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Phylogenetic Studies in the Commelinaceae Subfamily Commelinoideae Inferred from Nuclear Ribosomal and Chloroplast DNA Sequences

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Abstract—The Commelinaceae are a pantropical family of monocotyledonous herbs. Previous phylogenies in Commelinaceae have emphasized sampling among genera. We extended this previous work by sampling multiple species within some of the largest genera of Commelinaceae (especially *Commelina* and *Tradescantia*, and also including *Callisia*, *Cyanotis, Gibasis,* and *Murdannia*), and by sequencing non-coding regions both of the nuclear ribosomal DNA region, 5S NTS, and the chloroplast region, *trnL-trnF*. We generated a phylogenetic hypothesis for 68 Commelinaceae that partially tests previous morphological, taxonomic classifications. We found little evidence for conflict between nuclear and chloroplast regions for *Tradescantia*, *Murdannia*, and *Callisia*, and some evidence for conflict between the two regions for *Commelina*, though conflicting regions of the phylogeny were only weakly supported by bootstrap analyses. We found subtribe Tradescantieae to be paraphyletic, consistent with an *rbcL* study, though with a different topology than that produced by *rbcL*. In addition, subtribe Commelineae was monophyletic with strong support. We found *Callisia* to be polyphyletic, consistent with some previous molecular phylogenetic studies, and we found *Tradescantia*, *Gibasis*, *Cyanotis*, *Commelina*, and *Murdannia*, to be monophyletic. The molecular phylogenies presented here generally supported previous taxonomic classifications.

Keywords—*Callisia*, *Commelina*, cpDNA, *Murdannia*, nrDNA, *Tradescantia*.

Commelinaceae consists of approximately 650 species of monocotyledenous herbs in 41 genera (Faden 1998). Morphological taxonomy suggests that the family is split into two subfamilies, the Cartonematoideae (Pichon) Faden ex G. C. Tucker, with two genera, *Cartonema* R. Br. (11 species) and *Triceratella* Brenan (one species, *T. drummondii* Brenan), and the Commelinoideae Faden & D. R. Hunt, with the remaining 39 genera (Faden and Hunt 1991; Faden 1998). Taxonomists have split the Commelinoideae into two tribes, the primarily Old World Commelineae (Meisner) Faden & D. R. Hunt, with 13 genera, and the primarily New World Tradescantieae (Meisner) Faden & D. R. Hunt, with 25 genera (Faden and Hunt 1991; Faden 1998). The Commelineae includes *Murdannia* Royle, *Pollia* Thunb., and *Commelina* L., among others (Faden 1998). The Tradescantieae includes *Tradescantia* L., *Tinantia* Scheidw., *Thyrsanthemum* Pichon, *Gibasis* Raf., *Callisia* Loefl., *Tripogandra* Raf., *Cyanotis* D. Don, *Dichorisandra* Mikan, *Siderasis* Raf., *Spatholirion* Ridl., and *Palisota* Rchb. ex Endl., among others (Faden 1998). Within the Commelineae, *Commelina* (~170 species) and *Murdannia* (~50 species) have received morphological, taxonomic treatment. In his monograph of the family, Clarke (1881) provided a classification of *Commelina* based on capsule and seed characters, dividing the genus into two subgenera (*Didymoon* C. B. Clarke and *Monoon* C. B. Clarke). *Didymoon* species have two ovules per ventral locule, and *Monoon* species have one ovule per ventral locule (Clarke 1881). Brückner (1930) proposed four sections in *Murdannia*, including sect. *Pauciflorae* G. Brückn., with reduced inflorescences. Brückner's (1930) sect. *Terminatae* G. Brückn. ser. *Diovulatae* G. Brückn. includes *Murdannia simplex* and *M. nudiflora*.

Within the Tradescantieae, *Tradescantia*, with approximately 70 species, (Faden 1998) has been divided into twelve sections by morphological taxonomists, including sects. *Cymbispatha* (Pichon) D. R. Hunt, *Coholomia* D. R. Hunt, *Tradescantia* L., *Setcreasea* (K. Schum. & Sydow) D. R. Hunt, *Separotheca* (Waterf.) D. R. Hunt, *Mandonia* D. R. Hunt, *Parasetcreasea* D. R. Hunt, *Austrotradescantia* D. R. Hunt, (Hunt 1980), *Rhoeo* (Hance) D. R. Hunt, *Campelia* (Rich.) D. R. Hunt, *Zebrina* (Schnizlein) D. R. Hunt, and *Corinna* D. R. Hunt (Hunt 1986). Section *Cymbispatha* contains nine species distributed from Mexico to Brazil and Bolivia. Section *Coholomia* is a monotypic section containing the Guatemalan species *T. guatemalensis* C. B. Clarke ex Donn.Sm. Section *Tradescantia* was divided into four series by Hunt (1980), including ser. *Virginianae* D. R. Hunt, with approximately 17 species, which is endemic to North America. Section *Tradescantia* ser. *Virginianae* members typically have an upright habit, free petals, and have foliaceous spathes (Hunt 1980). Section *Tradescantia* ser. *Sillamontanae* D.R. Hunt contains two species, *T. sillamontana* and *T. rozynskii* Matuda (Hunt 1980). Section *Tradescantia* also contains ser. *Tuberosae* D. R. Hunt, with approximately 10 species, and ser. *Orchidophyllae* D. R. Hunt, with two species endemic to Mexico, *T. mirandae* Matuda and *T. orchidophylla* Rose & Hemsley ex Hemsley (Hunt 1980). Section *Setcreasea* has approximately five species, which are posited to be closely related based on their southern U. S. A. and Mexican distributions, petals with clawed bases, and persistent stem bases, among other characters (Hunt 1980). Section *Separotheca* is a monotypic Mexican section containing *T. pygmaea* D. R. Hunt. Section *Mandonia* is a Mexican and Guatemalan section containing seven species. Section *Parasetcreasea* is monotypic, and contains the Mexican species *T. andrieuxii* C. B. Clarke. The wholly South American sect. *Austrotradescantia* includes approximately five species, which are characterized by small chromosomes, a trailing habit (as opposed to upright), and a terminal inflorescence with spathaceous bracts (Hunt 1980). The monotypic sect. *Rhoeo* contains *T. spathacea*, the monotypic sect. *Campelia* contains *T. zanonia*, the monotypic sect. *Zebrina* contains *T. zebrina*, and the monotypic sect. *Corinna* contains *T. soconuscana*.

More recently, phylogenetic methods have been applied to the Commelinaceae using both morphological (Evans

et al. 2000) and molecular (Bergamo 2003; Evans et al. 2003; Wade et al. 2006) data. Evans et al. (2000; 2003) sampled most of the currently recognized genera, Wade et al. (2006) focused on the tribe Tradescantieae, and Bergamo's (2003) study was restricted to *Callisia*. Analyses with the chloroplast region *rbcL* provided strong support for a monophyletic Commelinaceae (Evans et al. 2003). Subtribal relationships in the Tradescantieae were also partly supported in Evans et al. (2003) and Wade et al. (2006), with the exceptions that subtribes Tradescantiinae Rohw., Thyrsantheminae Faden & D. R. Hunt, and Dichorisandrinae (Pichon) Faden & D. R. Hunt were not monophyletic in parsimony analyses of *rbcL* and *ndhF* (Wade et al. 2006). Within the Tradescantieae, *Gibasis* was nested within *Tradescantia*, and *Callisia* was not monophyletic (Evans et al. 2003), containing *Tripogandra* within it. Bergamo (2003) also found *Callisia* to be nonmonophyletic with respect to *Gibasis* and *Tripoganda*. Within the tribe Commelineae, Evans et al. (2003) found that *Commelina* and *Pollia* were part of a clade sister to *Murdannia* with strong support. However, the monophyly of most genera has not been tested, and little is known about phylogenetic relationships within most genera, including the largest, *Commelina* (~170 species) and *Tradescantia* (~70 species; Faden 1998). The family has been taxonomically difficult because of a high degree of morphological homoplasy (Evans et al. 2000, 2003).

The goal of this study was to develop a molecular phylogeny of Commelinaceae subfamily Commelinoideae, with particular focus on within genus sampling, especially for the largest two genera, *Commelina* and *Tradescantia*, using two DNA regions, the chloroplast intergenic spacer *trnL-trnF* and the 5S nuclear ribosomal nontranscribed spacer region (NTS). We sampled extensively within some genera and present a model-based (maximum likelihood, Bayesian) analysis of Commelinaceae subfamily Commelinoideae.

Materials and Methods

Taxon Sampling—We sampled 68 species of Commelinaceae (out of 650 species; Faden 1998), all of which are members of subfamily Commelinoideae, including representatives of 15 of the 41 genera in Commelinaceae (Faden 1998). We sampled six of these genera, *Commelina*, *Tradescantia*, *Murdannia*, *Cyanotis*, *Callisia*, and *Gibasis*, more intensively than other genera to aid in phylogenetic comparative analyses of these taxa (Burns 2006; Burns, Faden and Steppan, unpublished data). The present study did not sample members of the Cartonematoideae, which has only two genera, so it will not be able to address hypotheses about subfamily relationships (but see Evans et al. 2003).

Among the more densely sampled genera, we sequenced 22 *Commelina* out of ~170 species (Faden 1998). We also sequenced 17 out of ~70 species of *Tradescantia*, including *T. standleyi* (sect. *Cymbispatha*), and *T. zebrina* (sect. *Zebrina*), the monotypic sects. *Rhoeo* (*T. spathacea*), *Campelia* (*T. zanonia*), and *Corinna* (*T. soconuscana*), sect. *Cymbispatha* member *T. standleyi* (Hunt 1980), sect. *Setcreasea* members *T. brevifolia*, *T. buckleyi*, and *T. pallida*, sect. *Austrotradescantia* members *T. blossfeldiana* and *T. fluminensis*, and sect. *Tradescantia* ser. *Virginianae* members *T. bracteata*, *T. occidentalis*, *T. ohiensis*, *T. roseolens*, and *T. hirsutiflora.* Further, we sequenced five out of ~20 species of *Callisia,* five out of 50 species of *Murdannia*, five out of 50 *Cyanotis* species, and three out of 11 *Gibasis* (Faden 1998). We did not sequence additional species in *Callisia* to avoid duplicating the ongoing efforts of Bergamo (2003).

The choice of outgroup taxa was based on published accounts of higher order relationships among Commelinaceae and related families, including the Philydraceae, Pontederiaceae, and Haemodoraceae (Clark et al. 1993; Smith et al. 1993; Givnish et al. 1999; Kress et al. 2001; Evans et al. 2003; see also Chase et al. 2000). Outgroup sequences were downloaded from GenBank for the *trnL-trnF* region, including two species of Philydraceae, a single species of Pontederiaceae, and 17 species of Haemodoraceae (Givnish et al. 1999; Chase et al. 2000; Evans et al. 2003).

DNA Region Selection—DNA regions for phylogenetic analysis were chosen based on appropriate rate of molecular evolution, primer availability, and origin (e.g. cp vs. nuclear DNA) (Olmstead and Palmer 1994). The *trnL-trnF* intergenic spacer is in the large single-copy region of the chloroplast DNA, is 120–350 bp in length in Commelinaceae, and is noncoding. Because the size of *trnL-trnF* is fairly small for phylogenetic analysis, and because preliminary phylogenies indicated that *trnL-trnF* would provide insufficient resolution at the tips of the phylogeny, we also sequenced the nuclear ribosomal 5S NTS for 60 species of Commelinaceae to improve intrageneric resolution. The 5S NTS is a more quickly evolving noncoding region with sufficient variability to clarify intrageneric relationships in a variety of taxa (e.g. *Clivia* Lindl., Amaryllidaceae, Ran et al. 2001; *Ilex* L., Aquifoliaceae, Manen et al. 2002; *Lampranthus* N. E. Br., Aizoaceae, Klak et al. 2003), and has yielded suitable levels of variation in other taxa in Commelinaceae (Hardy 2001). The 5S NTS region is 98–424 bp in length in Commelinaceae.

DNA Extraction, Amplification, and Sequencing—Extractions were performed on fresh leaf material following Hsiao et al. (1994) up to the first digestion. Two rounds of phenol-chloroform extraction were performed using standard methods with modifications for phaselock tubes (2 ml Phase Lock Gel Light; Eppendorf 1999). Phenol-chloroform solutions were incubated in a water bath at 55°C for 5 min for each of two rounds of extraction. A third extraction was performed with chloroform and was also incubated at 55°C for 5 min. DNA was diluted in distilled water or TLE to a concentration of 25 ng/ml.

Amplification of *trnL-trnF* was conducted with primers "e" and "f" (Taberlet et al. 1991) or trnLF and trnLR (Sang et al. 1997). Amplification of *trnL-trnF* was modified from Taberlet et al. (1991), with dimethyl sulphoxide (DMSO) added to the PCR reactions to minimize the effects of secondary structure and enhance the sequence signal (Winship 1989). Amplification in 25 µL reactions included a mixture of 15.65 µL distilled water, 2.5 µL 10 × buffer, 1.5 µL $MgCl_2$ (25 mM), 1.25 µL DMSO, 0.1 µL dNTPs (25 mM), 0.5 µL Taq (Amplitaq Gold, Applied Biosystems, Foster City, California) polymerase (5 U/µL). Each reaction used 1.25 µL of each primer for a concentration of 10 mM per 25 µL reaction. For taxa that were difficult to amplify, 2.5 µL of BSA (10 µM) was added to the reaction mix. The PCR profile for *trnL-trnF* was 12 min at 94°C, then 40 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 30 sec, and extension at 72°C for 1 min, followed by 6 min at 72°C.

The *trnL-trnF* intergenic spacer was sequenced with the amplification primers, with the exception of *Pollia secundiflora*, which was sequenced in the forward direction with primer "e" and in the reverse direction with primer "trnLR", and *Callisia navicularis*, which was sequenced with "e" and "f". Initial chromatograms of *Commelina nairobiensis* contained multiple, overlapping peaks and could not be interpreted; this species was TA cloned using TA vector pCR2.1-TOPO (Invitrogen, Carlsbad, California) following the manufacturer's protocol, and four clones were sequenced per accession. Redundant clones of *Commelina nairobiensis* were excluded from analysis.

Universal primers (PI = forward, PII = reverse) were used to amplify 5S NTS (Cox et al. 1992). Amplification in a 25 µL reaction included: 16.65 µL water, 2.5 µL 10 × buffer, 1.25 µL DMSO, 0.5 µL $MgCl_2$, (25 mM), 0.1 µL dNTPs (25 mM), and 0.5 µL Taq (or Amplitaq Gold) (5 U/µL). 1.25 µL of each primer was used at a concentration of 10 mM. Bovine serum albumen was added to the reaction mix for taxa that were difficult to amplify. Amplification of 5S NTS used 27 cycles of denaturing at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min, followed by 6 mins at 72°C (modified from Cox et al. 1992). The Florida State University sequencing facility conducted sequencing on an Applied Biosystems 3100 Genetic Analyzer.

Because 5S NTS is a nuclear repeat, multiple copies (paralogues) exist in any individual. These multiple repeats could have divergent paralogues, in some cases potentially misleading phylogenetic analyses (Buckler et al. 1997). To ensure that sequences of 5S NTS were orthologous, PCR products of ~400 bp were isolated and cloned using TA cloning by the Florida State University cloning laboratory (vector pCR2.1-TOPO, from Invitrogen following the manufacturer's protocol). Clones of 5S NTS were sequenced using the cloning primers M13F and M13R. Multiple clones (two to four per accession) of five closely related *Commelina* species (based on preliminary *trnL-trnF* phylogenies) were sequenced to assess the monophyly of 5S NTS sequences within individuals. To determine whether 5S NTS copies yield a meaningful signal at the species level, maximum likelihood analyses were conducted with multiple clones per accession. The longest sequence per species was analyzed for species with multiple clones. Then a single arbitrary clone of 5S NTS was sequenced for the remaining species, for a total of 60 Commelinaceae species sequenced for 5S NTS (not all sequences were alignable or contained useful phylogenetic information,

and phylogenies from only 48 sequences are shown). Voucher information, GenBank accession numbers, and authorities for sampled species are reported in Appendix 1.

Data Analysis—Sequences of *trnL-trnF* were aligned in Clustal W (Version 1.82; Thompson et al. 1994; Chenna et al. 2003) using the default parameters: IUB pairwise mass matrix, 10.0 Gap opening penalty, 6.66 gap extension penalty, 0.50 transition weighing, 8 gap separation distance, 40% identity for delay, and the end gap separation penalty off. Sequences of 5S NTS were aligned using the default parameters (Clustal W Version 1.82; Thompson et al. 1994; Chenna et al. 2003) with a modified gap extension penalty of 2.5 following Hardy (2001). Because 5S NTS was quickly evolving and alignments across distantly related taxa proved difficult, separate alignments were conducted for *Murdannia*, *Tradescantia*, *Gibasis*, *Callisia*, *Cyanotis* and two clades of *Commelina (Commelina* 1 and *Commelina* 2; also see Swain 2009 for a similar approach). The choice to align within the genera *Murdannia*, *Tradescantia*, *Gibasis*, *Callisia*, and *Cyanotis* was independently supported by taxonomy and *trnL-trnF* analyses (Fig. 1). It was not possible to create a reliable alignment across all of the *Commelina* sampled for this data set, and we choose the subsets for *Commelina* using an iterative procedure. First, we attempted to align all *Commelina* and *Pollia,* chosen as an outgroup based on *trnL-trnF*, following the procedure described above (TreeBASE study number S10425). Then, we chose two blocks of taxa that the algorithm had aligned well. These initial alignments were then modified by eye and ambiguous regions were excluded for a *Callisia* alignment with 13.98% missing data (unalignable sequence which was excluded from analysis), a *Commelina* 1 alignment with 12.93% missing data, a *Commelina* 2 alignment with 19.33% missing data, a *Murdannia* alignment with 48.23% missing data, a *Tradescantia* alignment with 19.48% missing data, and a *Cyanotis* alignment with 14.40% missing data.

Two criteria were used to determine whether to combine data partitions. First, an incongruence length difference test (ILD) was used, which compares the length of the most parsimonious trees from two data partitions (Farris et al. 1995). The ILD tests were conducted in PAUP for each 5S NTS alignment (*Callisia*, *Commelina* 1 and *Commelina* 2, *Cyanotis*, *Murdannia*, and *Tradescantia*) with 1,000 replicates (HomPart command), and compared with a *trnL-trnF* phylogeny for that same subset of taxa. In addition, separate analyses were conducted for each DNA region, and the resulting phylogenies were examined for strongly supported (i.e. ≥ 80% bootstrap support) areas of conflict (Wiens 1998; see, e.g. Taylor and Hellberg 2005; but also see Bull et al. 1993; Cunningham 1997). We then combined alignments for *trnL-trnF* and 5S NTS partitions into a single alignment.

Each data partition and the combined data were analyzed with equal-weighting maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference. Modeltest (Version 3.7; Posada and Crandall 1998) was used to determine the optimal model for ML and Bayesian analyses using

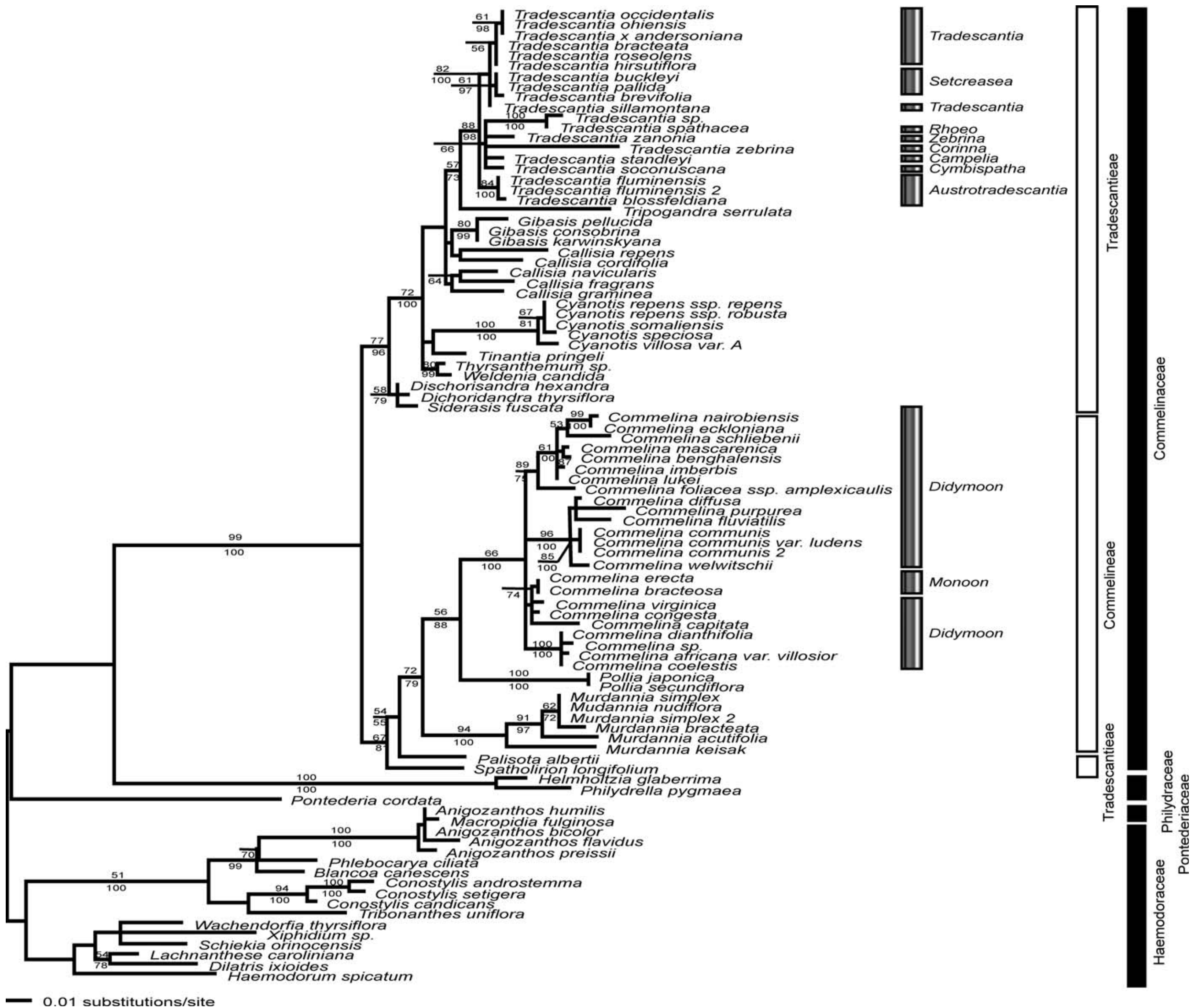

Fig. 1. One of the two maximum likelihood trees for the *trnL-trnF* data partition. Maximum likelihood bootstrap values are shown above the node and Bayesian posterior probabilities are shown below the node for values over 50%. Numbers refer to multiple genotypes sequenced per taxon. Section/subgenera (for *Tradescantia* and *Commelina*, respectively), subfamilies, and families are shown on the right.

Akaike's Information Criterion (AIC; Burnham and Anderson 2002). To determine whether there are multiple, equally parsimonious "islands" of trees, a multiple islands search approach was used, with five independent searches conducted with different random seeds (Maddison 1991). All MP and ML analyses used a heuristic search with five replicates, random addition sequences, TBR branch swapping, and a starting tree generated by stepwise addition. Bootstrap analyses were conducted using the same search parameters and 100 replicates. Each ML bootstrap replicate was stopped after ~50 hrs, about twice the amount of time necessary to reach the most likely tree in the full likelihood analysis. Maximum parsimony and ML phylogenies were generated using PAUP* version 4.0b10 for Unix (Swofford 1998).

Bayesian analyses were conducted in MrBayes (version 3.1.2; Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) with models chosen using Modeltest as described above. The *trnL-trnF* data set was run for 10 million generations sampled every 100 generations and the first 50% of the run was discarded. Data sets of 5S NTS were analyzed using four million generations sampled every 100 generations, with a discarded burnin of 10%. The combined data set was run for four million generations sampled every 100 generations, parameters estimated separately for the two data partitions, with branch lengths unlinked, with a discarded burnin of 10%. The chains were stopped when the average standard deviation of split frequencies (between two parallel chains) was less than 0.01 (Ronquist et al. 2005), and cumulative posterior probabilities of split frequencies had stabilized (Wilgenbusch et al. 2004). The *trnL-trnF* and combined analyses were rooted using members of Haemodoraceae, Philydraceae, and Pontederiaceae as outgroups. The 5S NTS analyses were rooted using the *trnL-trnF* topology to inform the rooting.

Results

trnL-trnF—The 93-sequence (68 Commelinaceae species, 20 outgroup species, with multiple assessions for some species, Appendix 1) alignment for *trnL-trnF* contained 818 characters, 225 of which were parsimony-informative (PI; TreeBASE study number S10425). Modeltest selected a TrN + G model, and two ML trees were recovered with a -ln likelihood score of 16,946.57076. Commelinaceae was monophyletic, with bootstrap support (BS) of 99% (Fig. 1). *Murdannia*, *Commelina*, *Pollia*, *Cyanotis*, *Tradescantia*, and *Gibasis* were all monophyletic, mostly with BS > 80%, whereas *Callisia* was polyphyletic. The two tribes Commelineae and Tradescantieae were mostly monophyletic in this analysis, with the exceptions of *Spatholirion longifolium* and *Palisota albertii*, which are putative members of the Tradescantieae (Faden and Hunt 1991; Faden 1998), and were placed near the base of the Commelineae, with BS of 67% and 54%, respectively (Fig. 1). Parsimony analyses were qualitatively similar to ML analyses and are not presented here.

5S NTS—Variation among clones within a species was moderate, with 7–12% variation among *Commelina communis* sequences, 0.5% for *C. benghalensis*, 0.5–1% for *C. erecta*, 0–5% for *C. diffusa*, with mostly point substitutions and no indels. As we sequenced in both directions, this variation was unlikely to be sequencing error.

The corresponding values for the 5S NTS taxon subsets were as follows: alignment for *Callisia/Gibasis/Tripogandra* (nine sequences, nine species, 230 bp, 103 PI characters), *Commelina* 1 (12 sequences, nine species, 660 bp, 80 PI characters), *Commelina* 2 (18 sequences, 11 species, 461 characters, 195 PI characters), *Cyanotis* (four sequences, four species, 257 bp, three PI characters), *Murdannia* (six sequences, five species, 668 bp, 26 PI characters), and *Tradescantia* (20 sequences, 18 species, 471 bp, 143 PI characters).

For the first *Commelina* partition, a TrN + G model was selected by AIC and a ML search found one tree with a -ln likelihood of 1,398.74467 (Fig. 2A). For the second *Commelina* partition, a GTR + G model was selected by AIC, and ML search found two most likely trees with a -ln likelihood of 3,069.83553 (Fig. 2B).

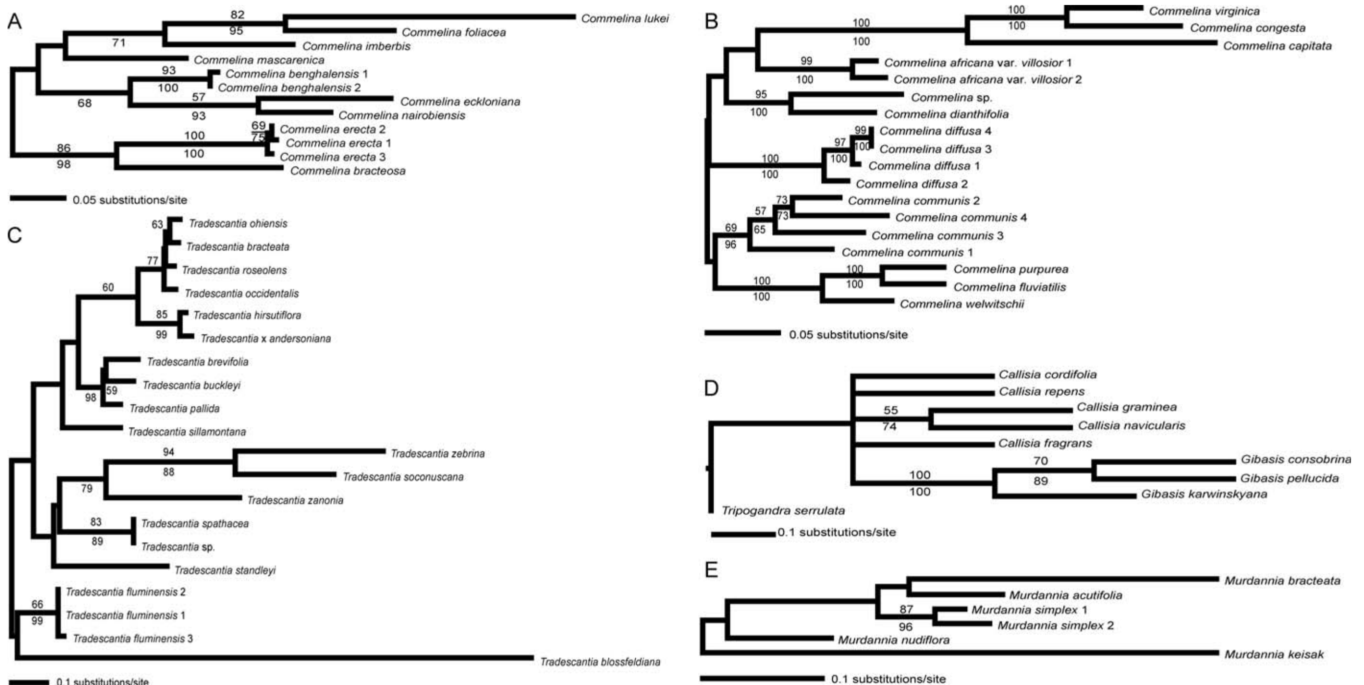

Fig. 2. Maximum likelihood (ML) trees for 5S NTS for each taxon subset. Maximum likelihood bootstrap values are shown above the branches (100 replicates) and Bayesian posterior probabilities below for values over 50%. Numbers refer to individuals with multiple clones. Trees were rooted based on the *trnL-trnF* tree (Fig. 1). (A) Maximum likelihood 5S NTS tree for the first partition of *Commelina* species. (B) One of two ML 5S NTS trees for the second partition of *Commelina* species. The two ML 5S NTS trees were topologically identical, and therefore only one is shown. (C) One ML 5S NTS tree (of 12 equally likely trees) of *Tradescantia*. (D) Single, ML 5S NTS *Callisia* tree. (E) The single ML 5S NTS tree of *Murdannia*.

The model for *Tradescantia* selected by AIC was TrN + I + G, resulting in 12 unrooted ML trees of *Tradescantia* with a -ln likelihood of 1,297.63275 (Fig. 2C). Among the 12 trees, the placement of *Tradescantia standleyi* was unclear, and in three trees, *T. blossfeldiana* was nested within *T. fluminensis*. All other clades remained stable in topology.

The model chosen for *Callisia* by AIC was GTR + G, and a single *Callisia* tree was found with a -ln likelihood score of 1489.77409 (Fig. 2D). For *Cyanotis*, a HKY model was selected by AIC, resulting in three trees with a -ln likelihood score of 662.60697. These phylogenies were unresolved and bootstrap analysis suggests that there was no useful phylogenetic information in 5S NTS among the *Cyanotis* species sampled, so the *Cyanotis* phylogeny for 5S NTS is not shown.

The model for *Murdannia* selected by AIC was K80 + I, and the single ML tree of *Murdannia* with a -ln likelihood of 1,425.61429 was rooted with *M. keisak* (Fig. 2E), based on strong support for this relationship in the *trnL-trnF* phylogeny (Fig. 1).

Analysis of Conflict Between cpDNA and nrDNA—The chloroplast region (*trnL-trnF*) and the nuclear region (5S NTS) yielded resolution at different levels of the phylogeny. The 5S NTS appeared to evolve much more quickly, and yielded a higher proportion of variable sites (64%) than did *trnL-trnF* (41%). The 5S NTS was so variable that alignments could not reliably be made across Commelinaceae, and separate alignments were made for different taxa partitions, as described in the methods. In general, *trnL-trnF* yielded better resolution at deeper nodes (Fig. 1), while 5S NTS yielded better resolution at the tips (Fig. 2). Due to this difference in the rate of evolution in the two regions, there was little well supported conflict between phylogenies generated by the two data sets.

The incongruence length difference (ILD) tests comparing phylogenies generated based on *trnL-trnF* and 5S NTS were significant for both *Commelina* data partitions (*Commelina* 1: $p = 0.02$; *Commelina* 2: $p < 0.01$). However, there was no well-supported topological conflict (> 70% BS for conflicting resolutions) between the two *Commelina* data partitions (Figs. 1, 2). There was no well-supported topological conflict between data sets within the Tradescantieae (Figs. 1, 2). Data partitions did not yield incongruent phylogenies for *Cyanotis* ($p > 0.25$), *Callisia* ($p > 0.25$), *Murdannia* ($p > 0.25$), or *Tradescantia* ($p = 0.22$). Due to the minimal conflict between data sets, a combined data set was analyzed.

Combined Analysis—Multiple copies of 5S NTS yielded monophyletic species with high BS, (≥ 69%, Fig. 2A, B), and there was no evidence of multiple paralogous copies of 5S NTS within species for which we sampled multiple clones. In addition, there was no sign of bimodality for 5S NTS in the paired differences in genetic distance between sequences, suggesting that either there were not multiple, paralogous copies of 5S NTS sequences or that there were too many to distinguish a signal of multiple copies (analysis not shown; Mitchell and Wen 2005). Therefore, a single copy of 5S NTS was used in the combined analyses.

The combined alignment contained 4,237 characters, 687 of which were PI. A combined alignment contained 100 accessions (68 Commelinaceae species, 20 outgroup species, with some sampling of multiple genotypes per species; Appendix 1). Redundancies for identical sequences were eliminated, as described above.

The combined phylogenetic analysis generally agrees with the separate analyses. A ML model of TrN + G was chosen by AIC. There were two equally likely trees in the ML analysis with a -ln likelihood score of 15756.25726, with identical ingroup topologies. Most genera were monophyletic: *Murdannia* with 76% BS, *Commelina* with 60% BS, *Pollia* with 100% BS, *Cyanotis* with 100% BS, *Tradescantia* with 99% Bayesian posterior probability (PP), and *Gibasis* with 80% BS. *Callisia* was not monophyletic (Fig. 3).

Pollia, *Commelina*, and *Murdannia*, all members of the tribe Commelineae, formed a clade (69% BS, 98% PP), and two members of the Tradescantieae, *Spatholirion* and *Palisota*, were placed sister to the Commelineae clade (Fig. 3). Members of the tribe Tradescantieae, including *Weldenia* Schult.f., *Thyrsanthemum*, *Tripogandra*, *Tradescantia*, *Gibasis*, *Callisia*, *Tinantia*, *Cyanotis*, *Dichorisandra*, and *Siderasis* (but excluding *Palisota albertii* and *Spatholirion longifolium*) also formed a clade: (55% BS, 98% PP) (Fig. 3).

Discussion

We found that Commelinaceae were a well-supported monophyletic group, consistent with previous molecular phylogenetic analysis based on the cpDNA region *rbcL* (100% BS; Evans et al. 2003) for our sample involving 68 species of the family. This was also consistent with morphological taxonomic evidence, which has long favored Commelinaceae as a natural group (e.g. Faden and Hunt 1991; Faden 1998). All members of Commelinaceae share a closed leaf sheath, succulent leaves, deliquescent flowers, and a lack of nectaries, among other characters, which appear to unite them morphologically (e.g. Faden and Hunt 1991; Faden 1998; Hardy and Faden 2004). However, morphological evidence has been inadequate to resolve relationships within the family, due to significant levels of morphological homoplasy or relatively few morphological characters (Evans et al. 2000, 2003).

The tribe Commelineae (Faden and Hunt 1991; Faden 1998) was strongly supported in this study, with 69% BS and 98% PP (Fig. 3), consistent with the molecular study of Evans et al. (2003). The Tradescantieae are nonmonophyletic (Fig. 3), though bootstrap support for this interpretation is weak. As in Evans et al. (2003), the placements of *Palisota* and *Spatholirion*, putative members of Tradescantieae (Faden and Hunt 1991; Faden 1998), were not well supported (Figs. 1, 3); although, contrary to Evans et al. (2003), they are here placed as sister to the Commelineae (Fig. 3). Nonmonophyly of Tradescantieae was also supported by some morphological evidence that places *Palisota* in Commelineae (Evans et al. 2000).

Within the tribe Tradescantieae, the close placement of *Dichorisandra* with *Siderasis* was consistent with their placement in the Dichorisandrinae, a New World subtribe (Faden and Hunt 1991; Faden 1998); however, there was no resolution in this phylogeny to evaluate the monophyly of *Dichorisandra* (Fig. 3). The placement of *Thyrsanthemum* sister to *Weldenia* was also consistent with both morphological taxonomic classifications (subtribe Thyrsantheminae; Faden and Hunt 1991; Faden 1998) and previous phylogenetic analyses (Evans et al. 2003; Wade et al. 2006), although another member of this subtribe, *Tinantia*, is not sister to *Thyrsanthemum* and *Weldenia* (Fig. 3), and its placement was poorly supported, both here (Fig. 3) and in *rbcL* analyses (Evans et al. 2003). However, the position of *Tinantia* outside the Thyrsantheminae was strongly supported by *rbcL/ndhF* data (Wade et al. 2006). *Elasis* D. R. Hunt (not sampled here) was also not monophyletic with other members of the Thyrsantheminae in Evans

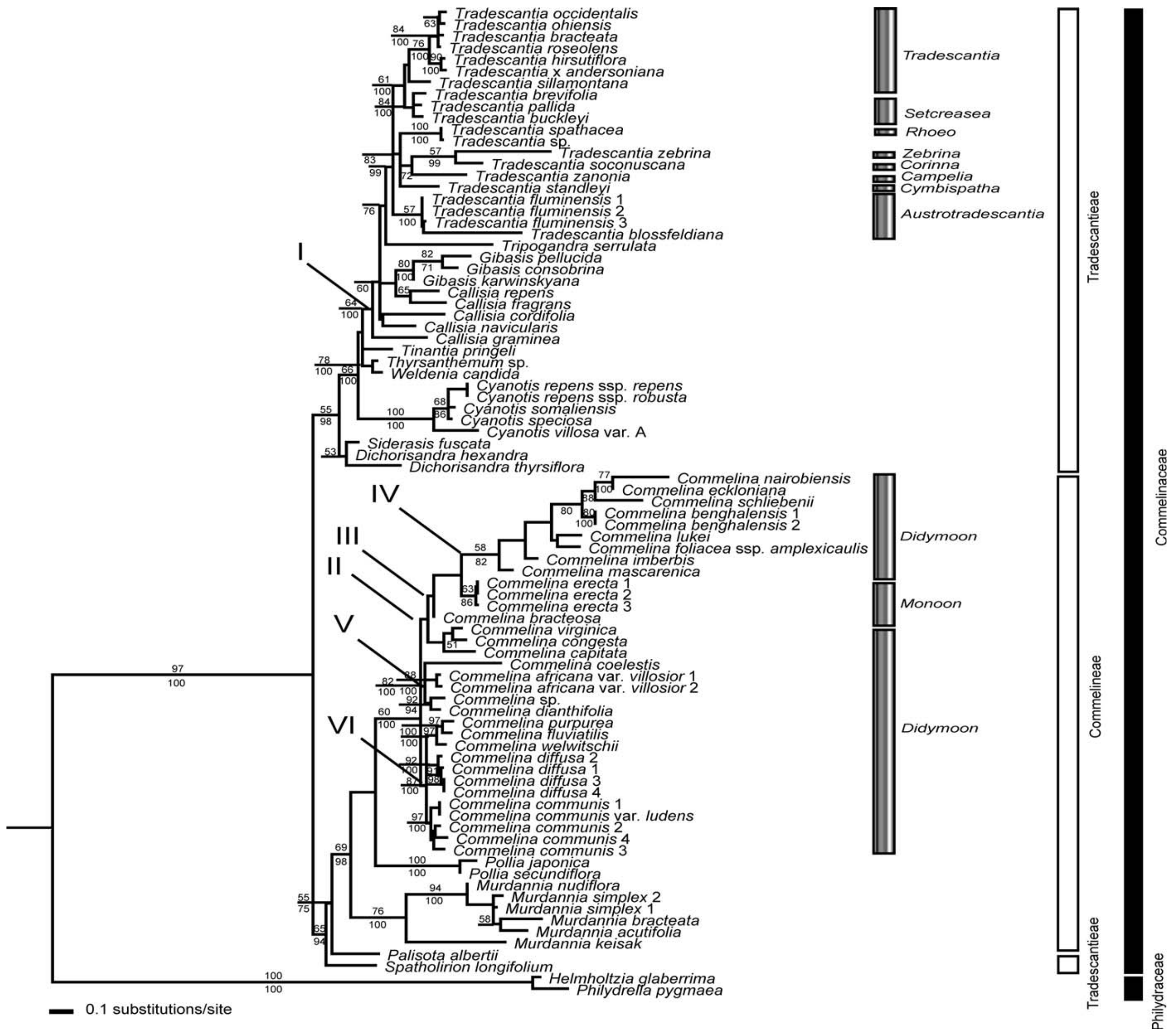

Fig. 3. Commelinaceae phylogeny generated using maximum likelihood for a combined data set with *trnL-trnF* and 5S NTS for 68 species of Commelinaceae, using Philydraceae as the outgroup. There were two topologically identical equally likely trees. Maximum likelihood bootstrap values are shown above the branches and Bayesian posterior probabilities are shown below the branches for values over 50%. We place *Commelina congesta* in Clarke's *Didymoon* for this figure due to the presence of two ovules per ventral locule. Section/subgenera (for *Tradescantia* and *Commelina*, respectively), subfamilies, and families are shown on the right. Roman numerals correspond to clades I-VI discussed in the text.

et al. (2003) and Wade et al. (2006), further questioning the status of this subtribe.

Within the subtribe Tradescantiinae (clade I; Fig. 3), *Callisia* was nonmonophyletic in these analyses (Fig. 3), confirming previous morphological and molecular studies (Evans et al. 2003; Bergamo 2003), which brings its current generic circumscription into serious question (Woodson 1942; Bergamo 2003). Contrary to Evans et al. (2003), the analyses presented here showed that *Tradescantia* is monophyletic (99% PP in the combined analysis, Fig. 3) and that *Gibasis* is not nested within *Tradescantia*, but instead within *Callisia,* though this nesting is weakly supported (Fig. 3). However, the support for *Gibasis* nested within *Tradescantia* was strong in a parsimony analysis of *rbcL* (Evans et al. 2003). Since the relationships among *Tradescantia*, *Callisia*, *Gibasis*, and *Tripogandra* were poorly supported, both here (Fig. 3) and elsewhere (Evans et al. 2003; Bergamo 2003), further work is needed to clarify the relationships among these genera; however, they were closely related (clade I; 64% BS; Fig. 3), consistent with previous taxonomic classifications (subtribe Tradescantiinae; Faden and Hunt 1991; Faden 1998) and molecular phylogenetic studies (Evans et al. 2003; Wade et al. 2006). The three sampled *Gibasis* species formed a monophyletic group (Fig. 3), and all three are members of Hunt's (1985) section *Gibasis*.

Within *Tradescantia*, our analysis was consistent with placement of *T. fluminensis* and *T. blossfeldiana* in *Tradescantia* sect. *Austrotradescantia* (Fig. 3; Hunt 1980). Woodson (1942) and others (Owens 1981) have suggested that sect. *Austrotradescantia* may warrant generic status, but only two out of six species were sampled here, so monophyly of the section remains to be demonstrated. Members of sect. *Austrotradescantia* share several morphological characteristics, including a sprawling

growth form (Hunt 1980) and unique stigmatic surface traits (Owens 1981). *Austrotradescantia* was sister to the remaining *Tradescantia* sampled here (Fig. 3).

Hunt's (1980) sect. *Tradescantia* series *Virginianae* was well-supported, with members *T. occidentalis*, *T. ohiensis*, *T. bracteata*, *T. roseolens,* and *T. hirsutiflora* monophyletic with 76% BS (Fig. 3). Also included in this clade was *T.* × *andersoniana*, a hybrid formed from members of sect. *Tradescantia* series *Virginianae* (*T. ohiensis* × (*T. subaspera* Ker Gawl. × *T. virginiana* L.)). Six out of 18 species in this series (Hunt 1980) were included in our study, therefore full validation of its monophyly requires further sampling. This analysis also suggested that *T. sillamontana* (sect. *Tradescantia* series *Sillamontanae*) is closely allied with members of sect. *Tradescantia* ser. *Virginianae*, consistent with Hunt's (1980) classification of these species in the same section, though this relationship was poorly supported by BS (≤ 50%, Fig. 3), and sect. *Tradescantia* was not monophyletic in the *trnL-trnF* analysis (Fig. 1).

Hunt's (1980) sect. *Setcreasea* was also well supported here, with *T. brevifolia*, *T. buckleyi*, and *T. pallida* forming a clade with 84% BS (Fig. 3). Additional members of sect. *Setcreasea* (*T. hirta* D. R. Hunt and *T. leiandra* Torr.) have yet to be sampled. We also found Hunt's (1986) sects. *Rhoeo*, *Zebrina*, *Corinna*, and *Campelia* and Hunt's (1980) *Cymbispatha* to form a single clade (83% PP, Fig. 3).

Within the tribe Commelineae, *Commelina* and *Pollia* were more closely related to one another than either is to *Murdannia* (Fig. 3), consistent with the *rbcL* topology (Evans et al. 2003). *Murdannia keisak* was sister to the other sampled *Murdannia* species, consistent with several morphological characteristics unique to this species (relative to the other species sampled here), including flowers in single-flowered cymes (Faden 2000) and large capsules (≥ 5 mm) (Hong and DeFilipps 2000). In addition, *Murdannia nudiflora*, *M. simplex*, *M. bracteata,* and *M. acutifolia* all have two fertile stamens and two seeds per locule (Hong and DeFilipps 2000), whereas *M. keisak* has three fertile stamens and two-six seeds per locule (Faden 2000). Brückner's (1930) series *Diovulatae* includes *Murdannia simplex* and *M. nudiflora*. In addition, we infer, based on the presence of two ovules per locule, that *Diovulatae* should include *M. bracteata* and *M. acutifolia*, and all four of these putative *Diovulatae* do form a clade with strong support (94% BS, Fig. 3), consistent with this interpretation (Brückner 1930).

No previous molecular studies have sampled extensively within *Commelina* (Evans et al. 2003). Most clades were consistently recovered by both *trnL-trnF* and 5S NTS analyses; however, *Commelina erecta* and *C. bracteosa* were placed in a clade with *C. virginica*, *C. congesta*, and *C. capitata* by *trnL-trnF* (< 50% BS; Fig. 1), and their placement within clade III in the combined analysis might partly result from the partitioning of 5S NTS (< 50% BS; Fig. 3; but note that neither placement is well supported by bootstrap analyses). The 5S NTS region did provide signal linking *C. erecta* and *C. bracteosa* together in a single clade (86% BS, Fig. 2); however, they did not form a clade in the combined analysis (Fig. 3). All other relationships were congruent between 5S NTS and *trnL-trnF*.

Our results did not support Clarke's (1881) subgeneric classifications for *Commelina*, as species of subgenus *Monoon*, *C. erecta* and *C. bracteosa*, are embedded within a larger clade, clade III, including members of subgenus *Didymoon* (Fig. 3). Within *Commelina*, a fused spathe margined clade, clade II, has arisen from a free-spathe margined ancestor, as all other *Commelina* species sampled here were free-spathe margined (Fig. 3). There were two main clades within the free-margined species, the major division seemingly between mostly New World species (clade V, which includes *C. coelestis*, *C. africana*, *C.* sp. and *C. dianthifolia*; Fig. 3; with the exception of *C. africana*, which is from Africa), and the Asian and African (and sometimes more widely distributed) species (clade VI, which includes *C. purpurea*, *C. fluviatilis*, *C. welwitschii*, *C. diffusa*, and *C. communis*; Fig. 3). Within clade VI, *Commelina welwitschii*, *C. purpurea*, and *C. fluviatilis* formed a well-supported clade (100% BS, Fig. 3), and all three species are African (Hyde and Wursten 2006) and have linear, often folded leaves (J. H. Burns, pers. obs.) and a highly unusual leaf anatomy (R. B. Faden, pers. obs.). *Commelina imberbis* and *C. mascarenica* are both in clade IV, consistent with their similar floral and vegetative morphology, which has made them difficult to distinguish in the field (Faden 2008). The yellow-flowered African species *C. welwitschii*, *C. capitata*, and *C. africana* were not closely related, in spite of the rarity of yellow flowers in the genus (R. B. Faden, pers. obs.). Moreover, *Commelina virginica* and *C. erecta,* two of the three North American species of *Commelina* indigenous north of Mexico, were not closely related to each other. Instead each was related to a different group of African species. *Commelina virginica* was closely related to *C. congesta* and *C. capitata,* with which it shares clustered, subsessile spathes. *Commelina virginica* and *C. capitata* also share the unusual character of having red hairs at the summits of the leaf sheaths. *Commelina erecta*, which is native to both Africa and the New World and was represented in our study by an American collection, was found in another predominantly African clade, clade III, which may suggest an African origin of the species and subsequent spread in the New World. Some *Commelina* species with reduced basic chromosome numbers (*C. nariobiensis*, *C. eckloniana*, and *C. benghalensis*) are closely related to one another, and reductions in basic chromosome number occurred only three times within the sampled *Commelina* species, once at the base of the *C. benghalensis, C. eckloniana*, *C. nariobiensis*, and *C. schliebenii* clade, one in *C. capitata,* and one in *C. communis*, suggesting that chromosome basic number might be taxonomically informative in the group.

We generated the first phylogenetic hypothesis in the Commelinaceae to sample multiple species within multiple genera for a nr- and a cpDNA region. We found little evidence for conflict between the two regions, with significant conflict only in *Commelina*, though with resolution at different levels of the phylogeny provided by the different DNA regions, and thus we might not have the power to detect such conflict if it does exist. We found strong support for a monophyletic Commelinaceae, a monophyletic subtribe Commelineae, and a paraphyletic subtribe Tradescantieae. We also found the genera *Commelina*, *Pollia*, *Murdannia*, *Tradescantia*, *Gibasis*, and *Cyanotis* to be monophyletic. In contrast to a previous *rbcL* study (Evans et al. 2003), we found a monophyletic *Tradescantia*, though this result is more strongly supported in the *trnL-trnF* phylogeny than in the combined *trnL-trnF* and 5S NTS phylogeny. *Callisia* was polyphyletic here, suggesting that its generic circumscription is questionable. We also found support for the sectional classifications of Hunt (1980, 1986) within *Tradescantia*, with the exception that sect. *Tradescantia* was paraphyletic in the *trnL-trnF* analysis and monophyletic in the combined analysis. This phylogeny will provide a framework for comparative studies in the Commelinaceae.

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Literature Cited

Bergamo, S. 2003. *A phylogenetic evaluation of* Callisia *Loefl. (Commelinaceae) based on molecular data*. Ph. D. dissertation. Athens: University of Georgia.

Brückner, G. 1930. Commelinaceae. Pp. 159–181 in *Die Natürlichen Pflanzenfamilien* Ed. 2. ed. A. Engler. Leipzig: Wilhelm Engelman.

Buckler, E.S. IV, A. Ippolito, and T.P. Holtsford. 1997. The evolution of ribosomal DNA: divergent paralogues and phylogenetic implications. *Genetics* 145: 821–832.

Bull, J.J., J.P. Huelsenbeck, C.W. Cunningham, D.L. Swofford, and P.J. Waddell. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42: 384–397.

Burnham, K.P. and D.R. Anderson. 2002. *Model selection and inference: a practical information–theoretic approach*. New York: Springer.

Burns, J.H. 2006. Relatedness and environment affect traits associated with invasive and noninvasive introduced Commelinaceae. *Ecological Applications* 16: 1367–1376.

Chase, M.W., D.E. Soltis, P.S. Soltis, P.J. Rudall, M.F. Fay, W.H. Hahn, S. Sullivan, J. Joseph, M. Molvray, P.J. Kores, T.J. Givnish, K.J. Sytsma, and J.C. Pires. 2000. Higher-level systematics of the monocotyledons: an assessment of current knowledge and a new classification. Pp. 3–16 in *Monocots: systematics and evolution*, eds. K.L. Wilson and D.A. Morrison. Melbourne: CSIRO.

Chenna, R., H. Sugawara, and T. Koike. 2003. Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Research* 31: 3497–3500.

Clark, W.D., B.S. Gaut, M.R. Duvall, and M.T. Clegg. 1993. Phylogenetic relationships of the Bromeliiflorae—Commeliniflorae—Zingiberiflorae complex of monocots based on *rbcL* sequence comparisons. *Annals of the Missouri Botanical Garden* 80: 987–998.

Clarke, C.B. 1881. Commelinaceae. Pp. 113–324 in *Monographiae Phanerogamarum*, Vol. 3, eds. A. de Candolle and C. de Candolle. Paris: Sumptibus G. Masson.

Cox, A.V., M.D. Bennett, and T.A. Dyer. 1992. Use of the polymerase chain reaction to detect spacer size heterogeneity in plant 5S-rRNA gene clusters and to locate such clusters in wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics* 83: 684–690.

Cunningham, C.W. 1997. Can three incongruence tests predict when data should be combined? *Molecular Biology and Evolution* 14: 733–740.

Eppendorf. 1999. *Phase lock gel (PLG) manual*. Westbury, New York: Eppendorf Scientific, Inc.

Evans, T.M., R. B. Faden, M.G. Simpson, and K.J. Sytsma. 2000. Phylogenetic relationships in Commelinaceae: I. a cladistic analysis of morphological data. *Systematic Botany* 25: 668–691.

Evans, T.M., K.J. Sytsma, R.B. Faden, and T.J. Givnish. 2003. Phylogenetic relationships in Commelinaceae: II. A cladistic analysis of *rbcL* sequences and morphology. *Systematic Botany* 28: 270–292.

Faden, R.B. 1998. Commelinaceae. Pp. 109–127 in *The families and genera of vascular plants* vol. 4, ed. K. Kubitzki. Berlin: Springer.

Faden, R.B. 2000. Commelinaceae R. Brown Spiderwort family. Pp. 170–197 in *Flora of North America*, eds. Flora of North America Editorial Committee. New York: Oxford University Press.

Faden, R.B. 2008. *Commelina mascarenica* (Commelinaceae): an overlooked Malagasy species in Africa. *Adansonia, sér* 3 30: 47–55.

Faden, R.B. and D. R. Hunt. 1991. The classification of the Commelinaceae. *Taxon* 40: 19–31.

Farris, J.S., M. Källersjö, A. Kluge, and C. Bult. 1995. Constructing a significance test for incongruence. *Systematic Biology* 44: 570–572.

Givnish, T.J., T.M. Evans, J.C. Pires, and K.J. Sytsma. 1999. Polyphyly and convergent morphological evolution in Commelinales and Commelinidea: evidence from *rbcL* sequence data. *Molecular Phylogenetics and Evolution* 12: 360–385.

Hardy, C.R. 2001. *Systematics of* Cochliostema, Geogenanthus, *and an undescribed genus in the spiderwort family, Commelinaceae*. Ph. D. dissertation. Ithaca, New York: Cornell University.

Hardy, C.R. and F.B. Faden. 2004. Commelinaceae (Spiderwort family). Pp. 425–427 in *Flowering plants of the Neotropics*. eds. N. Smith, S, Mori, A, Henderson, D.W. Stevenson, and S.V. Heald. Princeton: Princeton University Press.

Hong, D. and R.A. DeFilipps. 2000. Commelinaceae. Pp. 19–39 in *The flora of China*. *Vol. 24 (Flagellariaceae through Marantaceae)*. eds. Z.Y. Wu and P.H. Raven. Beijing: Science Press, and St. Louis: Missouri Botanical Garden Press.

Hsiao, C., N.J. Chatterton, K.H. Asay, and K.B. Jensen. 1994. Phylogenetic relationships of 10 grass species: an assessment of phylogentic utility of the internal transcribed spacer region in nuclear ribosomal DNA in monocots. *Genome* 37: 112–120.

Huelsenbeck, J.P. and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Hunt, D.R. 1980. Sections and series in *Tradescantia*. American Commelinaceae: IX. *Kew Bulletin* 35: 437–442.

Hunt, D.R. 1985. A revision of *Gibasis* Rafin. American Commelinaceae XII. *Kew Bulletin* 41: 107–129.

Hunt, D.R. 1986. *Campelia*, *Rhoeo* and *Zebrina* united with *Tradescantia*. American Commelinaceae: XIII. *Kew Bulletin* 41: 401–405.

Hyde, M.A. and B. Wursten. 2006. Commelinaceae in *Flora of Zimbabwe*. http://www.zimbabweflora.co.zw/index.php, retrieved 20 September 2006.

Klak, C., T.A. Hedderson, and H.P. Linder. 2003. A Molecular Systematic Study of the *Lampranthus* Group (Aizoaceae) Based on the Chloroplast *trnL-trnF* and Nuclear ITS and 5S NTS Sequence Data. *Systematic Botany* 28: 70–85.

Kress, W.J., L.M. Prince, W.J. Hahn, and E.A. Zimmer. 2001. Unraveling the evolutionary radiation of the families of the Zingiberales using morphological and molecular evidence. *Systematic Biology* 50: 926–944.

Maddison, D.R. 1991. The discovery and importance of multiple islands of most-parsimonious trees. *Systematic Zoology* 40: 315–328.

Manen, J.F., M.C. Boulter, and Y. Naciri-Graven. 2002. The complex history of the genus *Ilex* L. (Aquifoliaceae): evidence from the comparison of plastid and nuclear DNA sequences and from fossil data. *Plant Systematics and Evolution* 235: 79–98.

Mitchell, A. and J. Wen. 2005. Phylogeny of *Brassaiopsis* (Araliaceae) in Asia based on nuclear ITS and 5S-NTS sequences. *Systematic Botany* 30: 872–886.

Olmstead, R.G. and J.D. Palmer. 1994. Choroplast DNA systematics: a review of methods and data analysis. *American Journal of Botany* 81: 1205–1224.

Owens, S.J. 1981. Self-incompatibility in Commelinaceae. *Annals of Botany* 47: 567–581.

Posada, D. and K.A. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

Ran, Y.D., K.R.W. Hammett, and B.G. Murray. 2001. Phylogenetic analysis and karyotype evolution in the genus *Clivia* (Amaryllidaceae). *Annals of Botany* 87: 823–830.

Ronquist, F. and J.P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Ronquist, F., J.P. Huelsenbeck, and P. van der Mark. 2005. *Mr. Bayes 3.1 Manual*. Florida State University: School of Computational Science. http://mrbayes.csit.fsu.edu, retrieved 29 August 2006.

Sang, T., D.J. Crawford, and T.F. Stuessy. 1997. Chloroplast DNA phylogeny, reticulate evolution, and biogeography of *Paeonia* (Paeoniaceae). *American Journal of Botany* 84: 1120–1136.

Smith, J.F., W.J. Kress, and E.A. Zimmer. 1993. Phylogenetic analysis of the Zingiberales based on *rbcL* sequences. *Annals of the Missouri Botanical Garden* 80: 620–630.

Swain, T.D. 2009. Phylogeny-based species delimitations and the evolution of host associations in symbiotic zoanthids (Anthozoa, Zoanthidea) of the wider Caribbean region. *Zoological Journal of the Linnean Society* 156: 223–238.

Swofford, D.L. 1998. PAUP* Phylogenetic analysis using parsimony (*and other methods), V. 4.0 beta 10. Sunderland: Sinauer Associates.

Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplst DNA. *Plant Molecular Biology* 17: 1105–1109.

Taylor, M.S. and M.E. Hellberg. 2005. Marine radiations at small geographic scales: speciation in neotropical reef gobies (*Elacatinus*). *Evolution* 59: 374–385.

Thompson, J.D., D.G. Higgins, and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22: 4673–4680.

Wade, D.J., T.M. Evans, and R.B. Faden. 2006. Subtribal relationships in tribe Tradescantieae (Commelinaceae) based on molecular and morphological data. *Aliso* 22: 520–526.

Wiens, J.J. 1998. Combining data sets with different phylogenetic histories. *Systematic Biology* 47: 568–581.

Wilgenbusch, J.C., D.L. Warren, and D. L. Swofford. 2004. AWTY: A system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. Available at: http://ceb.csit.fsu.edu/awty.

Winship, P.R. 1989. An improved method for directly sequencing PCR amplified material using dimethyl-sulphoxide. *Nucleic Acids Research* 17: 1266.

Woodson, R.E. Jr. 1942. Commentary on the North American genera of Commelinaceae. *Annals of the Missouri Botanical Garden* 29: 141–154.

Appendix 1. Collections sampled for this study, presented in the following order: family: species, collecting locality, location of live collection (if applicable), voucher (herbarium acronyms follow Index Herbariorum), and GenBank assessions for *trnL-trnF* and 5S NTS. All GenBank accessions beginning in EF were generated new for this study.

Commelinaceae: *Callisia cordifolia* (Sw.) E. S. Anderson & Woodson, Florida, U. S. A., 83-197 (US), *Faden 83/37* (US), EF092889, EF101211. *Callisia fragrans* (Lindl.) Woodson, Unknown, 93-091 (US), *Chapman s. n. 1993* (US), EF092890, EF101216. *Callisia gramimea* (Small) G. C. Tucker, Unknown, Bergamo 99-189 (GA), *Giles 93L-1* (GA), EF092887, EF101213. *Callisia navicularis* (Ortgies) D. R. Hunt, Mexico, 80-410 (US), *Fryxell s. n. 1980* (GA), EF092888, EF101214. *Callisia repens* (Jacq.) L., Cultivation, Bergamo 82-291 (GA), *A. B. Graf. s. n. 1982* (US), EF092886, EF101212. *Commelina africana* L. var. *villosior* (C. B. Clarke) Brenan, Democratic Republic of Congo, Africa, 92-043 (US), *Mastromatteo s. n. 1992* (US), EF092863, EF101232, EF101233. *Commelina benghalensis* L. Florida, U. S. A., *J. H. Burns 246* (FSU), EF092854, EF101230, EF101231. *Commelina bracteosa* Hassk., Kenya, Africa, 86-207 (US), *Faden and Beentje 86/50* (US), EF092859, EF101222. *Commelina capitata* Benth., Cameroon, Africa, 90-026 (US), *Henke 90-6* (US), EF092864, EF101249. *Commelina coelestis* Willd., Andes, South America [usually this is considered to come from Mexico], R. B. Faden, *J. H. Burns 278* (FSU), EF092869, EF101252. *Commelina communis* L., Missouri, U. S. A., *J. H. Burns 250* (FSU), EF092866, EF101238, EF101239, EF101240, EF101241. *Commelina communis* L., Cultivation, R. B. Faden, *J. H. Burns 255* (FSU), EF092868. *Commelina communis* L. var. *ludens* L. (Miq.) C. B. Clarke, Cultivation, R. B. Faden, *J. H. Burns 277* (FSU), EF092867. *Commelina congesta* C. B. Clarke, Gabon, Africa, *W. Wilks 118* (US), EF092860, EF101247. *Commelina dianthifolia* DC., Cultivation, *J. H. Burns 280* (FSU), EF092857, EF101245. *Commelina diffusa* U. S. A. Burm. f., Florida, U. S. A., *J. H. Burns 259* (FSU), EF092861, EF101234, EF101235, EF101236, EF101237. *Commelina eckloniana* Kunth, Kenya, Africa, 96-456 (US), *Faden 96/546* (US), EF092853, EF101228. *Commelina erecta* L., Florida, U. S. A., *J. H. Burns 250* (FSU), EF092858 EF101223, EF101224, EF101225. *Commelina fluviatilis* Brenan, Tanzania, Africa, 96-457 (US), *Faden et al. 96/510* (US), EF092872, EF101242. *Commelina foliacea* Chiov. ssp. *amplexicaulis* Faden, Tanzania, Africa, 80-403 (US), *Faden 70/391* (US), EF092865, EF101226. *Commelina imberbis* Ehrenb. ex Hassk., Asia, Yemen (US), *Gillespie 4* (US), EF092850, EF101220. *Commelina lukei* Faden, Kenya, Africa, (US), *Luke 7080* (US), EF092855, EF101227. *Commelina mascarenica* C. B. Clarke, Somalia, Africa, *Faden & Kuchar 88/269* (US), EF092849 EF101221. *Commelina nairobiensis* Faden, Kenya, Africa, (US), *Faden 02/101 temp*. (US), EF092851, EF101229. *Commelina purpurea* C. B. Clarke, Kenya, Africa, 94-902 (US), *Faden and Ngweno 94/1* (US), EF092870, EF101243. *Commelina schliebenii* Mildbr., Tanzania, Africa, 96-462 (US), *Faden et al. 96/138* (US), EF092852, EF101253. *Commelina* L. sp., Ecuador, South America, 01-074 (US), *Grant 3983* (US), EF092862, EF101246. *Commelina virginica* L., Florida, U. S. A., *J. H. Burns 249* (FSU), EF092856, EF101248. *Commelina welwitschii* C. B. Clarke, Zimbabwe, Africa, 97-223 (US), *Faden and Drummond 97/24* (FSU), EF092871, EF101244. *Cyanotis repens* Faden & D. M. Cameron subsp. *repens*, Kenya, Africa, 80-337 (US), *Faden 74/1174* (US), EF092875, EF101257. *Cyanotis repens* subsp. *robusta* Faden & D. M. Cameron, Uganda, Africa, 80-336 (US), Faden 69/1069 (US), EF092876. *Cyanotis somaliensis* C. B. Clarke, Cultivation, Missouri Botanical Garden (Climatron) s. n. 1980 (US), EF092878, EF101254. *Cyanotis villosa* Schult. f. var. 'A', Sri Lanka, Asia, 80-331 (US), *Faden 76/555* (GA), EF092877, EF101256. *Cyanotis speciosa* (L. f.) Hassk., Cultivation, *J. H. Burns C61* (FSU), EF092879, EF101255. *Dichorisandra hexandra* (Aubl.) Kuntze ex Hand.-Mazz., French Guiana, South America, 89-070 (US), *J. J.de Granville s. n. 1989* (US), EF092883. *Dichorisandra thyrsiflora* J. C. Mikan, Brazil, South America, 80-340 (US), *Plowman 7614* (US), EF092884. *Gibasis consobrina* D. R. Hunt, Mexico, 18843 (K), EF092892, EF101217. *Gibasis karwinskyana* (Roem. & Schult. f.) Rohweder, Unknown, 18844 (K), EF092893, EF101218. *Gibasis pellucida* (Mart. & Gall.) D. R. Hunt, Florida, U. S. A., *J. H. Burns 248* (FSU), EF092891, EF101219. *Murdannia acutifolia* (Lauterb. & K.Schum.) Faden, Cultivation, *J. H. Burns 281* (FSU), EF092847, EF101260. *Murdannia bracteata* (C. B. Clarke) J. K. Morton ex D. Y. Hong, Cultivation, 94-293 (US), Missouri Botanical Garden 951919 (US), EF092846, EF101262. *Murdannia keisak* (Hassk.) Hand.-Mazz., Florida, U. S. A., *J. H. Burns 251* (FSU), EF092848, EF101261. *Murdannia nudiflora* (L.) Brenan, Florida, U. S. A., *J. H. Burns 252* (FSU), EF092844, EF101263. *Murdannia simplex* (Vahl) Brenan, Cameroon, Africa, 91-067 (US), *Kahn 90-18* (US), EF092843, EF101258. *Murdannia simplex* (Vahl) Brenan, Kenya, Africa, *Robertson 7389* (US), EF092845, EF101259. *Palisota albertii* L. Gentil, Cultivation, 18845 (K), EF092882. *Pollia japonica* Thunb., Cultivation, R. B. Faden, *J. H. Burns 266* (FSU), EF092873, EF101250. *Pollia secundiflora* (Blume) Bakh., [this collection is more properly called *P. siamensis* (Craib) D. Y. Hong], Philipines, 96-469 (US), *Bicknell 907* (US), EF092874, EF101251. *Siderasis fuscata* (Lodd.) H. E. Moore, Cultivation, Unknown (GA), EF092885. *Spatholirion longifolium* (Gagnep.) Dunn, Unknown, Unknown, AJ387744, NA. *Thyrsanthemum* sp., Unknown, (K), *Chase 606* (US), AJ387745. *Tinantia pringlei* (S. Watson) Rohweder, Unknown, R. B. Faden, *J. H. Burns 267* (FSU), EF092881. *Tradescantia × andersoniana* W. Ludw. & Rohweder nom inval. [*ohiensis* × (*subaspera* × *virginiana*)], Cultivation, *J. H. Burns 282* (FSU), EF092908, EF101268. *Tradescantia blossfeldiana* Mildbr., Cultivation, 80-362 (US), University of Chicago s. n. 1980 (US), EF092896, EF101282. *Tradescantia bracteata* Small, Cultivation, *J. H. Burns 283* (FSU), EF092906, EF101264. *Tradescantia brevifolia* (Torr.) Rose, Cultivation, R. B. Faden, *J. H. Burns 269* (FSU), EF092912, EF101272. *Tradescantia buckleyi* (I. M. Johnst.) D.R. Hunt, Unknown, 18846 (K), *18846* (K), EF092902, EF101270. *Tradescantia* sp. 'burmudensis' (horticultural variety), Cultivation, *J. H. Burns 284* (FSU), EF092900, EF101273. *Tradescantia fluminensis* Vell., Australia, 82-302 (US), *A. Faden 22/81* (US), EF092894, EF101280, EF101281. *Tradescantia fluminensis* Vell., Florida, U. S. A., *J. H. Burns 253* (FSU), EF092895. *Tradescantia fluminensis* Vell. 'variegata', Cultivation, *J. H. Burns 285* (FSU). *Tradescantia hirsutiflora* Bush, Florida, U. S. A., *J. H. Burns 279* (FSU), EF092910, EF101269. *Tradescantia occidentalis* (Britton) Smyth, Cultivation, *J. H. Burns 286* (FSU), EF092904, EF101266. *Tradescantia ohiensis* Raf., Florida, U.S.A, *J. H. Burns 247* (FSU), EF092907, EF101265. *Tradescantia pallida* (Rose) D. R. Hunt, Cultivation, *J. H. Burns 287* (FSU), EF092903, EF101271. *Tradescantia roseolens* Small, Florida, U. S. A., Bergamo 99-186 (GA), *Bergamo 99-186* (GA), EF092909, EF101267. *Tradescantia sillamontana* Matuda, Cultivation, *J. H. Burns 288* (FSU), EF092905, EF101275. *Tradescantia soconuscana* Matuda, Mexico, 80-365 (US), *Faden 76/98* (US), EF092911, EF101276. *Tradescantia spathacea* Sw., Cultivation, *Bergamo 99-201* (GA), EF092901, EF101274. *Tradescantia standleyi* Steyerm., Unknown, 18847 (K), EF092899, EF101279. *Tradescantia zanonia* (L.) Sw., Costa Rica, Central America, 91-056, *J. Grant s. n. 1995* (US), EF092897, EF101278. *Tradescantia zebrina* Heynh. ex Bosse, Unknown, 82-303, Munchen Bot. Gard. 995/65 (US), EF092898, EF101277. *Tripogandra serrulata* (Vahl) Handlos, Cultivation, *J. H. Burns 290* (FSU), EF092880, EF101215. *Weldenia candida* Schult. f., Unknown, *Chase 592* (US), AJ387746. Haemodoraceae: *Anigozanthos bicolor* Endl., Western Australia, *Demarz 9866* (KPBG), AJ387724, NA. *Anigozanthos flavidus* Redouté, Western Australia, *Chase 3082* (K), AJ387725, NA. *Anigozanthos humilis* Lindl., Western Australia, *Demarz 9866* (KPBG), AJ387726, NA. *Anigozanthos preissii* Endl., Western Australia, Ex cult. (KPBG), AJ387727, NA. *Blancoa canescens* Lindl., Western Australia, *Chase 2232* (K), AJ387728, NA. *Conostylis androstemma* F. Muell. Western Australia, *Hopper 8335* (KPBG), AJ387729, NA. *Conostylis candicans* Endl., Western Australia, *Chase 185* (NCU), AJ387730, NA. *Conostylis setigera* R. Br., Western Australia, *Demarz 11519* (KPBG), AJ387731, NA. *Dilatris ixioides* Lam., South Africa, *Goldblatt 9402* (MO), AJ387732, NA. *Haemodorum spicatum* R. Br., Australia, Unknown *Dixon s. n.* (KPBG), AJ387733, NA. *Lachnanthes caroliniana* (Lam.) Dandy, Unknown, *Chase 2910* (K), AJ387734, NA. *Macropidia fuliginosa* (Hook.) Druce, Western Australia, *Dixon s. n.* (KPBG), AJ387735, NA. *Phlebocarya ciliata* R. Br., Western Australia, *Chase 2233* (K), AJ387736, NA. *Schiekia orinocensis* (Kunth) Meisn., Unknown, *Chase 2918* (K), AJ387737, NA. *Tribonanthes uniflora* Lindl., Australia, *Chase 485* (K), AJ387738, NA. *Xiphidium* Aubl. sp., Unknown, *Chase 221* (NCU), AJ387740, NA. *Wachendorfia thyrsiflora* Burm., South Africa, *IRVC 8572* (IRVC), AJ387739, NA. Philydraceae: *Helmholtzia glaberrima* (Hook. f.) Caruel, Unknown, *Chase 452* (K), AJ387741, NA. *Philydrella pygmaea* R. Br., Unknown, *Chase 2236* (K), AJ387742, NA. Pontederiaceae: *Pontederia cordata* L., Unknown, *Chase 2996* (K), AJ387743, NA.