSV = 100/100). The mitochondrial tree supported a clade including *Gilliesia*, *Allium cepa*, and *Allium fistulosum* with low bootstrap support (BSV = 62), whereas the plastid tree supports these three samples plus *Tulbaghia* in a clade with strong bootstrap support (BSV = 100/100).

Nuclear ribosomal DNA data—Pairwise identity for the three concatenated genes (18S + 5.8S + 26S) is 96.7%, and the percentage of identical sites 63.3% (Appendix S3). The three-gene concatenated alignment for 48 taxa was 2445 bp in length (Table 1). The tree resulting from the ML analysis of the nrDNA marker resulted in most branches supported by BSVs below 50 (Appendix S3). Those relationships with BSVs greater than 50 agreed with the plastid tree, with two exceptions. The nrDNA tree supported a clade containing the four samples in Amaryllidaceae; however, relationships between genera varied from that inferred from the plastid dataset but with lower bootstrap support. The nrDNA tree supported a sister relationship between *Asphodeline* and *Kniphofia* (BSV = 99) with *Haworthia* and *Aloe* occurring elsewhere in the tree, whereas the plastid tree grouped these four samples from Asphodeloideae in a clade (BSV = 100/100) with *Haworthia* sister to *Aloe*, these two sister to *Kniphofia*, and these three sister to *Asphodeline*, all with BSV = 100/100.

Combined data—The plastid marker of 79 genes, the mitochondrial marker of five genes, and the nuclear ribosomal marker of three genes were concatenated into a combined data set with an aligned length of 79 286 bp (Table 1). The ML tree topology is identical to the plastid tree (Fig. 4) and returned similar bootstrap support.

Quality assessment of plastome assemblies in Poaceae—For the grass taxa, the number of reads from one sample (representing one sixth of an Illumina lane) varied from 1.82 million (*Zea* CML52) to almost 5.46 million (*Sorghum*, Table 2). The percentage of Illumina reads used in plastome reference-based

TABLE 1. Alignment characteristics and tree statistics for all data sets.

Statistic	All plastid genes	All mitochondrial genes	All nrDNA genes	All data combined
Aligned length (bp)	73 585	3256	2445	79 286
No. samples included	50	46	48	50
–ln L	370 523.39	8841.38	9512.01	393 246.51
No. of MP trees	1	–	–	1
Tree length	47 266	–	–	49515
CI	0.4570	–	–	0.4565
RI	0.6511	–	–	0.6478
No. p.i. characters	12344	–	–	12 828
% p.i. characters	16.7%	–	–	16.2%

Notes: MP = maximum parsominy; p.i. = parsimony informative. CI and RI exclude uninformative characters.

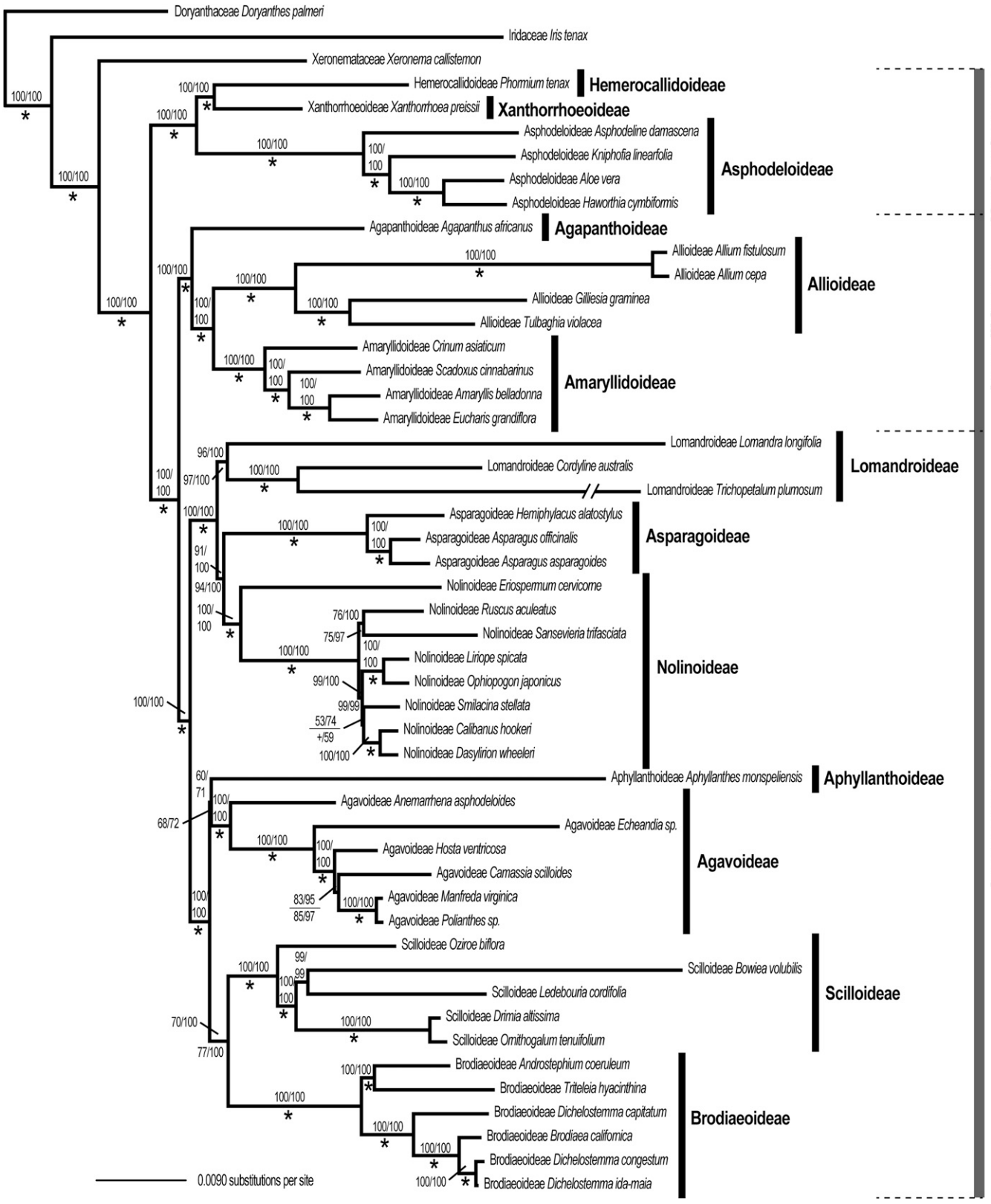

Fig. 4. Maximum likelihood (ML) tree inferred from the 79-gene plastid marker (−ln L = 370 523.39) and from the combined plastid, mitochondrial, and nuclear ribosomal gene data set (−ln L = 393 246.59). Data set includes 50 samples, with *Doryanthes palmeri* and *Iris tenax* used to root the tree. Tree is identical to the single maximum parsimony (MP) tree inferred from the plastid marker (length = 47 266; CI = 0.46; RI = 0.65) and from the combined data set (length = 49 515; CI = 0.46; RI = 0.65). Numbers above branches indicate MP/ML bootstrap values for the plastid marker. Numbers below branches indicate MP/ML bootstrap values for the combined data set. When bootstrap values are identical for both data sets, an asterisk (*) is shown below the branch.

TABLE 2. Summary information for Poaceae taxa used in this study and both reference-based and de novo plastome assemblies.

Taxon	Number of reads	% plastome (coverage)	Reference (GenBank accession)
Oryza sativa subsp. *japonica* cv. Nipponbare	4 095 296	2.18 (76.9×)	*Oryza sativa* subsp. *japonica* (×15901.1)
	3 503 395	2.29 (65×)	de novo, 1 contig
Sorghum bicolor cv. B Tx642	5 457 273	4.37 (196.5×)	*Sorghum bicolor* (EF115542.1)
	5 073 632	4.41 (177×)	de novo, 2 contigs
Zea mays subsp. *mays* cv. B73	5 158 725	0.56 (23.7×)	*Zea mays* (X86563.2)
	4 581 252	0.53 (27×)	de novo, 97 contigs

assembly ranged from 0.56 (*Zea* B73) to 4.37% (*Sorghum*). The average depth of coverage for the plastome ranged from 14.6 (*Zea* CML52) to 196.5× (*Sorghum*). The largest GLR resulted from assembling *Sorghum* as a target with the *Oryza* genome (1.21, target longer than reference sequencel; online Appendix S2). The smallest GLR resulted from assembling *Oryza* with *Cycas* as the reference (0.82, target shorter than reference). Each grass target assembled with a reference sequence from the same species resulted in identity over 99%. The lowest percentage identity (94.1%) between the reference and assembled target was *Sorghum* (target) and *Cycas* (reference). *Oryza* targets assembled with their control reference sequences both resulted in a single contig spanning the entire range of the reference. The highest number of contigs (70) resulted from assembling *Oryza* with *Amborella*.

We tested for correlations between variables for each of three Poaceae taxa separately. Because there were no a priori reasons to assume nonlinearity, all correlations presented are linear. In some comparisons, R^2 improved with exponential curves, but these modifications did not change the interpretation of our results (data not shown). As percentage identity between the reference and target taxon increased, both percentage plastome and plastome coverage increased (Fig. 5A, B). As percentage plastome and plastome coverage increased, the number of contigs decreased (Fig. 5C, D). There was no relationship between either percentage plastome or plastome coverage and the relative size of the target and reference genomes (GLR, Fig. 5E, F). As percentage identity increased, the number of contigs decreased (Fig. 5G). Finally, GLR was weakly and positively correlated with percentage identity (Fig. 5H), indicating for taxa sharing sequence identity, reference and target genomes tended to be of similar sizes.

De novo assemblies resulted in similar percentage of plastome reads and depth of coverage as reference-based methods (Table 2). *Oryza* and *Sorghum* resulted in one and two contigs from de novo methods, respectively, but lower depth of coverage across the plastome in *Zea* B73 yielded a large number of contigs. Assembled sequences may differ from published plastomes because of sequencing/assembly error and/or natural variation in plant genomes. Large numbers of contigs preclude accurate comparisons between assemblies and reference genomes, especially in tests between reference sequences (Appendix S2), but there are several trends concerning the nature of sequence variation. Larger contigs from reference-based assemblies, especially those above ~1000 bp in length, more accurately reconstruct plastome sequence with higher percentage similarity to the "correct," published sequence (Appendix S2).

Variation in the number of single nucleotide polymorphisms (SNPs) and insertion–deletion polymorphisms (indels) between assemblies accounted for less than 0.05% of the plastome (data not shown). Indels generally involved single nucleotides, except in the case of a few large indels in *Oryza*. In this case, we found that Illumina reads are too short to assemble over large indels (>50 bp) relative to reference sequences. SNPs indicated expected levels of variation within taxa relative to other published studies of intraspecific taxon variation in grasses (Nock et al., 2011).

DISCUSSION

We demonstrate the utility of genome survey sequence (GSS) data for plant phylogenomics. Phylogenetic analyses of plastomes, mitochondrial genes, and nuclear ribosomal RNA gene assemblies derived from this low-cost approach inform problems concerning the delineation of subfamilies within core Asparagales and their relationships.

***Evolution of Asparagales*—**For the purpose of estimating the phylogeny of core Asparagales, we included only coding regions of the three cellular genomes because incorporating noncoding regions introduced sequence alignment difficulties. This tree is the same as that estimated by Pires et al. (2006), except for the placement of *Aphyllanthes* and increased bootstrap support at all nodes. The combined ML tree supports all subfamilial relationships identified by APG III (2009). The placement of *Aphyllanthes* confounded previous analyses of Asparagales (Fay et al., 2000; Pires et al., 2006; Kim et al., 2010), perhaps because of rapid diversification, an elevated substitution rate, and lack of extant diversity. Our results present moderate support for *Aphyllanthes* sister to Agavoideae (BSV = 72).

None of the plastomes in Asparagales have yet been found to have a rearranged structure; however, M. R. McKain et al. (unpublished data) discovered genes or exons absent from some members of Agavoideae—*rps16* exon 2 (*Hosta* and *Beschorneria*) and *rps19* (*Echeandia*, *Chlorophytum,* and *Camassia*). Our assembled plastome sequences reveal five genes (verified by PCR and Sanger sequencing; *clpP*, *ndhF*, *rpL32*, *rps16*, and *rps19*) are missing from various taxa when compared to the typical land plant plastid genome. These results confirm absence of plastid genes documented by earlier systematic studies (McPherson et al., 2004; Meerow, 2010). The loss of *rpL32* is seen in all included members of Asphodeloideae, but the gene is present in Hemerocallidoideae and Xanthorrhoeoideae, implying that the loss occurred along the branch leading to Asphodeloideae. The placements of other gene losses are not as clear since they occur in only one or few members of clades.

***Sequence assembly from GSS*—**Previous plastome sequencing from total genomic DNA highlighted the necessity of selecting particular taxa (and subsequent DNA extractions) based on genome size and relative amount of plastid in the DNA sample (here represented as Ct value, Nock et al., 2011). Our results

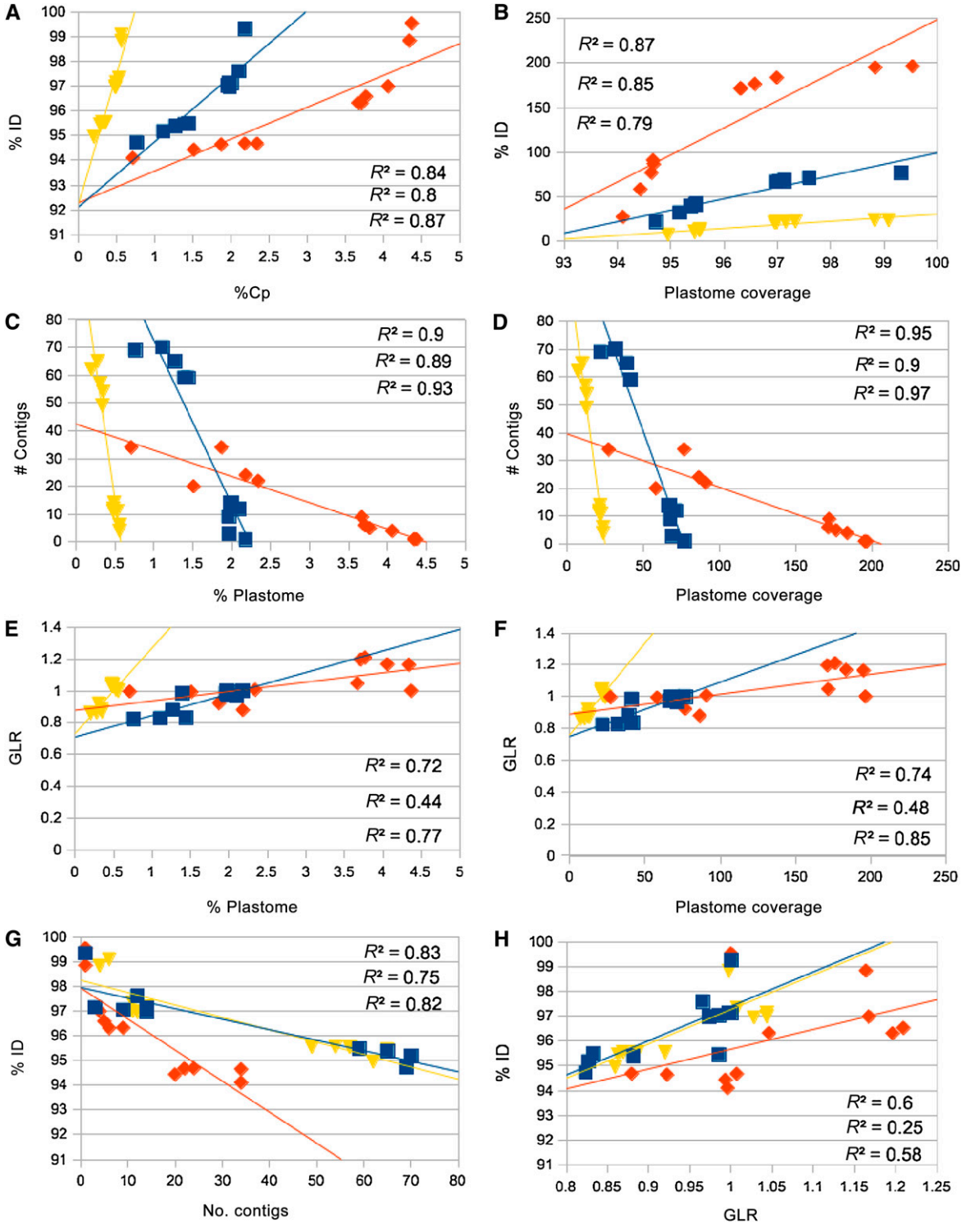

Fig. 5. Effect of phylogenetic distance between target and reference taxa on plastome assembly in Poaceae. All relationships reported are linear. Blue is *Oryza*, red is *Sorghum*, and yellow is *Zea*. R^2 values are from *Oryza*, *Sorghum*, and *Zea* listed from top to bottom. (A) Percentage of Illumina reads from the plastome and percentage identity between reference and target genomes. (B) Average depth of coverage in plastome assembly and percentage identity between reference and target genomes. (C) Percentage of Illumina reads from the plastome and number of contigs resulting from first YASRA assembly. (D) Average depth of coverage in plastome assembly and number of contigs resulting from first YASRA assembly. (E) Percentage of Illumina reads from the plastome and ratio of target to reference genome length. (F) Average depth of coverage in plastome assembly and ratio of target to reference genome length. (G) Number of contigs resulting from first YASRA assembly and percentage identity between reference and target genomes. (H) Ratio of target to reference genome length and percentage identity between reference and target genomes.

suggest the percentage of total reads (and as a result, assembly coverage) from the plastome is only weakly correlated with Ct value or genome size. Our assessment of quantity of plastomes in a DNA sample using RT-PCR, here represented as Ct value, does not accurately predict the proportion of reads assembled into a plastome sequence. Similarly, large and/or polyploid genomes need not prohibit data assembly from GSS, because our results agree with knowledge about cellular alterations that accompany genome size changes. Because cell size increases with genome size, the number of organelles, including plastids, per cell increases (Ellis and Leech, 1985).

Our results indicate that reference sequences from closely related taxa (i.e., same family) are not necessary to obtain sequence information from GSS. However, decreased similarity (and therefore, phylogenetic distance) can complicate attempts to assemble large contigs. Breakpoints in assemblies, illustrated by increased numbers of contigs, result from two situations: first, rearrangements relative to the reference sequence or second, low depth of sequencing coverage. De novo assembly methods can alleviate the first issue by presenting an independent assessment of sequence order. These breakpoints, however, can also reveal the magnitude of structural changes of the target sample relative to the reference taxon. For all grasses, the number of contigs from assemblies using references within Poaceae ranged from one to 14. The number of contigs from assemblies using *Typha* as a reference, however, ranged from 22 to 59, which is similar to assemblies from references outside of Poales (Appendix S2). Analysis of the *Typha* plastome indicates a number of rearrangements relative to Poaceae plastomes (Guisinger et al., 2010), which is consistent with the high number of contigs for a relatively closely related species.

The second reason behind partial or difficult assemblies is low depth of coverage. *Zea* B73 had noticeably fewer reads than the other sequenced grasses, resulting in lower coverage plastomes composed of many short contigs (Table 2). We contend that reference-based assemblies are an appropriate application for systematic studies, because they capitalize on the nature of Illumina GSS to reliably construct coding regions useful in phylogenetic reconstructions. Most angiosperm orders contain at least one published plastome reference sequence (Straub et al., 2012), so reference-based assembly methods can be applied with relative ease to most plant groups. Our results reinforce the need for appropriate quality control measures to ensure robustness of assemblies and removal of artifacts, especially for low coverage GSS. Like any other sequencing method, Illumina technology inherently contains biases (e.g., caused by hexamer binding, Hansen et al., 2010) and types of error (e.g., frequencies of incorrect base pair calls, Dohm et al., 2008) that can inhibit robust reconstructions of genomic sequences, especially in organisms with large genomes (Schatz et al., 2010). Our results indicate the utility of comparing methods for sequence assembly and a priori quality control for trimming reads (as used for de novo sequence assembly). Errors occur in all sequencing and assembly procedures, and checking for consistency of results is essential, especially when working in under-studied systems.

A number of other iterative procedures using reference-based and de novo sequence assembly have effectively assembled plastomes (Straub et al., 2011; Wang and Messing, 2011; Wolf et al., 2011). Our results suggest the less labor-intensive reference-based method yields results similar to the more stringent de novo method. However, the general consensus from our results, as well as other implementations (Straub et al., 2011; Wolf et al., 2011), indicate YASRA alone is insufficient to yield robust, accurate data for full plastome sequences; manual curation, de novo assembly, and/or Sanger sequencing to fill in gaps is also required. We contend, however, that our method of multiplexed Illumina sequencing from total genomic DNA followed by quality-controlled reference-based assembly provides data suitable for phylogenetic analysis at least at higher taxonomic levels. The effects of error in both sequencing and assembly are likely more problematic at lower taxonomic levels.

***Applications*—**At the time of this study, the cost for sequencing these data on the Illumina Genome Analyzer (GAII) platform (multiplex, single-pass, 6 sequences per lane) was ca. US$250-300 per sample, including library preparation. This resulted in 133 894 bp of assembled sequence in *Asparagus officinalis* (chloroplast minus 2nd inverted repeat – 128 193 bp + mitochondrial genes – 3256 bp + nuclear ribosomal genes – 2445 bp). While the cost per sample is ca. 10 times that of Sanger sequencing of an individual locus, the result is a ca. 100-fold increase in quantity of data. Additionally, the savings in time for conducting laboratory work are extensive, as GSS does not require as much optimization of laboratory procedures for different samples (designing primers, adjusting PCR cocktails, and modifying thermal cycle routines). The result is a large data set including enough informative characters to resolve even recalcitrant relationships between taxa, which are especially useful at lower taxonomic levels. The drawbacks of computational time and expertise to assemble sequences are also constantly decreasing. In fact, this same procedure has been used to successfully sequence the entire mitochondrial genome in water bugs, katydids, elephants, and bears from DNA extracted from blood or other tissue (laboratory of J. C. Pires, unpublished data).

We have shown the feasibility of obtaining large amounts of both coding and noncoding DNA sequence data from three genomic compartments, allowing phylogenetic reconstruction between even problematic groups with recent divergence (Parks et al., 2009). Our method of Illumina GSS is especially attractive for systematic studies, where large numbers of taxa and many genes are optimal for phylogeny estimation. Ideally, databases for plastomes, mitogenomes, and nuclear ribosomal repeats should be prioritized for systematists, as well as support for online tools that make assembly and annotation easier. Consolidation and standardization of these types of analysis will allow broader applications for both taxonomy and molecular evolution. Plastomes, for example, have potential as a single-locus DNA barcode for identification of plants (Nock et al., 2011), and we contend that mitogenomes and nuclear ribosomal loci have similar potential for confirming problematic taxa. Furthermore, a broader sampling of plastomes from across the plant kingdom will help inform the relevance and frequency of structural changes in organellar genomes and provide a framework for comparative biology of organellar evolution.

Finally, this paper presents assemblies of plastome, mitogenome, and nuclear ribosomal sequences in plants, but these data still only account for, at most, 10% of Illumina GSS reads. The majority of reads are presumably from the nuclear genome, and further work should investigate the feasibility of assembling repetitive elements (REs) from these data. For example, deeper Illumina GSS sequences have been applied effectively in barley to characterize REs in a genome (Wicker et al., 2008). Further research should explore the effectiveness of very low coverage GSS to recover REs in nonmodel systems, or where the RE compliment is unknown.

Massively parallel sequencing technology is becoming more efficient and cost-effective. Scientific researchers and computational biologists are working to streamline sample preparation and data analysis. The procedural pipeline used in this study demonstrates how the technique and software already available can be used to sequence complete plastid genomes, mitochondrial genes, and nuclear ribosomal genes in plants for use in phylogenetics. The promise of mining Illumina GSS for plastome, mitogenomic, and ribosomal nuclear elements makes developing genomic tools across diverse organisms possible.

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APPENDIX 1. Voucher information for Asparagales taxa and GenBank accession numbers for sequences used in phylogenetic analysis.

Column headings:

Taxon,Voucher,Mitochondrial *atp4*,Mitochondrial *atp6*,Mitochondrial *cox3*,Mitochondrial *nad9*,Mitochondrial *rps13*,Nuclear 18S,Nuclear 26S,Plastid *accD*,Plastid *atpA*,Plastid *atpB*,Plastid *atpE*,Plastid *atpF*,Plastid *atpH*,Plastid *atpI*,Plastid *ccsA*,Plastid *cemA*,Plastid *clpP*,Plastid *matK*,Plastid *ndhA*,Plastid *ndhB*,Plastid *ndhC*,Plastid *ndhD*,Plastid *ndhE*,Plastid *ndhF*,Plastid *ndhG*,Plastid *ndhH*,Plastid *ndhI*,Plastid *ndhJ*,Plastid *ndhK*,Plastid *petN*,Plastid *psaA*,Plastid *psaB*,Plastid *psaC*,Plastid *psaI*,Plastid *psaJ*,Plastid *psbA*,Plastid *psbB*,Plastid *psbC*,Plastid *psbD*,Plastid *psbE*,Plastid *psbF*,Plastid *psbH*,Plastid *psbI*,Plastid *psbJ*,Plastid *psbK*,Plastid *psbL*,Plastid *psbN*,Plastid *psbT*,Plastid *psbZ*,Plastid *rbcL*,Plastid *rpL14*,Plastid *rpL16*,Plastid *rpL2*,Plastid *rpL20*,Plastid *rpL22*,Plastid *rpL23*,Plastid *rpL32*,Plastid *rpL33*,Plastid *rpL36*,Plastid *rpoA*,Plastid *rpoB*,Plastid *rpoC1*,Plastid *rpoC2*,Plastid *rps11*,Plastid *rps12*,Plastid *rps14*,Plastid *rps15*,Plastid *rps16*,Plastid *rps18*,Plastid *rps19*,Plastid *rps2*,Plastid *rps3*,Plastid *rps4*,Plastid *rps7*,Plastid *rps8*,Plastid *ycf1*,Plastid *ycf2*,Plastid *ycf3*,Plastid *ycf4*,Plastid *ycf68*

Agapanthus africanus,Steele 1099,–,–,–,–,–,JQ283896,–,JQ277064,JQ273544,JQ273593,JQ273642,JQ273691,JQ273789,JQ273740,JQ276285,JQ276334,JQ276723,JQ276383,JQ276432,JQ276481,JQ276530,JQ276579,JQ276628,JQ276771,JQ276819,JQ276868,JQ276917,JQ276966,JQ277015,JQ276236,JQ276187,JQ276138,JQ276089,JQ276040,JQ275991,JQ275942,JQ275893,JQ275844,JQ275795,JQ275746,JQ275697,JQ275648,JQ275599,JQ275550,JQ275501,JQ275452,JQ275403,JQ275354,JQ275305,JQ273888,JQ275157,JQ274620,JQ275256,JQ275206,JQ274669,JQ275108,JQ274526,JQ275059,JQ275010,JQ274477,JQ274428,JQ274571,JQ274961,JQ274912,JQ274183,JQ274863,JQ274814,JQ276677,JQ274765,JQ274718,JQ274379,JQ274330,JQ274281,JQ274232,JQ274134,JQ274084,JQ274035,JQ273986,JQ273937,JQ273838

Allium cepa,JC Pires 2011 - 04,–,JQ283728,JQ283798,JQ283762,–,JQ283941,JQ283849,JQ277072,JQ273552,JQ273601,JQ273650,JQ273699,JQ273797,JQ273748,JQ276293,JQ276342,JQ276731,JQ276391,JQ276440,JQ276489,JQ276538,JQ276587,JQ276636,JQ276779,JQ276827,JQ276876,JQ276925,JQ276974,JQ277023,JQ276244,JQ276195,JQ276146,JQ276097,JQ276048,JQ275999,JQ275950,JQ275901,JQ275852,JQ275803,JQ275754,JQ275705,JQ275656,JQ275607,JQ275558,JQ275509,JQ275460,JQ275411,JQ275362,JQ275313,JQ273896,JQ275165,JQ274628,JQ275264,JQ275214,JQ274677,JQ275116,JQ274534,JQ275067,JQ275018,JQ274485,JQ274436,JQ274579,JQ274969,JQ274920,JQ274191,JQ274871,JQ274822,JQ276685,JQ274773,JQ274724,JQ274387,JQ274338,JQ274289,JQ274240,JQ274142,JQ274092,JQ274043,JQ273994,JQ273945,JQ273846

Allium fistulosum,Steele 1094, JQ283699,JQ283733,JQ283805,JQ283771,–,JQ283942,JQ283850,JQ277071,JQ273551,JQ273600,JQ273649,JQ273698,JQ273796,JQ273747,JQ276292,JQ276341,JQ276730,JQ276390,JQ276439,JQ276488,JQ276537,JQ276586,JQ276635,JQ276778,JQ276826,JQ276875,JQ276924,JQ276973,JQ277022,JQ276243,JQ276194,JQ276145,JQ276096,JQ276047,JQ275998,JQ275949,JQ275900,JQ275851,JQ275802,JQ275753,JQ275704,JQ275655,JQ275606,JQ275557,JQ275508,JQ275459,JQ275410,JQ275361,JQ275312,JQ273895,JQ275164,JQ274627,JQ275263,JQ275213,JQ274676,JQ275115,JQ274533,JQ275066,JQ275017,JQ274484,JQ274435,JQ274578,JQ274968,JQ274919,JQ274190,JQ274870,JQ274821,JQ276684,JQ274772,JQ274723,JQ274386,JQ274337,JQ274288,JQ274239,JQ274141,JQ274091,JQ274042,JQ273993,JQ273944,JQ273845

Aloe vera,Steele 1098,JQ283710,–,–,–,–,JQ283935,JQ283851,JQ277083,JQ273563,JQ273612,JQ273661,JQ273710,JQ273808,JQ273759,JQ276304,JQ276353,JQ276742,JQ276402,JQ276451,JQ276500,JQ276549,JQ276598,JQ276647,JQ276789,JQ276838,JQ276887,JQ276936,JQ276985,JQ277034,JQ276255,JQ276206,JQ276157,JQ276108,JQ276059,JQ276010,JQ275961,JQ275912,JQ275863,JQ275814,JQ275765,JQ275716,JQ275667,JQ275618,JQ275569,JQ275520,JQ275471,JQ275422,JQ275373,JQ275324,JQ273907,JQ275176,JQ274639,JQ275275,JQ275225,JQ274688,JQ275127,–,JQ275078,JQ275029,JQ274496,JQ274447,JQ274590,JQ274980,JQ274931,JQ274202,JQ274882,JQ274833,JQ276696,JQ274784,JQ274735,JQ274398,JQ274349,JQ274300,JQ274251,JQ274153,JQ274103,JQ274054,JQ274005,JQ273956,JQ273857

Amaryllis belladonna,Steele 1103, JQ283711,JQ283743,JQ283814,JQ283783,JQ283844,JQ283909,JQ283852,JQ277075,JQ273555,JQ273604,JQ273653,JQ273702,JQ273800,JQ273751,JQ276296,JQ276345,JQ276734,JQ276394,JQ276443,JQ276492,JQ276541,JQ276590,JQ276639,JQ276782,JQ276830,JQ276879,JQ276928,JQ276977,JQ277026,JQ276247,JQ276198,JQ276149,JQ276100,JQ276051,JQ276002,JQ275953,JQ275904,JQ275855,JQ275806,JQ275757,JQ275708,JQ275659,JQ275610,JQ275561,JQ275512,JQ275463,JQ275414,JQ275365,JQ275316,JQ273899,JQ275168,JQ274631,JQ275267,JQ275217,JQ274680,JQ275119,JQ274537,JQ275070,JQ275021,JQ274488,JQ274439,JQ274582,JQ274972,JQ274923,JQ274194,JQ274874,JQ274825,JQ276688,JQ274776,JQ274727,JQ274390,JQ274341,JQ274292,JQ274243,JQ274145,JQ274095,JQ274046,JQ273997,JQ273948,JQ273849

Androstephium coeruleum,Pires and Sytsma 2002 AJB 01,JQ283696,–,JQ283802,JQ283766,–,JQ283897,JQ283853,JQ277105,JQ273585,JQ273634,JQ273683,JQ273732,JQ273830,JQ273781,JQ276326,JQ276375,JQ276763,JQ276424,JQ276473,JQ276522,JQ276571,JQ276620,JQ276669,JQ276811,JQ276860,JQ276909,JQ276958,JQ277007,JQ277056,JQ276277,JQ276228,JQ276179,JQ276130,JQ276081,JQ276032,JQ275983,JQ275934,JQ275885,JQ275836,JQ275787,JQ275738,JQ275689,JQ275640,JQ275591,JQ275542,JQ275493,JQ275444,JQ275395,JQ275346,JQ273929,JQ275198,JQ274661,JQ275297,JQ275248,JQ274710,JQ275149,JQ274564,JQ275100,JQ275051,JQ274518,JQ274469,JQ274612,JQ275002,JQ274953,JQ274224,JQ274904,JQ274855,JQ276717,JQ274806,JQ274757,JQ274420,JQ274371,JQ274322,JQ274273,JQ274175,JQ274126,JQ274076,JQ274027,JQ273978,JQ273880

Anemarrhena asphodeloides,Steele 1089,–,JQ283716,–,–,JQ283819,JQ283939,JQ283894,JQ277065,JQ273545,JQ273594,JQ273643,JQ273692,JQ273790,JQ273741,JQ276286,JQ276335,JQ276724,JQ276384,JQ276433,JQ276482,JQ276531,JQ276580,JQ276629,JQ276772,JQ276820,JQ276869,JQ276918,JQ276967,JQ277016,JQ276237,JQ276188,JQ276139,JQ276090,JQ276041,JQ275992,JQ275943,JQ275894,JQ275845,JQ275796,JQ275747,JQ275698,JQ275649,JQ275600,JQ275551,JQ275502,JQ275453,JQ275404,JQ275355,JQ275306,JQ273889,JQ275158,JQ274621,JQ275257,JQ275207,JQ274670,JQ275109,JQ274527,JQ275060,JQ275011,JQ274478,JQ274429,JQ274572,JQ274962,JQ274913,JQ274184,JQ274864,JQ274815,JQ276678,JQ274766,JQ274719,JQ274380,JQ274331,JQ274282,JQ274233,JQ274135,JQ274085,JQ274036,JQ273987,JQ273938,JQ273839

Aphyllanthes monspeliensis,Seberg 1600/2008,JQ283705,JQ283739,JQ283811,JQ283778,JQ283840,JQ283919,JQ283869,JQ277079,JQ273559,JQ273608,JQ273657,JQ273706,JQ273804,JQ273755,JQ276300,JQ276349,JQ276738,JQ276398,JQ276447,JQ276496,JQ276545,JQ276594,JQ276643,JQ276785,JQ276834,JQ276883,JQ276932,JQ276981,JQ277030,JQ276251,JQ276202,JQ276153,JQ276104,JQ276055,JQ276006,JQ275957,JQ275908,JQ275859,JQ275810,JQ275761,JQ275712,JQ275663,JQ275614,JQ275565,JQ275516,JQ275467,JQ275418,JQ275369,JQ275320,JQ273903,JQ275172,JQ274635,JQ275271,JQ275221,JQ274684,JQ275123,JQ274541,JQ275074,JQ275025,JQ274492,JQ274443,JQ274586,JQ274976,JQ274927,JQ274198,JQ274878,JQ274829,JQ276692,JQ274780,JQ274731,JQ274394,JQ274345,JQ274296,JQ274247,JQ274149,JQ274099,JQ274050,JQ274001,JQ273952,JQ273853

Asparagus asparagoides,worldplants.com,JQ283706,JQ283740,–,JQ283779,JQ283841,JQ283899,JQ283881,JQ277081,JQ273561,JQ273610,JQ273659,JQ273708,JQ273806,JQ273757,JQ276302,JQ276351,JQ276740,JQ276400,JQ276449,JQ276498,JQ276547,JQ276596,JQ276645,JQ276787,JQ276836,JQ276885,JQ276934,JQ276983,JQ277032,JQ276253,JQ276204,JQ276155,JQ276106,JQ276057,JQ276008,JQ275959,JQ275910,JQ275861,JQ275812,JQ275763,JQ275714,JQ275665,JQ275616,JQ275567,JQ275518,JQ275469,JQ275420,JQ275371,JQ275322,JQ273905,JQ275174,JQ274637,JQ275273,JQ275223,JQ274686,JQ275125,JQ274543,JQ275076,JQ275027,JQ274494,JQ274445,JQ274588,JQ274978,JQ274929,JQ274200,JQ274880,JQ274831,JQ276694,JQ274782,JQ274733,JQ274396,JQ274347,JQ274298,JQ274249,JQ274151,JQ274101,JQ274052,JQ274003,JQ273954,JQ273855

APPENDIX 1. Continued.

Asparagus officinalis,Leebens-Mack DH00/086, JQ283694,JQ283730,JQ283800,JQ283764,JQ283830,JQ283900,JQ283882,JQ277080,JQ273560,JQ273609,JQ273658,JQ273707,JQ273805,JQ273756,JQ276301,JQ276350,JQ276739,JQ276399,JQ276448,JQ276497,JQ276546,JQ276595,JQ276644,JQ276786,JQ276835,JQ276884,JQ276933,JQ276982,JQ277031,JQ276252,JQ276203,JQ276154,JQ276105,JQ276056,JQ276007,JQ275958,JQ275909,JQ275860,JQ275811,JQ275762,JQ275713,JQ275664,JQ275615,JQ275566,JQ275517,JQ275468,JQ275419,JQ275370,JQ275321,JQ273904,JQ275173,JQ274636,JQ275272,JQ275222,JQ274685,JQ275124,JQ274542,JQ275075,JQ275026,JQ274493,JQ274444,JQ274587,JQ274977,JQ274928,JQ274199,JQ274879,JQ274830,JQ276693,JQ274781,JQ274732,JQ274395,JQ274346,JQ274297,JQ274248,JQ274150,JQ274100,JQ274051,JQ274002,JQ273953,JQ273854

Asphodeline damascena,J.L. Hudson Seeds, La Honda, CA,JQ283707,–,JQ283812,JQ283780,–,JQ283940,JQ283873,JQ277084,JQ273564,JQ273613,JQ273662,JQ273711,JQ273809,JQ273760,JQ276305,JQ276354,JQ276743,JQ276403,JQ276452,JQ276501,JQ276550,JQ276599,JQ276648,JQ276790,JQ276839,JQ276888,JQ276937,JQ276986,JQ277035,JQ276256,JQ276207,JQ276158,JQ276109,JQ276060,JQ276011,JQ275962,JQ275913,JQ275864,JQ275815,JQ275766,JQ275717,JQ275668,JQ275619,JQ275570,JQ275521,JQ275472,JQ275423,JQ275374,JQ275325,JQ273908,JQ275177,JQ274640,JQ275276,JQ275226,JQ274689,JQ275128,–,JQ275079,JQ275030,JQ274497,JQ274448,JQ274591,JQ274981,JQ274932,JQ274203,JQ274883,JQ274834,JQ276697,JQ274785,JQ274736,JQ274399,JQ274350,JQ274301,JQ274252,JQ274154,JQ274104,JQ274055,JQ274006,JQ273957,JQ273858

Bowiea volubilis,JC Pires 2011 - 06,JQ283691,JQ283725,JQ283796,JQ283758,JQ283826,JQ283902,JQ283870,JQ277089,JQ273569,JQ273618,JQ273667,JQ273716,JQ273814,JQ273765,JQ276310,JQ276359,JQ276748,JQ276408,JQ276457,JQ276506,JQ276555,JQ276604,JQ276653,JQ276795,JQ276844,JQ276893,JQ276942,JQ276991,JQ277040,JQ276261,JQ276212,JQ276163,JQ276114,JQ276065,JQ276016,JQ275967,JQ275918,JQ275869,JQ275820,JQ275771,JQ275722,JQ275673,JQ275624,JQ275575,JQ275526,JQ275477,JQ275428,JQ275379,JQ275330,JQ273913,JQ275182,JQ274645,JQ275281,JQ275231,JQ274694,JQ275133,JQ274547,JQ275084,JQ275035,JQ274502,JQ274453,JQ274596,JQ274986,JQ274937,JQ274208,JQ274888,JQ274839,JQ276702,JQ274790,JQ274741,JQ274404,JQ274355,JQ274306,JQ274257,JQ274159,JQ274109,JQ274060,JQ274011,JQ273962,JQ273863

Brodiaea californica,Pires and Sytsma 2002 AJB 02,JQ283688,JQ283722,JQ283793,JQ283754,JQ283823,JQ283923,JQ283865,JQ277106,JQ273586,JQ273635,JQ273684,JQ273733,JQ273831,JQ273782,JQ276327,JQ276376,JQ276764,JQ276425,JQ276474,JQ276523,JQ276572,JQ276621,JQ276670,JQ276812,JQ276861,JQ276910,JQ276959,JQ277008,JQ277057,JQ276278,JQ276229,JQ276180,JQ276131,JQ276082,JQ276033,JQ275984,JQ275935,JQ275886,JQ275837,JQ275788,JQ275739,JQ275690,JQ275641,JQ275592,JQ275543,JQ275494,JQ275445,JQ275396,JQ275347,JQ273930,JQ275199,JQ274662,JQ275298,JQ275249,JQ274711,JQ275150,JQ274565,JQ275101,JQ275052,JQ274519,JQ274470,JQ274613,JQ275003,JQ274954,JQ274225,JQ274905,JQ274856,–,JQ274807,JQ274758,JQ274421,JQ274372,JQ274323,JQ274274,JQ274176,JQ274127,JQ274077,JQ274028,JQ273979,JQ273881

Calibanus hookeri,Steele 1095,JQ283684,JQ283717,–,JQ283749,–,JQ283912,JQ283875,JQ277097,JQ273577,JQ273626,JQ273675,JQ273724,JQ273822,JQ273773,JQ276318,JQ276367,JQ276755,JQ276416,JQ276465,JQ276514,JQ276563,JQ276612,JQ276661,JQ276803,JQ276852,JQ276901,JQ276950,JQ276999,JQ277048,JQ276269,JQ276220,JQ276171,JQ276122,JQ276073,JQ276024,JQ275975,JQ275926,JQ275877,JQ275828,JQ275779,JQ275730,JQ275681,JQ275632,JQ275583,JQ275534,JQ275485,JQ275436,JQ275387,JQ275338,JQ273921,JQ275190,JQ274653,JQ275289,JQ275240,JQ274702,JQ275141,JQ274556,JQ275092,JQ275043,JQ274510,JQ274461,JQ274604,JQ274994,JQ274945,JQ274216,JQ274896,JQ274847,JQ276710,JQ274798,JQ274749,JQ274412,JQ274363,JQ274314,JQ274265,JQ274167,JQ274118,JQ274068,JQ274019,JQ273970,JQ273872

Camassia scilloides,McKain 107,–,–,–,–,–,–,–,JQ277066,JQ273546,JQ273595,JQ273644,JQ273693,JQ273791,JQ273742,JQ276287,JQ276336,JQ276725,JQ276385,JQ276434,JQ276483,JQ276532,JQ276581,JQ276630,JQ276773,JQ276821,JQ276870,JQ276919,JQ276968,JQ277017,JQ276238,JQ276189,JQ276140,JQ276091,JQ276042,JQ275993,JQ275944,JQ275895,JQ275846,JQ275797,JQ275748,JQ275699,JQ275650,JQ275601,JQ275552,JQ275503,JQ275454,JQ275405,JQ275356,JQ275307,JQ273890,JQ275159,JQ274622,JQ275258,JQ275208,JQ274671,JQ275110,JQ274528,JQ275061,JQ275012,JQ274479,JQ274430,JQ274573,JQ274963,JQ274914,JQ274185,JQ274865,JQ274816,JQ276679,JQ274767,–,JQ274381,JQ274332,JQ274283,JQ274234,JQ274136,JQ274086,JQ274037,JQ273988,JQ273939,JQ273840

Cordyline australis,J.L. Hudson Seeds, La Honda, CA,JQ283704,JQ283738,JQ283810,JQ283777,JQ283839,JQ283903,JQ283863,JQ277095,JQ273575,JQ273624,JQ273673,JQ273722,JQ273820,JQ273771,JQ276316,JQ276365,–,JQ276414,JQ276463,JQ276512,JQ276561,JQ276610,JQ276659,JQ276801,JQ276850,JQ276899,JQ276948,JQ276997,JQ277046,JQ276267,JQ276218,JQ276169,JQ276120,JQ276071,JQ276022,JQ275973,JQ275924,JQ275875,JQ275826,JQ275777,JQ275728,JQ275679,JQ275630,JQ275581,JQ275532,JQ275483,JQ275434,JQ275385,JQ275336,JQ273919,JQ275188,JQ274651,JQ275287,JQ275237,JQ274700,JQ275139,JQ274553,JQ275090,JQ275041,JQ274508,JQ274459,JQ274602,JQ274992,JQ274943,JQ274214,JQ274894,JQ274845,JQ276708,JQ274796,JQ274747,JQ274410,JQ274361,JQ274312,JQ274263,JQ274165,JQ274115,JQ274066,JQ274017,JQ273968,JQ273869

Crinum asiaticum,Asiatica Nursery, Raleigh, NC,–,–,–,JQ283755,–,JQ283938,JQ283893,JQ277076,JQ273556,JQ273605,JQ273654,JQ273703,JQ273801,JQ273752,JQ276297,JQ276346,JQ276735,JQ276395,JQ276444,JQ276493,JQ276542,JQ276591,JQ276640,JQ276783,JQ276831,JQ276880,JQ276929,JQ276978,JQ277027,JQ276248,JQ276199,JQ276150,JQ276101,JQ276052,JQ276003,JQ275954,JQ275905,JQ275856,JQ275807,JQ275758,JQ275709,JQ275660,JQ275611,JQ275562,JQ275513,JQ275464,JQ275415,JQ275366,JQ275317,JQ273900,JQ275169,JQ274632,JQ275268,JQ275218,JQ274681,JQ275120,JQ274538,JQ275071,JQ275022,JQ274489,JQ274440,JQ274583,JQ274973,JQ274924,JQ274195,JQ274875,JQ274826,JQ276689,JQ274777,JQ274728,JQ274391,JQ274342,JQ274293,JQ274244,JQ274146,JQ274096,JQ274047,JQ273998,JQ273949,JQ273850

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Dichelostemma capitatum,Pires and Sytsma 2002 AJB 03,JQ283687,–,JQ283792,–,JQ283822,JQ283926,JQ283866,JQ277107,JQ273587,JQ273636,JQ273685,JQ273734,JQ273832,JQ273783,JQ276328,JQ276377,JQ276765,JQ276426,JQ276475,JQ276524,JQ276573,JQ276622,JQ276671,JQ276813,JQ276862,JQ276911,JQ276960,JQ277009,JQ277058,JQ276279,JQ276230,JQ276181,JQ276132,JQ276083,JQ276034,JQ275985,JQ275936,JQ275887,JQ275838,JQ275789,JQ275740,JQ275691,JQ275642,JQ275593,JQ275544,JQ275495,JQ275446,JQ275397,JQ275348,JQ273931,JQ275200,JQ274663,JQ275299,JQ275250,JQ274712,JQ275151,JQ274566,JQ275102,JQ275053,JQ274520,JQ274471,JQ274614,JQ275004,JQ274955,JQ274226,JQ274906,JQ274857,JQ276718,JQ274808,JQ274759,JQ274422,JQ274373,JQ274324,JQ274275,JQ274177,JQ274128,JQ274078,JQ274029,JQ273980,JQ273882

Dichelostemma congestum,Pires 09-023,–,–,–,–,–,JQ283924,JQ283867,JQ277108,JQ273588,JQ273637,JQ273686,JQ273735,JQ273833,JQ273784,JQ276329,JQ276378,JQ276766,JQ276427,JQ276476,JQ276525,JQ276574,JQ276623,JQ276672,JQ276814,JQ276863,JQ276912,JQ276961,JQ277010,JQ277059,JQ276280,JQ276231,JQ276182,JQ276133,JQ276084,

APPENDIX 1. Continued.

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Dichelostemma ida-maia,R. Preston 2682,JQ283686,JQ283721,JQ283791,JQ283753,JQ283821,JQ283925,JQ283868,JQ277109,JQ273589,JQ273638,JQ273687,JQ273736,JQ273834,JQ273785,JQ276330,JQ276379,JQ276767,JQ276428,JQ276477,JQ276526,JQ276575,JQ276624,JQ276673,JQ276815,JQ276864,JQ276913,JQ276962,JQ277011,JQ277060,JQ276281,JQ276232,JQ276183,JQ276134,JQ276085,JQ276036,JQ275987,JQ275938,JQ275889,JQ275840,JQ275791,JQ275742,JQ275693,JQ275644,JQ275595,JQ275546,JQ275497,JQ275448,JQ275399,JQ275350,JQ273933,JQ275202,JQ274665,JQ275301,JQ275252,JQ274714,JQ275153,JQ274568,JQ275104,JQ275055,JQ274522,JQ274473,JQ274616,JQ275006,JQ274957,JQ274228,JQ274908,JQ274859,JQ276720,JQ274810,JQ274761,JQ274424,JQ274375,JQ274326,JQ274277,JQ274179,JQ274130,JQ274080,JQ274031,JQ273982,JQ273884

Doryanthes palmeri,Pires 2010-009,JQ283713,JQ283746,JQ283817,JQ283786,JQ283847,JQ283943,JQ283858,JQ277087,JQ273567,JQ273616,JQ273665,JQ273714,JQ273812,JQ273763,JQ276308,JQ276357,JQ276746,JQ276406,JQ276455,JQ276504,JQ276553,JQ276602,JQ276651,JQ276793,JQ276842,JQ276891,JQ276940,JQ276989,JQ277038,JQ276259,JQ276210,JQ276161,JQ276112,JQ276063,JQ276014,JQ275965,JQ275916,JQ275867,JQ275818,JQ275769,JQ275720,JQ275671,JQ275622,JQ275573,JQ275524,JQ275475,JQ275426,JQ275377,JQ275328,JQ273911,JQ275180,JQ274643,JQ275279,JQ275229,JQ274692,JQ275131,JQ274545,JQ275082,JQ275033,JQ274500,JQ274451,JQ274594,JQ274984,JQ274935,JQ274206,JQ274886,JQ274837,JQ276700,JQ274788,JQ274739,JQ274402,JQ274353,JQ274304,JQ274255,JQ274157,JQ274107,JQ274058,JQ274009,JQ273960,JQ273861

Drimia altissima,Steele 1092,JQ283695,JQ283731,JQ283801,JQ283765,JQ283831,JQ283906,JQ283878,JQ277090,JQ273570,JQ273619,JQ273668,JQ273717,JQ273815,JQ273766,JQ276311,JQ276360,JQ276749,JQ276409,JQ276458,JQ276507,JQ276556,JQ276605,JQ276654,JQ276796,JQ276845,JQ276894,JQ276943,JQ276992,JQ277041,JQ276262,JQ276213,JQ276164,JQ276115,JQ276066,JQ276017,JQ275968,JQ275919,JQ275870,JQ275821,JQ275772,JQ275723,JQ275674,JQ275625,JQ275576,JQ275527,JQ275478,JQ275429,JQ275380,JQ275331,JQ273914,JQ275183,JQ274646,JQ275282,JQ275232,JQ274695,JQ275134,JQ274548,JQ275085,JQ275036,JQ274503,JQ274454,JQ274597,JQ274987,JQ274938,JQ274209,JQ274889,JQ274840,JQ276703,JQ274791,JQ274742,JQ274405,JQ274356,JQ274307,JQ274258,JQ274160,JQ274110,JQ274061,JQ274012,JQ273963,JQ273864

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Eriospermum cervicorne,Hannon 97568,–,JQ283720,–,JQ283752,–,JQ283932,JQ283855,JQ277099,JQ273579,JQ273628,JQ273677,JQ273726,JQ273824,JQ273775,JQ276320,JQ276369,JQ276757,JQ276418,JQ276467,JQ276516,JQ276565,JQ276614,JQ276663,JQ276805,JQ276854,JQ276903,JQ276952,JQ277001,JQ277050,JQ276271,JQ276222,JQ276173,JQ276124,JQ276075,JQ276026,JQ275977,JQ275928,JQ275879,JQ275830,JQ275781,JQ275732,JQ275683,JQ275634,JQ275585,JQ275536,JQ275487,JQ275438,JQ275389,JQ275340,JQ273923,JQ275192,JQ274655,JQ275291,JQ275242,JQ274704,JQ275143,JQ274558,JQ275094,JQ275045,JQ274512,JQ274463,JQ274606,JQ274996,JQ274947,JQ274218,JQ274898,JQ274849,JQ276712,JQ274800,JQ274751,JQ274414,JQ274365,JQ274316,JQ274267,JQ274169,JQ274120,JQ274070,JQ274021,JQ273972,JQ273874

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Haworthia cymbiformis,Steele 1093,JQ283702,JQ283736,JQ283808,JQ283774,JQ283836,JQ283936,JQ283861,JQ277085,JQ273565,JQ273614,JQ273663,JQ273712,JQ273810,JQ273761,JQ276306,JQ276355,JQ276744,JQ276404,JQ276453,JQ276502,JQ276551,JQ276600,JQ276649,JQ276791,JQ276840,JQ276889,JQ276938,JQ276987,JQ277036,JQ276257,JQ276208,JQ276159,JQ276110,JQ276061,JQ276012,JQ275963,JQ275914,JQ275865,JQ275816,JQ275767,JQ275718,JQ275669,JQ275620,JQ275571,JQ275522,JQ275473,JQ275424,JQ275375,JQ275326,JQ273909,JQ275178,JQ274641,JQ275277,JQ275227,JQ274690,JQ275129,–,JQ275080,JQ275031,JQ274498,JQ274449,JQ274592,JQ274982,JQ274933,JQ274204,JQ274884,JQ274835,JQ276698,JQ274786,JQ274737,JQ274400,JQ274351,JQ274302,JQ274253,JQ274155,JQ274105,JQ274056,JQ274007,JQ273958,JQ273859

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Hosta ventricosa,McKain 106,–,–,–,–,–,–,–,JQ277068,JQ273548,JQ273597,JQ273646,JQ273695,JQ273793,JQ273744,JQ276289,JQ276338,JQ276727,JQ276387,JQ276436,JQ276485,JQ276534,JQ276583,JQ276632,JQ276775,JQ276823,JQ276872,JQ276921,JQ276970,JQ277019,JQ276240,JQ276191,JQ276142,JQ276093,JQ276044,JQ275995,JQ275946,JQ275897,JQ275848,JQ275799,JQ275750,JQ275701,JQ275652,JQ275603,JQ275554,JQ275505,JQ275456,JQ275407,JQ275358,JQ275309,JQ273892,JQ275161,JQ274624,JQ275260,JQ275210,JQ274673,JQ275112,JQ274530,JQ275063,JQ275014,JQ274481,JQ274432,JQ274575,JQ274965,JQ2

APPENDIX 1. Continued.

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Kniphofia linearifolia,J.L. Hudson Seeds, La Honda, CA,–,JQ283744,–,JQ283784,JQ283845,JQ283934,JQ283874,JQ277086,JQ273566,JQ273615,JQ273664,JQ273713,JQ273811,JQ273762,JQ276307,JQ276356,JQ276745,JQ276405,JQ276454,JQ276503,JQ276552,JQ276601,JQ276650,JQ276792,JQ276841,JQ276890,JQ276939,JQ276988,JQ277037,JQ276258,JQ276209,JQ276160,JQ276111,JQ276062,JQ276013,JQ275964,JQ275915,JQ275866,JQ275817,JQ275768,JQ275719,JQ275670,JQ275621,JQ275572,JQ275523,JQ275474,JQ275425,JQ275376,JQ275327,JQ273910,JQ275179,JQ274642,JQ275278,JQ275228,JQ274691,JQ275130,–,JQ275081,JQ275032,JQ274499,JQ274450,JQ274593,JQ274983,JQ274934,JQ274205,JQ274885,JQ274836,JQ276699,JQ274787,JQ274738,JQ274401,JQ274352,JQ274303,JQ274254,JQ274156,JQ274106,JQ274057,JQ274008,JQ273959,JQ273860

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***Liriope spicat*a**,Steele 1082,JQ283690,JQ283724,JQ283795,JQ283757,JQ283825,JQ283914,JQ283877,JQ277100,JQ273580,JQ273629,JQ273678,JQ273727,JQ273825,JQ273776,JQ276321,JQ276370,JQ276758,JQ276419,JQ276468,JQ276517,JQ276566,JQ276615,JQ276664,JQ276806,JQ276855,JQ276904,JQ276953,JQ277002,JQ277051,JQ276272,JQ276223,JQ276174,JQ276125,JQ276076,JQ276027,JQ275978,JQ275929,JQ275880,JQ275831,JQ275782,JQ275733,JQ275684,JQ275635,JQ275586,JQ275537,JQ275488,JQ275439,JQ275390,JQ275341,JQ273924,JQ275193,JQ274656,JQ275292,JQ275243,JQ274705,JQ275144,JQ274559,JQ275095,JQ275046,JQ274513,JQ274464,JQ274607,JQ274997,JQ274948,JQ274219,JQ274899,JQ274850,JQ276713,JQ274801,JQ274752,JQ274415,JQ274366,JQ274317,JQ274268,JQ274170,JQ274121,JQ274071,JQ274022,JQ273973,JQ273875

Lomandra longifolia,Steele 1087,JQ283685,JQ283719,JQ283790,JQ283751,JQ283820,JQ283905,JQ283856,JQ277096,–,JQ275238,–,–,JQ274554,–,–,–,–,–,–,–,–,–,–,–,–,–,–,–,–,–,–,JQ274116,–,–,–,JQ273870

Manfreda virginica,Missouri Native Plants, Jefferson City, MO,JQ283712,JQ283745,JQ283816,JQ283785,JQ283846,JQ283921,JQ283871,JQ277069,JQ273549,JQ273598,JQ273647,JQ273696,JQ273794,JQ273745,JQ276290,JQ276339,JQ276728,JQ276388,JQ276437,JQ276486,JQ276535,JQ276584,JQ276633,JQ276776,JQ276824,JQ276873,JQ276922,JQ276971,JQ277020,JQ276241,JQ276192,JQ276143,JQ276094,JQ276045,JQ275996,JQ275947,JQ275898,JQ275849,JQ275800,JQ275751,JQ275702,JQ275653,JQ275604,JQ275555,JQ275506,JQ275457,JQ275408,JQ275359,JQ275310,JQ273893,JQ275162,JQ274625,JQ275261,JQ275211,JQ274674,JQ275113,JQ274531,JQ275064,JQ275015,JQ274482,JQ274433,JQ274576,JQ274966,JQ274917,JQ274188,JQ274868,JQ274819,JQ276682,JQ274770,JQ274721,JQ274384,JQ274335,JQ274286,JQ274237,JQ274139,JQ274089,JQ274040,JQ273991,JQ273942,JQ273843

Ophiopogon japonicus,Steele 1090,–,JQ283718,JQ283789,JQ283750,–,JQ283915,JQ283876,JQ277101,JQ273581,JQ273630,JQ273679,JQ273728,JQ273826,JQ273777,JQ276322,JQ276371,JQ276759,JQ276420,JQ276469,JQ276518,JQ276567,JQ276616,JQ276665,JQ276807,JQ276856,JQ276905,JQ276954,JQ277003,JQ277052,JQ276273,JQ276224,JQ276175,JQ276126,JQ276077,JQ276028,JQ275979,JQ275930,JQ275881,JQ275832,JQ275783,JQ275734,JQ275685,JQ275636,JQ275587,JQ275538,JQ275489,JQ275440,JQ275391,JQ275342,JQ273925,JQ275194,JQ274657,JQ275293,JQ275244,JQ274706,JQ275145,JQ274560,JQ275096,JQ275047,JQ274514,JQ274465,JQ274608,JQ274998,JQ274949,JQ274220,JQ274900,JQ274851,JQ276714,JQ274802,JQ274753,JQ274416,JQ274367,JQ274318,JQ274269,JQ274171,JQ274122,JQ274072,JQ274023,JQ273974,JQ273876

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Oziroe biflora,Arroyo 28509,–,–,–,–,–,JQ283931,JQ283887,JQ277093,JQ273573,JQ273622,JQ273671,JQ273720,JQ273818,JQ273769,JQ276314,JQ276363,JQ276752,JQ276412,JQ276461,JQ276510,JQ276559,JQ276608,JQ276657,JQ276799,JQ276848,JQ276897,JQ276946,JQ276995,JQ277044,JQ276265,JQ276216,JQ276167,JQ276118,JQ276069,JQ276020,JQ275971,JQ275922,JQ275873,JQ275824,JQ275775,JQ275726,JQ275677,JQ275628,JQ275579,JQ275530,JQ275481,JQ275432,JQ275383,JQ275334,JQ273917,JQ275186,JQ274649,JQ275285,JQ275235,JQ274698,JQ275137,JQ274551,JQ275088,JQ275039,JQ274506,JQ274457,JQ274600,JQ274990,JQ274941,JQ274212,JQ274892,JQ274843,JQ276706,JQ274794,JQ274745,JQ274408,JQ274359,JQ274310,JQ274261,JQ274163,JQ274113,JQ274064,JQ274015,JQ273966,JQ273867

Phormium tenax,Logee's Greenhouse, Danielson, CT,JQ283693,JQ283729,JQ283799,JQ283763,JQ283829,JQ283929,JQ283885,JQ277088,JQ273568,JQ273617,JQ273666,JQ273715,JQ273813,JQ273764,JQ276309,JQ276358,JQ276747,JQ276407,JQ276456,JQ276505,JQ276554,JQ276603,JQ276652,JQ276794,JQ276843,JQ276892,JQ276941,JQ276990,JQ277039,JQ276260,JQ276211,JQ276162,JQ276113,JQ276064,JQ276015,JQ275966,JQ275917,JQ275868,JQ275819,JQ275770,JQ275721,JQ275672,JQ275623,JQ275574,JQ275525,JQ275476,JQ275427,JQ275378,JQ275329,JQ273912,JQ275181,JQ274644,JQ275280,JQ275230,JQ274693,JQ275132,JQ274546,JQ275083,JQ275034,JQ274501,JQ274452,JQ274595,JQ274985,JQ274936,JQ274207,JQ274887,JQ274838,JQ276701,JQ274789,JQ274740,JQ274403,JQ274354,JQ274305,JQ274256,JQ274158,JQ274108,JQ274059,JQ274010,JQ273961,JQ273862

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Ruscus aculeatus,B&T World Seeds, France,JQ283703,JQ283737,JQ283809,JQ283776,JQ283838,JQ283918,JQ283892,JQ277102,JQ273582,JQ273631,JQ273680,JQ273729,JQ273827,JQ273778,JQ276323,JQ276372,JQ276760,JQ276421,JQ276470,JQ276519,JQ276568,JQ276617,JQ276666,JQ276808,JQ276857,JQ276906,JQ276955,JQ277004,JQ277053,JQ276274,JQ276225,JQ276176,JQ276127,JQ276078,JQ276029,JQ275980,JQ275931,JQ275882,JQ275833,JQ275784,JQ275735,JQ275686,JQ275637,JQ275588,JQ275539,JQ275490,JQ275441,JQ275392,JQ275343,JQ273926,JQ275195,JQ274658,JQ275294,JQ275245,JQ274707,JQ275146,JQ274561,JQ275097,JQ275048,JQ274515,JQ274466,JQ274609,JQ274999,JQ274950,JQ274221,JQ274901,JQ274852,–,JQ274803,JQ274754,JQ274417,JQ274368,JQ274319,JQ274270,JQ274172,JQ274123,JQ274073,JQ274024,JQ273975,JQ273877

Sansevieria trifasciata,Steele 1085,JQ283692,JQ283727,JQ283797,JQ283761,JQ283828,JQ283917,JQ283880,JQ277103,JQ273583,JQ273632,JQ273681,JQ273730,JQ273828,JQ273779,JQ276324,JQ276373,JQ276761,JQ276422,JQ276471,JQ276520,JQ276569,JQ276618,JQ276667,JQ276809,JQ276858,JQ276907,JQ276956,JQ277005,JQ277054,JQ276275,JQ276226,JQ276177,JQ276128,JQ276079,JQ276030,JQ275981,JQ275932,JQ275883,JQ275834,JQ275785,JQ275736,JQ275687,JQ275638,JQ275589,JQ275540,JQ275491,JQ275442,JQ275393,JQ275344,JQ273927,JQ275196,JQ274659,JQ275295,JQ275246,JQ274708,JQ275147,JQ274562,JQ275098,JQ275049,JQ274516,JQ274467,JQ274610,JQ275000,JQ274951,JQ274222,JQ274902,JQ274853,JQ276715,JQ274804,JQ274755,JQ274418,JQ274369,JQ274320,JQ274271,JQ274173,JQ274124,JQ274074,JQ274025,JQ273976,JQ273878

Scadoxus cinnabarinus,JC Pires 2011 - 04,–,–,–,–,–,JQ283911,JQ283889,JQ277078,JQ273558,JQ273607,JQ273656,JQ273705,JQ273803,JQ273754,JQ276299,JQ276348,JQ276737,JQ276397,JQ276446,JQ276495,JQ276544,JQ276593,JQ276642,JQ276784,JQ276833,JQ276882,JQ276931,JQ276980,JQ277029,JQ276250,JQ276201,JQ276152,JQ276103,JQ276054,JQ276005,JQ275956,JQ275907,JQ275858,JQ275809,JQ275760,JQ275711,JQ275662,JQ275613,JQ275564,JQ275515,JQ275466,JQ275417,JQ275368,JQ275319,JQ273902,JQ275171,JQ274634,JQ275270,JQ275220,JQ274683,JQ275122,JQ274540,JQ275073,JQ275024,JQ274491,JQ274442,JQ274585,JQ274975,JQ274926,JQ274197,JQ274877,JQ274828,JQ276691,JQ274779,JQ274730,JQ274393,JQ274344,JQ274295,JQ274246,JQ274148,JQ274098,JQ274049,JQ274000,JQ273951,JQ273852

Smilacinastellata,Plant Delight's Nursery (Raleigh, NC),JQ283715,JQ283748,–,JQ283788,–,JQ283913,JQ283860,JQ277104,JQ273584,JQ273633,JQ273682,JQ273731,JQ273829,JQ273780,JQ276325,JQ276374,JQ276762,JQ276423,JQ276472,JQ276521,JQ276570,JQ276619,JQ276668,JQ276810,JQ276859,JQ276908,JQ276957,JQ277006,JQ277055,JQ276276,JQ276227,JQ276178,JQ276129,JQ276080,JQ276031,JQ275982,JQ275933,JQ275884,JQ275835,JQ275786,JQ275737,JQ275688,JQ275639,JQ275590,JQ275541,JQ275492,JQ275443,JQ275394,JQ275345,JQ273928,JQ275197,JQ274660,JQ275296,JQ275247,JQ274709,JQ275148,JQ274563,JQ275099,JQ275050,JQ274517,JQ274468,JQ274611,JQ275001,JQ274952,JQ274223,JQ274903,JQ274854,JQ276716,JQ274805,JQ274756,JQ274419,JQ274370,JQ274321,JQ274272,JQ274174,JQ274125,JQ274075,JQ274026,JQ273977,JQ273879

Trichopetalum plumosum,Arroyo & Montalva 28505,JQ283709,JQ283742,–,JQ283782,JQ283843,JQ283904,JQ283886,–,JQ273576,JQ273625,JQ273674,JQ273723,JQ273821,JQ273772,JQ276317,JQ276366,JQ276754,JQ276415,JQ276464,JQ276513,JQ276562,JQ276611,JQ276660,JQ276802,JQ276851,JQ276900,JQ276949,JQ276998,JQ277047,JQ276268,JQ276219,JQ276170,JQ276121,JQ276072,JQ276023,JQ275974,JQ275925,JQ275876,JQ275827,JQ275778,JQ275729,JQ275680,JQ275631,JQ275582,JQ275533,JQ275484,JQ275435,JQ275386,JQ275337,JQ273920,JQ275189,JQ274652,JQ275288,JQ275239,JQ274701,JQ275140,JQ274555,JQ275091,JQ275042,JQ274509,JQ274460,JQ274603,JQ274993,JQ274944,JQ274215,JQ274895,JQ274846,JQ276709,JQ274797,JQ274748,JQ274411,JQ274362,JQ274313,JQ274264,JQ274166,JQ274117,JQ274067,JQ274018,JQ273969,JQ273871

Triteleia hyacinthina,Pires and Sytsma 2002 AJB 04,–,–,JQ283803,JQ283768,JQ283832,JQ283898,JQ283854,JQ277110,JQ273590,JQ273639,JQ273688,JQ273737,JQ273835,JQ273786,JQ276331,JQ276380,JQ276768,JQ276429,JQ276478,JQ276527,JQ276576,JQ276625,JQ276674,JQ276816,JQ276865,JQ276914,JQ276963,JQ277012,JQ277061,JQ276282,JQ276233,JQ276184,JQ276135,JQ276086,JQ276037,JQ275988,JQ275939,JQ275890,JQ275841,JQ275792,JQ275743,JQ275694,JQ275645,JQ275596,JQ275547,JQ275498,JQ275449,JQ275400,JQ275351,JQ273934,JQ275203,JQ274666,JQ275302,JQ275253,JQ274715,JQ275154,JQ274569,JQ275105,JQ275056,JQ274523,JQ274474,JQ274617,JQ275007,JQ274958,JQ274229,JQ274909,JQ274860,–,JQ274811,JQ274762,JQ274425,JQ274376,JQ274327,JQ274278,JQ274180,JQ274131,JQ274081,JQ274032,JQ273983,JQ273885

Tulbaghia violacea,JC Pires 2011 - 03,–,–,–,JQ283775,JQ283837,JQ283928,JQ283888,JQ277074,JQ273554,JQ273603,JQ273652,JQ273701,JQ273799,JQ273750,JQ276295,JQ276344,JQ276733,JQ276393,JQ276442,JQ276491,JQ276540,JQ276589,JQ276638,JQ276781,JQ276829,JQ276878,JQ276927,JQ276976,JQ277025,JQ276246,JQ276197,JQ276148,JQ276099,JQ276050,JQ276001,JQ275952,JQ275903,JQ275854,JQ275805,JQ275756,JQ275707,JQ275658,JQ275609,JQ275560,JQ275511,JQ275462,JQ275413,JQ275364,JQ275315,JQ273898,JQ275167,JQ274630,JQ275266,JQ275216,JQ274679,JQ275118,JQ274536,JQ275069,JQ275020,JQ274487,JQ274438,JQ274581,JQ274971,JQ274922,JQ274193,JQ274873,JQ274824,JQ276687,JQ274775,JQ274726,JQ274389,JQ274340,JQ274291,JQ274242,JQ274144,JQ274094,JQ274045,JQ273996,JQ273947,JQ273848

Xanthorrhoea preissii,Pires 017-4June2010,JQ283714,JQ283747,JQ283818,JQ283787,JQ283848,JQ283930,JQ283890,JQ277111,JQ273591,JQ273640,JQ273689,JQ273738,JQ273836,JQ273787,JQ276332,JQ276381,JQ276769,JQ276430,JQ276479,JQ276528,JQ276577,JQ276626,JQ276675,JQ276817,JQ276866,JQ276915,JQ276964,JQ277013,JQ277062,JQ276283,JQ276234,JQ276185,JQ276136,JQ276087,JQ276038,JQ275989,JQ275940,JQ275891,JQ275842,JQ275793,JQ275744,JQ275695,JQ275646,JQ275597,JQ275548,JQ275499,JQ275450,JQ275401,JQ275352,JQ273935,–,JQ274667,JQ275303,JQ275254,JQ274716,JQ275155,–,JQ275106,JQ275057,JQ274524,JQ274475,JQ274618,JQ275008,JQ274959,JQ274230,JQ274910,JQ274861,JQ276721,JQ274812,JQ274763,JQ274426,JQ274377,JQ274328,JQ274279,JQ274181,JQ274132,JQ274082,JQ274033,JQ273984,JQ273886

Xeronema callistemon,worldplants.com, JQ283700,JQ283734,JQ283806,JQ283772,JQ283834,JQ283920,JQ283862,JQ277112,JQ273592,JQ273641,JQ273690,JQ273739,JQ273837,JQ273788,JQ276333,JQ276382,JQ276770,JQ276431,JQ276480,JQ276529,JQ276578,JQ276627,JQ276676,JQ276818,JQ276867,JQ276916,JQ276965,JQ277014,JQ277063,JQ276284,JQ276235,JQ276186,JQ276137,JQ276088,JQ276039,JQ275990,JQ275941,JQ275892,JQ275843,JQ275794,JQ275745,JQ275696,JQ275647,JQ275598,JQ275549,JQ275500,JQ275451,JQ275402,JQ275353,JQ273936,JQ275204,JQ274668,JQ275304,JQ275255,JQ274717,JQ275156,JQ274570,JQ275107,JQ275058,JQ274525,JQ274476,JQ274619,JQ275009,JQ274960,JQ274231,JQ274911,JQ274862,JQ276722,JQ274813,JQ274764,JQ274427,JQ274378,JQ274329,JQ274280,JQ274182,JQ274133,JQ274083,JQ274034,JQ273985,JQ273887

Note: "–" indicates sequence was absent, except in the case of *Lomandra*, where submitted sequences were identical to those already present in GenBank.

APPENDIX 2. Summary of Asparagales sequencing and plastome assembly. Taxonomic assignations follow Chase et al. (2009). Ct value is an estimate from RT-PCR of the amount of plastome in total genomic DNA. Number of reads is total quantity of Illumina reads per sample after removing reads with >5Ns. Percentage plastome is the proportion of total reads assembled into plastomes (at least 85% similarity), and coverage is average read depth across the whole plastome. Gene variants indicate genes that were not present in assembled plastomes, or were present as pseudogenes.

Lineage	Taxon	Genome size (pg/2C)	Ct value	Length of reads	No. of reads	% Plastome (coverage)	Gene variants
Amaryllidaceae/ Agapanthoideae	*Agapanthus africanus* Beauverd	20.95	14.6	80	1|281|941	3.1 (25.3×)	
Amaryllidaceae/ Allioideae	*Allium cepa* L.	16.8[b]	16.3	80	2|795|386	4.38 (78.2×)	*rps2*
Amaryllidaceae/ Allioideae	*Allium fistulosum* L.	26.4	17.52	80	5|098|306	1.89 (62.1×)	*psaB*[e] *rps2*[e] *ycf1*[e]
Amaryllidaceae/ Allioideae	*Gilliesia graminea* Lindl.	N/A	17.25	80	2|915|826	1.91 (35.5×)	
Amaryllidaceae/ Allioideae	*Tulbaghia violacea* Harv.	45.1	18.45	80	2|381|172	3.08 (46.8×)	
Amaryllidaceae/ Amaryllidoideae	*Amaryllis belladonna* L.	50.9[a]	18.1	80	2|972|595	7.47 (136.3×)	
Amaryllidaceae/ Amaryllidoideae	*Crinum asiaticum* L.	45.7[a]	17.1	80	2|230|364	2.7 (38.1×)	
Amaryllidaceae/ Amaryllidoideae	*Eucharis grandiflora* Planch. & Linden	30.9[a]	18.3	80	2|745|718	0.69 (12.5×)	*ndhF*[d] *ndhD*[e] *ndhH*[e]
Amaryllidaceae/ Amaryllidoideae	*Scadoxus cinnabarinus* (Decne.) Friis & Nordal	44.2[bc]	18.6	120	5|942|909	0.51 (18×)	
Asparagaceae/ Agavoideae	*Anemarrhena asphodeloides* Bunge	6.21	17.5	80	6|425|759	1.82 (69.3×)	
Asparagaceae/ Agavoideae	*Camassia scilloides* (Raf.) Cory	N/A	N/A		1|091|459	95.79 (643.2×)[f]	*rps19*[d]
Asparagaceae/ Agavoideae	*Echeandia* sp. Ortega	18.63	18.6	80	2|368|193	4.83 (71.3×)	*rps19*[d] *rps2*[e]
Asparagaceae/ Agavoideae	*Hosta ventricosa* (Salisb.) Stearn	N/A	N/A		4|768|151	82.88 (2324.5×)[f]	*rps16 exon2*[d]
Asparagaceae/ Agavoideae	*Manfreda virginica* (L.) Rose	12.71	19.7	80	3|055|209	3.81 (71.3×)	
Asparagaceae/ Agavoideae	*Polianthes* sp. L.	4.58a[b]	17.85	80	3|274|771	2.44 (49.4×)	
Asparagaceae/ Aphyllanthoideae	*Aphyllanthes monspeliensis* L.	N/A	N/A		2|658|794	9.84 (160.8×)	
Asparagaceae/ Asparagoideae	*Asparagus asparagoides* (L.) W. Wight	4.02[a]	24.1	80	1|601|185	4.73 (47.1×)	
Asparagaceae/ Asparagoideae	*Asparagus officinalis* L.	2.73	17.5	120	4|996|275	6.37 (190.3×)	
Asparagaceae/ Asparagoideae	*Hemiphylacus alatostylus* L.Hern.	4.18[a]	17.5	80	2|876|326	7.02 (124.5×)	
Asparagaceae/ Brodiaeoideae	*Androstephium coeruleum* Greene	14.9	18.2	80	2|633|504	2.36 (39×)	
Asparagaceae/ Brodiaeoideae	*Brodiaea californica* Jeps.	13.1[a]	N/A	120	6|863|043	1.25 (51.2×)	*rps16*[d]
Asparagaceae/ Brodiaeoideae	*Dichelostemma capitatum* (Benth.) Alph.Wood	18.1	19.37	120	3|915|145	2.47 (58.2×)	*rps16 exon1*[d]
Asparagaceae/ Brodiaeoideae	*Dichelostemma congestum* Kunth	15.3[a]	16.6	120	2|492|563	2.14 (31.6×)	
Asparagaceae/ Brodiaeoideae	*Dichelostemma ida-maia* Greene	18.7[a]	16.3	120	3|933|031	2.93 (68.9×)	
Asparagaceae/ Brodiaeoideae	*Triteleia hyacinthina* Greene	12.8	17.64	120	3|559|280	1.91 (41.5×)	*rps16*[d]
Asparagaceae/ Lomandroideae	*Cordyline australis* Hook.f	1.6	14.64	80	5|855|724	13.32 (482.8×)	*clpP*[d]
Asparagaceae/ Lomandroideae	*Lomandra longifolia* Labill.	2.3	16.7	80	4|465|309&	2.04 (57.6×)	
Asparagaceae/ Lomandroideae	*Trichopetalum plumosum* J.F.Macbr.	N/A	14.2	80	2|753|011	9.92 (171.4×)	*rps16 exon1*[d]
Asparagaceae/ Nolinoideae	*Calibanus hookeri* Trel.	24	16.28	80	2|417|131	2.2 (31.9×)	
Asparagaceae/ Nolinoideae	*Dasylirion wheeleri* S.Watson	4	16.07	80	3|116|974	2.79 (55×)	
Asparagaceae/ Nolinoideae	*Eriospermum cervicorne* Marloth	N/A	16.2	120	3|037|618	2.05 (37.2×)	
Asparagaceae/ Nolinoideae	*Liriope spicata* Lour.	21	17	120	3|321|934	1.78 (35.5×)	
Asparagaceae/ Nolinoideae	*Ophiopogon japonicus* Ker Gawl.	10.2	16.43	80	2|942|473	1.29 (22.9×)	
Asparagaceae/ Nolinoideae	*Ruscus aculeatus* L.	8.8[bc]	18.37	80	3|352|547	1.86 (39.7×)	*rps16*[d] *ycf1*[e]
Asparagaceae/ Nolinoideae	*Sansevieria trifasciata* Prain	2.5	18.4	120	4|865|400	5.1 (148.4×)	
Asparagaceae/ Nolinoideae	*Smilacina stellata* Desf.	13.3[b]	N/A	120	3|171|872	2.37 (45.1×)	
Asparagaceae/ Scilloideae	*Bowiea volubilis* Harve.ex Hook.f.	4.6[b]	17.8	80	2|965|244	6.7 (124.3×)	
Asparagaceae/ Scilloideae	*Drimia altissima* (L.f.) Ker Gawl.	15.4[a]	18.78	80	3|670|644	3.72 (84.9×)	
Asparagaceae/ Scilloideae	*Ledebouria* cf. *cordifolia* (Baker) Stedje & Thulin	17.7	15.3	80	4|137|538	4.74 (121.8×)	
Asparagaceae/ Scilloideae	*Ornithogalum tenuifolium* Redouté	36.9	17.48	80	2|374|018&	3.58 (52.4×)	
Asparagaceae/ Scilloideae	*Oziroe biflora* (Ruiz & Pav.) Speta	N/A	17.5	80	1|996|258	1.99 (25×)	
Doryanthaceae	*Doryanthes palmeri* W.Hill ex Benth	6.4	16.7	120	4|446|830	4.09 (106.5×)	
Iridaceae	*Iris tenax* Douglas ex Lindl.	N/A	17.74	80	4|917|819	3.19 (100.7×)	
Xanthorrhoeaceae/ Asphodeloideae	*Aloe vera* (L.) Burm.f.	39.6	18.9	80	2|451|314	1.77 (27.7×)	*rpL32*[d] *ycf1*[e]
Xanthorrhoeaceae/ Asphodeloideae	*Asphodeline damascena* Baker	5.8[a]	14.2	80	1|608|643	10.55 (105.3×)	*rpL32*[d]
Xanthorrhoeaceae/ Asphodeloideae	*Haworthia cymbiformis* Duval	30.4	18.72	80	4|618|960	1.35 (40.2×)	*rpL32*[d]
Xanthorrhoeaceae/ Asphodeloideae	*Kniphofia linearifolia* Baker	27	21.6	80	3|078|437	1.65 (31.6×)	*rpL32*[d] *atpB*[e]
Xanthorrhoeaceae/ Hemerocallidoideae	*Phormium tenax* J.R.Forst & G.Forst	2.1	14.9	80	3|424|451	9.61 (202.9×)	
Xanthorrhoeaceae/ Hemerocallidoideae	*Xanthorrhoea preissii* Endl.	3.7[bc]	N/A	120	4|148|994	1.94 (49.6×)	
Xeronemataceae	*Xeronema callistemon* W.R.B.Oliv.	6.8	17.04	120	4|506|941	5.17 (140.8×)	*psaA*[e]

[a] Genome size obtained from averaging multiple records

[b] Genome size previously published in Kew's Plant DNA C-value Database

[c] Genome size estimate was substituted from a closely related taxon

[d] Not present in plastome, confirmed by PCR

[e] Pseudogenes

[f] Sequenced from chloroplast isolations

[g] Not included in phylogeny