Molecular systematics of targeted flat sedges (*Cyperus,* Cyperaceae) of the Americas

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Background and aims – *Cyperus* is a large, ecologically diverse, and important sedge genus. Recent systematic work resolved problems with generic delimitation and implicated C_4 photosynthesis as a possible key innovation spurring diversification. Our research addressed several systematic, taxonomic, and biogeographic questions at the levels of species, species groups, and taxonomic sections. By targeting American taxa, this work helped fill a geographic sampling gap.

Methods – Phylogenetic relationships of 121 ingroup samples representing 93 taxa were inferred using both Bayesian and maximum likelihood approaches in a multi-locus framework with nucleotide data from nuclear ITS, and plastid markers *matK*, *ndhF*, *rpl32-trnL$^{(UAG)}$*, and *trnH-psbA*.

Key results – This study was the first to estimate the phylogenetic position of *Karinia*, a monotypic genus endemic to Mexico with a varied taxonomic history. *Karinia mexicana* is herein transferred to *Scirpoides* based on its phylogenetic position and morphology. *Cyperus seslerioides* and *C. andinus*, members of "section *Dichostylis*", belong in the C_3 section *Leucocephali*. Previous work resolving *Leucocephali* as sister to C_4 *Cyperus* was corroborated. Molecular evidence provided taxonomic insight in some focal taxa but was inconclusive in others. Results support an amphitropical disjunction within the *Cyperus rigens* group, centred in subtropical South America, accounting for the presence of *C. cephalanthus* in southeastern North America where it is of conservation concern. An emerging biogeographic pattern is that many North American endemic C_4 *Cyperus* are derived from Mesoamerican ancestors.

Conclusions – Phylogenetic analyses with greater taxon sampling may solidify taxonomic decisions receiving preliminary support from this research. Loci with greater phylogenetic signal are required to resolve the *Cyperus retrorsus* and *C. rigens* complexes. Further testing of the integrity of section *Leucocephali* should be of high priority considering its compelling morphology and inferred relationship to C_4 *Cyperus*. This study builds on previous work with novel taxon and increased locus sampling and contributes to ongoing efforts to build a comprehensive phylogeny of *Cyperus*.

Key words – Cyperaceae, *Cyperus*, phylogeny, C_4.

INTRODUCTION

Cyperus L. is a large, ecologically diverse, and economically important sedge genus (Simpson & Inglis 2001, Bryson & Carter 2008). For many years, the circumscription of *Cyperus* was problematic. Early molecular studies resolved a well-supported clade that included *Cyperus* s. str., but also inferred a paraphyletic genus with the inclusion of up to thir-teen other embedded genera (Simpson et al. 2007, Muasya et al. 2009a, 2009b). However, recent work has broadened the circumscription of *Cyperus* to include previously segregated genera, rendering *Cyperus* monophyletic (Larridon et al. 2011b, Larridon et al. 2014, Bauters et al. 2014).

Phylogenetic hypotheses of *Cyperus* consistently resolve a grade composed of taxa with C_3 photosynthesis leading to a strongly supported C_4 clade (Muasya et al. 2001, 2002,

Larridon et al. 2011c, 2013, Reid et al. 2014). Having apparently evolved once, the C_4 photosynthetic pathway is a synapomorphy in *Cyperus*. The evolution of C_4 photosynthesis may have spurred rapid diversification in *Cyperus* by allowing colonization of drier, sunny, and fire-driven habitats (Larridon et al. 2013). Most taxa included in this study have C_4 photosynthesis. Many sampled taxa, particularly those endemic to eastern North America, occur in dry, open habitats subject to recurring fire.

Most of the c. 950 *Cyperus* species have not been included in phylogenetic studies. Due to the massive size of the genus, a comprehensive phylogeny for *Cyperus* is not imminent. This study was motivated by several systematic, taxonomic and biogeographic questions and seeks: (1) to estimate the phylogenetic position of *Karinia* Reznicek & McVaugh, which has previously been included in *Cyperus*; (2) to estimate relationships of *Cyperus* sections *Leucocephali* Chermezon ex Kük. and "*Dichostylis* (P.Beauv.) Baillon" to C_4 *Cyperus*; (3) to test the monophyly of *Cyperus* section *Diclidium* (Schrad. ex Nees) Griseb.; (4) to assess taxonomic problems in *Cyperus* sections *Strigosi* Kük. and *Umbellati* (C.B.Clarke) Kük. (nom. illeg.; Reynders et al. 2011); and (5) to elucidate relationships within the *Cyperus rigens* species group. This study includes samples of many taxa outside of primary groups of interest, adding an exploratory aspect to this work with the intentions of gaining additional phylogenetic and biogeographic insights and identifying future research problems.

Scirpus, *Cyperus*, or *Karinia?*

Karinia is a monotypic genus endemic to Mexico. Its only species, *Karinia mexicana*, was previously included in *Scirpus* L. as *S. mexicanus* C.B.Clarke ex Britton (and *S. orbicephala* Beetle) on the basis of having spirally arranged floral scales, a character state typical of tribe Scirpeae. Koyama & McVaugh (1963) transferred this species to *Cyperus* because of its basally disposed leaves and presence of empty floral scales at the base of each spikelet (lowest floral scales in *Scirpus* are flower-bearing). Later, Reznicek and McVaugh (McVaugh 1993) determined this Mexican endemic was sufficiently unique, having dense spherical spikes and spirally-arranged floral scales, to warrant erection of a new genus, *Karinia*, which they placed in tribe Cypereae due to its *Cyperus*-type embryo. *Karinia* was subsequently validated by Reznicek & McVaugh (1994). The phylogenetic position of *Karinia* has not previously been assessed and its taxonomic classification has not been evaluated using molecular evidence.

Which C_3 taxa are most closely related to the C_4 clade?

As mentioned above, C_4 photosynthesis is a synapomorphy in *Cyperus*. Larridon et al. (2011c) resolved a clade containing *Cyperus schomburgkianus* plus three species of *Kyllingiella* R.W.Haynes & Lye as sister to C_4 *Cyperus*. Larridon et al. (2011b) lumped *Kyllingiella* with *Cyperus* on the basis of morphological and molecular evidence and placed these species in section *Leucocephali*, to which *C. schomburgkianus* had previously been assigned. The pantropical section *Leucocephali* is diagnosed by the presence of small to medium sized plants with dense head-like inflorescences, having pale-coloured floral scales, and growing in open grasslands subject to seasonal drying (Simpson 1990, Larridon et al. 2011b, 2011c). The ecological adaptation to drier open habitats of section *Leucocephali* may be transitional toward the evolution of C_4 photosynthesis, a photosynthetic pathway that enhances drought tolerance (Larridon et al. 2011b).

Included in this study are *Cyperus seslerioides* and *C. andinus*, which are distinguished by Kükenthal (1935–1936) but considered conspecific by more recent authors (Tucker 1994, Gómez-Laurito 2003, Govaerts et. al 2016). *Cyperus seslerioides* (including *C. andinus*) is common in Mexico (Tucker 1994) and extends into South America to Argentina (Govaerts et al. 2016). *Cyperus seslerioides* and *C. andinus* were included in Kükenthal's (1935–1936) "section *Dichostylis*". This sectional name is illegitimate (Huygh et al. 2010) and no name currently exists for this group. This group is likely artificial since it contains at least two species, *Cyperus meeboldii* Kük. and *C. michelianus* (L.) Link, confirmed to be C_4 plants (Bruhl & Wilson 2007, Larridon et al. 2011c). *Cyperus andinus* and *C. seslerioides* resemble members of section *Leucocephali* in having pale, head-like inflorescences, minutely papillose achenes (papillae lacking in the sample of *C. andinus* included in this study), and by growing in upland habitats (Simpson 1990, Tucker 1994). The morphological similarity between *Cyperus andinus* and *C. schomburgkianus* is evident in figs 1 & 2. This study examines the relationship between the included representatives of "section *Dichostylis*" and section *Leucocephali*, and relationships of both taxa to C_4 *Cyperus*.

Cyperus section *Diclidium*

Cyperus section *Diclidium* is characterized by a unique mode of spikelet shattering with the spikelet breaking into

Figure 1 – *Cyperus andinus* in Arizona, U.S.A. Photo by Max Licher, Northern Arizona University. Used with permission.

one-fruited segments (Kükenthal 1935–1936, Tucker 1994, Tucker et al. 2002). This research tests the null hypothesis that section *Diclidium* is monophyletic. *Cyperus odoratus* shares a corky spikelet rachilla with *Cyperus pedunculatus*, a species not previously classified in section *Diclidium*. This feature may have allowed water dispersal of fruits, accounting at least partly for the large, transoceanic ranges of these two species. Further investigation of a possible close relationship between these two taxa was suggested by Larridon et al. (2013). Due to their overall morphological dissimilarity, the null hypothesis is that the corky rachilla is a homoplastic trait.

Distinctness of *Cyperus stenolepis*

There has been inconsistency in the taxonomic treatment of *Cyperus stenolepis*, a wetland species endemic to the southeastern United States. Some authors (Tucker et al. 2002, Govaerts et al. 2016) do not recognize *C. stenolepis*, treating it as a synonym of *C. strigosus*. Kükenthal (1935–1936) treated *C. stenolepis* as *C. strigosus* var. *stenolepis* (Torr.) Kük., a treatment retained by Horvat (1941) with reservation. *Cyperus stenolepis* is a larger, more robust plant than

Figure 2 – *Cyperus schomburgkianus* in the State of Minas Gerais, Brazil. Photo by William Milliken, RBG/Kew. Used with permission.

C. strigosus (electronic appendix 1A & B) and has longer and more remote floral scales (fig. 3). *Cyperus stenolepis* also differs ecologically from *C. strigosus* by inhabiting sites with organic, peaty substrates and long hydroperiods. *Cyperus strigosus* typically occurs on seasonally wet mineral soils subject to acute drying in late summer (Reid, pers. obs.). The relationship between *C. strigosus* and *C. stenolepis*, both members of section *Strigosi*, has not previously been tested using molecular evidence, but the morphological similarity suggests these two taxa are sister species.

Problems in *Cyperus* section *Umbellati*

There are several focal taxonomic problems in *Cyperus* section *Umbellati*. This apparently artificial group (Reid et al. 2014) is diagnosed by the following traits: perennial duration, cormose plant bases, simple (unbranched) spikes, and spikelets usually possessing only one to several fertile floral scales (Kükenthal 1935–1936). Images of several focal *Umbellati* taxa are presented in fig.4. The taxonomic and nomenclatural histories of some taxa within this group are complex. The goals of this study relative to members of this group are to provide additional information for taxonomic revision and to elucidate evolutionary relationships. Focal taxa of section *Umbellati* in this research are *Cyperus blodgetii*, *C. echinatus*, and *C. retrorsus*.

Emphasizing similarities in floral scale posture and general inflorescence architecture, Carter (1984) relegated plants with dense spikes, ascending scales, and divaricate to reflexed primary inflorescence bracts to *Cyperus ovatus* (fig. 4C). These plants were treated variously by Kükenthal (1935–1936) and Horvat (1941) as *Cyperus blodgettii*, *C. pollardii* Britton ex Small, *C. retrorsus* var. *curtisii* (C.B.Clarke) Kük., and *C. winkleri* Britton ex Small. Plants called *Cyperus blodgettii* (fig 4E), having greenish to reddish brown floral scales and fewer pedunculate spikes and primary inflorescence bracts, and being endemic to southern peninsular Florida, U.S.A., were provisionally given infraspecific rank under *C. ovatus* (Carter 1984). Desiring further study, Carter never published this new combination. *Cyperus blodgettii* was not accounted for by Tucker et al. (2002) in their *Flora of North America* treatment of *Cyperus*. This species is treated as a synonym of *C. retrorsus* by Govaerts et al. (2016).

Carter & Kral (1990) determined that *Cyperus echinatus* was the correct name for *C. ovularis* (Michx.) Torr. *Cyperus echinatus*, which is endemic to eastern North America, is easily recognized by its dense, spherical spikes and divaricate bracts (fig. 4A, electronic appendix 1C) (Carter 1984). Kükenthal (1935–1936) treated *Cyperus ovularis* var. *sphaericus* Boeckeler as a distinct taxon characterized by fewer and shorter rays, shorter spikelets, and more intensely reddish pigmented floral scales than the nominal variety now called *C. echinatus* (electronic appendix 1D). This variety was regarded by Carter (1984) as an ecotype not warranting formal rank. No combination has been published including "var. *sphaericus*" as an infraspecific taxon under *Cyperus echinatus*. Additional morphological evidence (Carter, unpubl. data) has prompted re-evaluation of this entity. Ecological evidence also suggests that "var. *sphaericus*" warrants

formal recognition. Whereas typical *Cyperus echinatus* tends to grow in disturbed areas on loamy and clayey soils, "var. *sphaericus*" occurs in sandy soils of high-quality, fire-maintained pine grasslands in the southeastern United States.

Cyperus retrorsus is nearly endemic to the Atlantic and Gulf of Mexico coastal plains of the southern and eastern United States. This species is a tufted perennial with cylindric spikes and strongly ascending bracts (fig. 4D) that grows in dry to mesic, often disturbed, sites. Several varieties of *Cyperus retrorsus* have been described, but these are often not recognized, with recent authors taking a broad view of *C. retrorsus* (e.g. Tucker et al. 2002, Govaerts et al. 2016). *Cyperus retrorsus* var. *nashii* differs from the nominal variety by having divaricate versus ascending bracts, spikes with less densely-packed spikelets, lustrous, fuscus floral scales with faint nerves, longer anthers, broader and usually longer achenes, and smaller differences between floral scale lengths and achene lengths (Carter 1984). Ecologically, *C. retrorsus* var. *nashii* is faithful to undisturbed xeric sand ridges, whereas var. *retrorsus* is a weedy element of more mesic soils. *Cyperus plankii* represents another taxon included in this study which is not usually recognized. Tucker et al. (2002) list this species as a synonym of *Cyperus croceus* while Govaerts et al. (2016) include it within *C. retrorsus*. Also included in this study is an undescribed species, referred to herein as *Cyperus* sp. ined. (fig. 4F & G), which is suspected to be a close relative of *Cyperus retrorsus* (fig. 4D). Like *Cyperus retrorsus* var. *nashii*, this entity occurs in undisturbed xeric sand ridges on the southeastern United States coastal plains. The goals of this study with respect to *Cyperus retrorsus* are to examine its taxonomy, using a molecular approach to determine whether a broad circumscription of this species is warranted, and to estimate the relationship of *Cyperus* sp. ined. to *C. retrorsus*.

Cyperus cephalanthus and the *C. rigens* group

Cyperus cephalanthus was first collected from coastal Texas in the southern United States and was described in 1836 (Torrey 1836). This sedge is now known also from wet coastal tall-grass prairies in Louisiana. Its relationship to similar plants in South America, including *Cyperus rigens*, has long been suspected (Pedersen 1972) and was recently confirmed (Reid et al. 2014). Close similarity between North American *Cyperus cephalanthus* and specimens collected from humid grasslands in subtropical South America (Argentina, Brazil, and Paraguay) has been recognized. Some South American specimens have been determined as *C. cephalanthus* (Tucker et al. 2002). Pedersen (1972) provided the most detailed taxonomic revision of the *Cyperus rigens* group, making several new combinations and providing a key to hypothetically related species. Pedersen (1972) treated *Cyperus cephalanthus* as a subspecies of *C. rigens* (*C. rigens* subsp. *cephalanthus* (Torr. & Hook.) T.M.Ped.). In Pedersen's key *C. rigens* subsp. *cephalanthus* keys out with taxa having nine nerves on the floral scales and being restricted to southeastern North America. Floral scales of North American *Cyperus cephalanthus* typically have seven nerves, and plants usually feature scabrous culm angles. Therefore, North American *Cyperus cephalanthus* key to *Cyperus impolitus* in Pedersen's treatment, suggesting these species are morphologically the same. Morphometric study by Reid (2016) aimed at distinguishing *Cyperus cephalanthus* and *C. impolitus* revealed significant differences in several characters between North and South American specimens. Based on those results, North American samples are identified herein as *Cyperus cephalanthus* and South American samples as *C. impolitus*. Hefler (2010) addressed the taxonomy of the *Cyperus rigens* group by lumping several *Cyperus rigens* subspecies, includ-

Figure 3 – Comparison between spikes of *Cyperus stenolepis* (left) and *C. strigosus* (right) showing typically longer and more remotely spaced floral scales on the former species as compared to the latter. Photo of *Cyperus stenolepis* by Randy Mears; photo of *C. strigosus* by J.R. Carter.
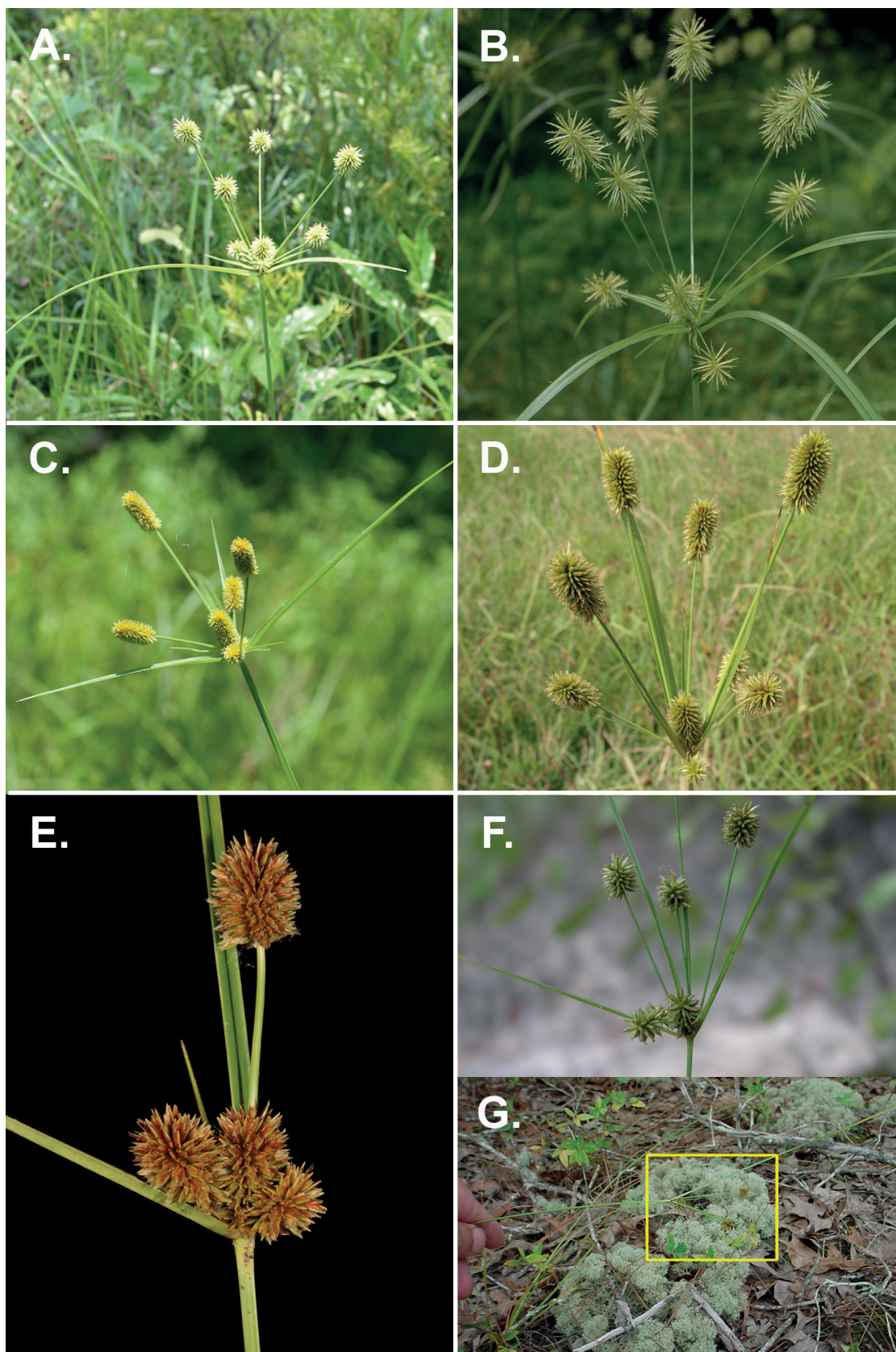
Figure 4 – Images of representative members of *Cyperus* section *Umbellati*. A, *Cyperus echinatus*; B, *Cyperus lancastriensis*; C, *Cyperus ovatus*; D, *Cyperus retrorsus*; E, *Cyperus blodgettii*; F, *Cyperus* sp. ined.; G, *Cyperus* sp. ined. with anthelum highlighted, associated with the fruticose lichen *Cladonia* P. Browne, an indicator of xeric sandy soils. A–D & F–G by J.R. Carter; E by Randy Mears.

ing subsp. *serrae*, which is included in this study, with *C. rigens* var. *rigens*. She also treated *Cyperus impolitus* as a variety of *C. rigens*, creating the new combination *C. rigens* var. *impolitus* (Kunth) Hefler & Longhi-Wagner (Hefler 2010). Hefler did not address *Cyperus cephalanthus*. The research presented here seeks to elucidate relationships among the *Cyperus rigens* group (fig. 5) and to determine if evidence shows molecular divergence of North American *Cyperus cephalanthus* from South American *Cyperus impolitus*.

MATERIALS AND METHODS

Taxonomic sampling

Sampling of taxa for this study was guided mainly by the research goals described above. An attempt was also made to acquire samples of as many *Cyperus* species as possible during field work. Most samples were collected by the authors from the southeastern United States, Argentina, and Uruguay. Several samples were obtained from herbarium speci-

Figure 5 – Representative members of the *Cyperus rigens* group. A, *Cyperus cephalanthus* in Louisiana, U.S.A.; B, *Cyperus cephalanthus* habit; C, *Cyperus rigens* subsp. *rigens* growing on margins of freshwater pond in Uruguay; D, *Cyperus rigens* subsp. *rigens* (arenic variant) growing in sandy *Butia yatay* (Mart.) Becc. savanna in Corrientes, Argentina. Photographs by C.S. Reid.

mens and some were kindly provided by colleagues in other regions. Sequences for some ingroup and most outgroup taxa were obtained from GenBank (www.ncbi.nlm.nih.gov/genbank/). Taxon names, voucher specimen information, and GenBank accession numbers are supplied in electronic appendix 2. Scientific names are those accepted by Govaerts et al. (2016) in most cases. Alternative names are used to highlight taxa which may have merit, but that have been lumped with other taxa or not recognized, often without substantial evidence. For these taxa, names accepted by Govaerts et al. (2016) are also provided in electronic appendix 2. Most specimens are deposited at the Shirley C. Tucker Herbarium at Louisiana State University (LSU). Once databased and filed at LSU, specimen images can be viewed online at http://data.cyberfloralouisiana.com/lsu/. The herbarium code (Thiers continuously updated) is given only for specimens deposited elsewhere. Images of specimens deposited at VSC and FLAS can be viewed online at http://herb.valdosta.edu/database.php and https://www.flmnh.ufl.edu/herbarium/cat/catsearch.htm, respectively. To enable evaluation of biogeographic patterns, native ranges of taxa were determined by consulting Govaerts et al. (2016) and Kartesz (2015).

DNA extraction and marker amplification

Tissue samples were placed in silica gel upon collection in the field. Approximately 20 mg of dry leaf material was ground using the Mini-Beadbeater 8 (BioSpec Products, Bartlesville, OK, U.S.A.). Genomic DNA was extracted and purified using the DNeasy Plant Mini kit (Qiagen, Valencia, CA, U.S.A.) following manufacturer's protocol. Amplifications were performed in 25 ml reactions containing 1 ml of template, 12.5 ml of Master AMP™, 0.5 ml of Tfl DNA Polymerase (Epicentre Biotechnologies), and 1 ml of each 10 mM forward and reverse primer.

Two chloroplast genes, two chloroplast intergenic spacers, and the nuclear ribosomal internal transcribed spacer were amplified. The gene *matK* was amplified using forward primer *matK-1F* and reverse primer *matK-5R* (Gilmour et al. 2013). The *ndhF* region was amplified using *ndhF-A* and *ndh-D1* forward and reverse primers (Gilmour et al. 2013). Cycling conditions for amplification of *matK* and *ndhF* were as described in Gilmour et al. (2013). The intergenic spacers *rpl32-trnL*$^{(UAG)}$ and *trnH-psbA* were amplified using the primers and cycling conditions described in Shaw et al. (2007). Amplification of the nuclear internal transcribed spacer (ITS) (ITS1-5.8SrDNA-ITS*2*) followed the protocol described in Reid et al. (2014) using forward primer sef 17 and reverse primer ser 26 (Sun et al. 1994). Gel electrophoresis was performed to confirm success of PCR reactions. PCR products were shipped on dry ice to Beckman Coulter Genomics, Danvers, MA, U.S.A, for sequencing using the amplification primers. Returned sequences were compared with the NCBI nucleotide database using a query with the blastn algorithm (Altschul et al. 1990) to verify that the sequences belonged to the target organism and not a contaminant. Sequences were edited using Sequencher ver. 4.2.2 (Gene Codes Corporation). Multiple sequence alignments (MSA) of edited sequences were estimated with MAFFT ver. 7.017 (Katoh et al. 2002) using the MAFFT plug-in of Geneious ver. 6.1.5

(Drummond et al. 2013) using default settings (algorithm = auto; scoring matrix = 200PAM; gap open penalty = 1.53; offset value = 0.123).

Phylogenetic analyses

Phylogenetic hypotheses were inferred using Bayesian (BI) and maximum likelihood (ML) approaches. The best-fit model of nucleotide sequence evolution was selected using jModelTest ver. 2.1.7 (Darriba et al. 2012). Models selected using the corrected Akaike Information Criterion (AICc) were K80+G for ITS1, TPM3+I+G for 5.8SrDNA, JC for ITS2, TVM+I+G for *matK*, TPM3uf+I+G for *ndhF*, TVM+G for *rpl32-trnL*$^{(UAG)}$, and TIM3+G for *trnH-psbA*.

Bayesian analyses were conducted using MrBayes ver. 3.1.2 (Ronquist & Huelsenbeck 2003) via the CIPRES Science Gateway (Miller et al. 2010) and ML analyses were performed using Garli ver. 2.01 (Zwickl 2006) for independent locus and concatenated MSAs. The number of sequences of individual regions varied based on variable success of PCR reactions performed for this research and availability of sequences from GenBank. Missing data is indicated in electronic appendix 2. Following confirmation of general agreement among single locus trees, concatenated MSAs including all five loci and only the four plastid loci were assembled using SequenceMatrix ver. 1.8 (Gaurav et al. 2011) with partitions corresponding to individual loci. To be included in the concatenated MSA containing all five markers, a taxon had to have sequences available for at least three out of five loci with ITS data present, or four out of five loci when the ITS sequence was lacking. Taxa having data present for at least two of the four plastid regions were included in the plastid concatenated MSA.

The BI analysis of the five-marker concatenated MSA consisted of four parallel runs using one heated (temp = 0.1) and three cold chains per run for 50,000,000 generations, sampling every 1 000 generations. Bayesian analysis of the plastid MSA was run for 20 000 000 generations, sampling every 2 000 generations. MrBayes implements fewer models than are tested by jModelTest and implemented in Garli. Therefore, the best-fit model selected by jModelTest that is implemented in MrBayes was applied to each partition, which corresponded to individual genic loci. All parameters of sequence evolution were unlinked across partitions. Convergence of each run was assessed by ensuring that potential scale reduction factors (PSRF) reported in MrBayes were close to 1 and effective sample size (ESS) values were greater than 200 in Tracer ver. 1.5 (Rambaut et al. 2014). Bayesian majority-rule consensus trees for five-locus and plastid concatenated MSAs were produced from stationary distributions after discarding the first 25 % of sampled trees as burn-in.

The ML topology and parameters of the partitioned concatenated MSAs were estimated from 100 replicate searches per dataset with a starting tree generated by stepwise addition with fifty attachments evaluated per taxon. For both concatenated MSAs, node support was estimated from ML analysis of 1 008 pseudoreplicate bootstrapped datasets with the best tree from two search replicates stored per bootstrap dataset for the calculation of node frequencies. Support values (both

posterior probabilities and node frequencies) were mapped onto the ML trees with SumTrees ver. 3.3.1 using the Dendro-Py Phylogenetic Computing Library ver. 3.12.0 (Sukumaran & Holder 2010). The final trees were inspected in FigTree ver. 1.4.2 (Rambaut 2006–2009) and prepared for publication using Adobe® Illustrator® CS3 and Photoshop® CS3.

RESULTS

The results of individual-locus phylogenetic analyses showed considerable variation in resolution across the five molecular markers; however, they were generally congruent (electronic appendix 3B–F). The five-locus concatenated MSA included 121 ingroup samples representing 93 taxa, and 13 outgroup taxa. The aligned concatenated five-marker matrix, consisting of seven partitions corresponding to individual loci (ITS containing three partitions), had a total of 5 844 characters. Despite 100 ML search replicates, the best tree was not found more than once. The ML tree with the highest likelihood resulting from the five-marker concatenated analysis is presented in figs 6 & 7. Outgroup taxa *Fuirena robusta*, *Schoenoplectus lacustris*, and *S. tabernaemontani*, were pruned from the final tree using Mesquite ver. 3.04 (Maddison & Maddison 2015) to reduce compression of branches.

The plastid concatenated matrix included 125 ingroup samples representing 97 taxa, and 13 outgroup taxa. The aligned concatenated plastid matrix had four partitions and a total of 5 119 characters. In the analysis of the plastid MSA, the best ML tree was found twice in 100 search replicates. The same outgroup taxa pruned from the five-marker phy-

logenetic tree, plus *Calliscirpus brachythrix*, were similarly pruned from the plastid tree, which is presented in electronic appendix 3A.

DISCUSSION

This discussion is based on the five-marker phylogenetic analysis and the resulting hypothesis presented in figs 6 & 7 since this is the most robust result generated from this research. The plastid tree (electronic appendix 3A) was inferred to include *Cyperus schoburgkianus* in this study to gain insight into its relationship with morphologically similar taxa in "section *Dichostylis*" (figs 1 & 2). Material of this species was not available for DNA extraction. It is included on the basis of *rpl32-trnL$^{(UAG)}$* and *trnH-psbA* sequences available from GenBank, which were not adequate to permit inclusion of this taxon in the five-marker analysis based on criteria explained above.

Cyperus is monophyletic and strongly supported (bootstrap proportion (bsp)/posterior probability (pp) = 95/1.00) (fig. 6). Taxa possessing C$_3$ photosynthesis form a grade (fig. 6) that leads to a C$_4$ clade (bsp/pp = 100/1.00) (fig. 7). As with other studies (Larridon et al. 2013, Reid et al. 2014), the C$_4$ clade is poorly resolved at deeper nodes and branch lengths are short, but resolution in the C$_4$ clade improves, in many cases, at shallower nodes in the phylogeny.

Several C$_3$ clades (fig. 6) correspond to taxonomic sections within *Cyperus* (Kükenthal 1935–1936, Larridon et al. 2011c) and are so labelled. Clades correspond to sections *Haspani* (Kunth) C.B.Clarke, *Fusci* (Kunth) C.B.Clarke,
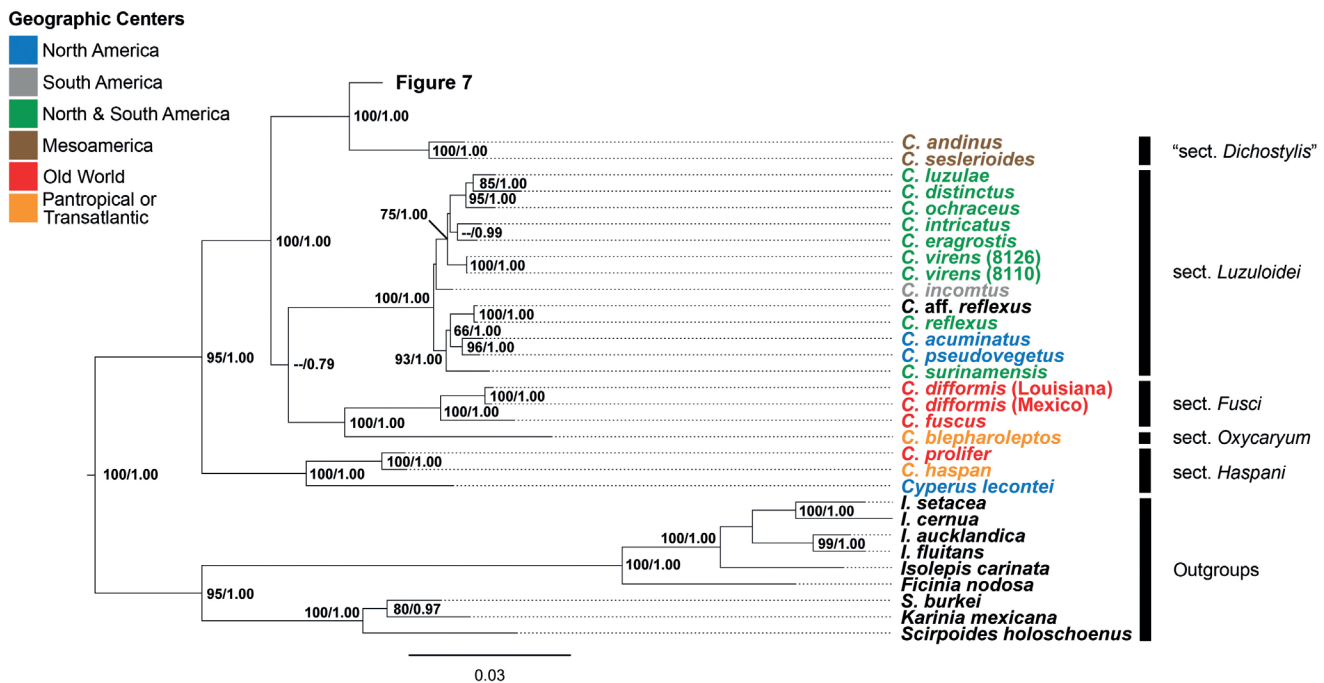

Figure 6 – Outgroups and *Cyperus* C$_3$ grade. Maximum likelihood tree inferred from a concatenated multiple sequence alignment (MSA) consisting of sequences from nuclear ITS, and plastid markers *matK*, *ndhF*, *rpl32-trnL$^{(UAG)}$*, and *trnH-psbA*. Node support values are given when ML bootstrap proportion is ≥ 75 or BI posterior probability is ≥ 0.85 (values for nodes approaching these thresholds are also given). Font color of taxon names corresponds to the taxon's native center of distribution according to the legend. Native ranges of taxa were determined by consulting Govaerts et al. (2016) and Kartesz (2015).

Oxycaryum (Nees) Larridon (Larridon et al. 2011b) and *Luzuloidei* (Kunth) C.B.Clarke (fig. 6). Results support the inclusion of *Cyperus incomtus* within section *Luzuloidei*, as suggested in Larridon et al. (2011c). Included in section *Luzuloidei* by Kükenthal (1935–1936), this species was excluded from a taxonomic study of the Luzulae group by Denton (1978) because of her view that it belonged in section *Elegantes* C.B.Clarke (sect. *Glutinosi* Boeckeler *sensu* Kükenthal 1935–1936). This study included five members of section *Luzuloidei* (*Cyperus acuminatus*, *C. incomtus*, *C. intricatus*, *C. reflexus*, and *C. virens*) that were not included in the phylogenetic analysis of Larridon et al. (2011c), increasing confidence that this group is monophyletic.

Within the C_4 clade, *Cyperus cuspidatus* is sister to the remaining taxa in this clade (fig. 7), a phylogenetic position consistently inferred in previous studies (Muasya et al. 2001, 2002, Larridon et al. 2011c, Reid et al. 2014). The phylogenetic position of *Cyperus hyalinus* (*Queenslandiella hyalina* (Vahl) Ballard) as sister to a large clade containing the vast majority of C_4 taxa has fairly strong support (bsp/pp = 77/1.00; fig. 7). Taxa formerly included in the genus *Lipocarpha* R.Br. comprise Clade 1. *Cyperus subsquarrosus* (formerly *Lipocarpha micrantha* (Vahl) G.C.Tucker and *Hemicarpha micrantha* (Vahl) Pax) is a member of the newly erected *Cyperus* section *Neohemicarpha* Bauters (Bauters et al. 2014). *Cyperus neotropicalis* and *C. sellowianus* are placed in *Cyperus* section *Lipocarpha* (R.Br.) Bauters (Bauters et al. 2014). Clade 3 includes species formerly of the genus *Kyllinga*. Clade 5, which has little support, includes species formerly treated as *Pycreus* P.Beauv. The subclade within Clade 5 containing *Cyperus flavescens*, *C. lanceolatus*, *C. niger*, *C. sanguinolentus*, and an undetermined species is very strongly supported (fig. 7). The *Cyperus polystachyos* group, including *C. filicinus*, is also strongly supported (Clade 5, fig. 7).

Clade 7 (fig. 7) includes members of five sections, as employed by Kükenthal (1935–1936): *Brevifoliati* C.B.Clarke (nom. illeg., cf. Larridon et al. 2011a), *Exaltati* (Kunth) C.B.Clarke, *Fastigiati* Kük., *Papyrus* (Willd.) Thouars, and *Rotundi* C.B.Clarke (= sect. *Cyperus*, cf. Larridon et al. 2011a). Members of these groups share long or medium length styles and winged rachillas (Kükenthal 1935–1936). *Cyperus articulatus* and *C. rotundus* form a sub-clade within Clade 7. The gestalt morphological similarity between these two species is striking.

Phylogenetic position of *Karinia*

This study is the first to estimate the phylogenetic position of *Karinia*, a monotypic genus endemic to Mexico. *Karinia* is nested within *Scirpoides* Ség. with strong support (Outgroups, fig. 6). *Scirpoides* has seven species and subspecific taxa distributed in the Old World from southern Africa to Europe and Eurasia (Govaerts et al. 2016). A specimen of *Karinia mexicana* failed to key to *Scirpoides* using Goetghebeur's (1998) generic key mainly because *Karinia* has terminal rather than pseudolateral inflorescences. The *Karinia* specimen would have keyed to *Cyperus*, terminating at couplet 164 in Goetghebeur's (1998) key, were it not for its perennial duration. However, other aspects of the morphology

of *Karinia*, including a tenacious perennial habit, capitate inflorescence with numerous spikelets, and spirally-arranged floral scales having many parallel nerves, fit the diagnosis of *Scirpoides*. Goetghebeur (1998) tentatively combined *Karinia* with *Scirpoides* stating that additional information was needed to support such placement. In light of the novel molecular evidence presented here, the following new combination is proposed:

Scirpoides mexicanus (Reznicek & McVaugh) Goetghebeur ex C.S.Reid & J.R.Carter, **comb. nov.**

Scirpus mexicanus C.B.Clarke ex Britton, Transactions of the New York Academy of Sciences 11: 77 (Britton 1892). – Type: Mexico, region of San Luis Potosi, *C.C. Parry & E. Palmer* 905 (lecto-: K, **designated here**).

Notes – Reznicek & McVaugh in McVaugh (1993: 387) cited this specimen as lectotype without indicating who designated it as such. In the absence of an explicit designation, the lectotype is designated here.

Images of the lectotype and an isolectotype are available through:
- Royal Botanic Gardens, Kew (lectotype: https://apps.kew.org/herbcat/getImage.do?imageBarcode=K000632052)
- JSTOR Plant Science (isolectotype: https://plants.jstor.org/stable/10.5555/al.ap.specimen.ny00051712)

Closest relatives of C_4 *Cyperus*

In the phylogenetic tree based on analysis of all five molecular makers, *Cyperus seslerioides* and *C. andinus* are resolved as sister to C_4 *Cyperus* (fig. 6). While they are synonymous in current taxonomy, the samples of these two taxa used in this study differ markedly in floral scale colour, venation, and size, and in achene size, shape, and surface texture. Whether *Cyperus andinus* should be resurrected is beyond the scope of this study. The taxonomic status of this species is currently being addressed by other workers (M. González-Elizondo, Instituto Politécnico Nacional, CIIDIR Unidad Durango, Durango, Mexico, pers. comm.). Lack of sequences did not permit inclusion of *Cyperus schomburgkianus* in the five-marker analysis. However, *Cyperus schomburgkianus* was included in the analysis of four plastid markers.

The plastid tree includes *Cyperus andinus*, *C. schomburgkianus*, and *C. sesslerioides*; these three taxa are in a well-supported clade that is sister to C_4 *Cyperus* (electronic appendix 3A). Phylogenetic analysis of the ITS region for this study resolved a clade composed of *Cyperus andinus*, *C. pulchellus*, and *C. steudneri* as sister to C_4 *Cyperus*, with moderately strong support (bsp/pp = 65/0.87) (electronic appendix 3B). *Cyperus pulchellus* and *C. steudneri* are Old World tropical members of section *Leucocephali* (Larridon et al. 2011b, Simpson 1990). Simpson (1990) observed that *Cyperus seslerioides* superficially resembled *C. tenerrimus* J.Presl & C.Presl, a member of section *Leucocephali*, but noted that *C. seslerioides* differed from members of section *Leucocephali* by the combination of outward curving (versus inrolled) floral scale margins, excurrent scale apices, and an ellipsoidal (versus shortly cylindrical to globose) achene shape. Considering the phylogenetic results presented herein
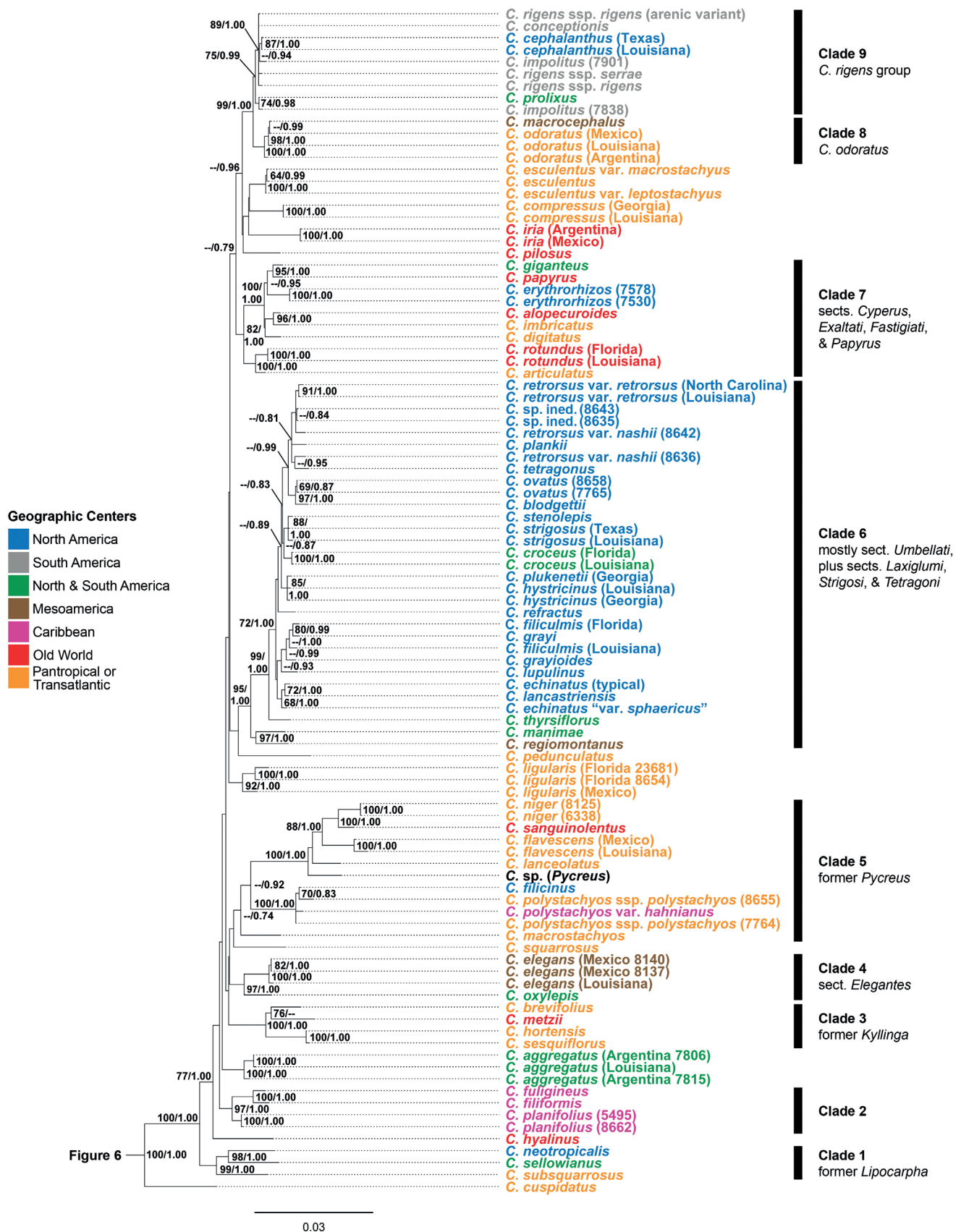
Figure 7 – *Cyperus* C₄ clade. Maximum likelihood tree inferred from a concatenated multiple sequence alignment (MSA) consisting of sequences from nuclear ITS, and plastid markers *matK*, *ndhF*, *rpl32-trnL*$^{(UAG)}$, and *trnH-psbA* (continued).

and the range of variation in Simpson's (1990) sectional description of *Leucocephali*, assignment of *C. seslerioides* and *C. andinus* to section *Leucocephali* should not be controversial. Other C$_3$ members of "section *Dichostylis*" such as *Cyperus humilis* Kunth, *C. microbrunneus* G.C.Tucker, and *C. uncinulatus* Schrad. ex Nees should be subjects for molecular studies in the near future. As with *Cyperus seslerioides* and *C. andinus*, and currently recognized members of section *Leucocephali*, they possess spherical to hemispherical inflorescences and occupy seasonally dry grasslands (figs 1 & 2) (Tucker 1994).

Cyperus section *Diclidium*

Three species of section *Diclidium* were included in this study: *Cyperus filiformis*, *C. macrocephalus*, and *C. odoratus*. *Cyperus macrocephalus* is considered synonymous with *C. odoratus* by some (Tucker 1994, Govaerts et al. 2016) and recognized as distinct by others (Adams 1994 as *Torulinium macrocephalum* (Liebm.) C.B.Clarke, Jones et al. 1996). *Cyperus macrocephalus* and *C. odoratus* compose Clade 8 (fig. 7), while *C. filiformis* is well-removed in a strongly supported sister relationship with *C. fuligineus* (Clade 2, fig. 7). Therefore, section *Diclidium* is polyphyletic. In addition to *Cyperus filiformis*, Clade 2 contains *C. planifolius* and *C. fuligineus* which belong to sections *Thunbergiani* (C.B.Clarke) Kük. and *Laxiglumi* (C.B.Clarke) Kük., respectively. Section *Thunbergiani* is also represented by *Cyperus cephalanthus*, *C. impolitus*, and *C. rigens* in Clade 9 (fig. 7). Additional representatives of section *Laxiglumi* include *Cyperus filiculmis*, *C. grayi*, *C. grayioides*, and *C. lupulinus* that fall in Clade 6. Sections *Thunbergiani* and *Laxiglumi* are polyphyletic based on these results. While Clade 2 (fig. 7) does not give credence to an existing classification, its members all share Caribbean distributions.

Cyperus odoratus (Clade 8) is only distantly related to *C. pedunculatus*, which is weakly supported as sister to Clade 6 (fig. 7). This result would seem to indicate two independent origins of the corky rachilla. However, while Clade 8 is strongly supported as sister to the *Cyperus rigens* group (Clade 9, fig. 7), the phylogenetic placement of *C. pedunculatus* is not strongly supported. Given the weak support along the backbone of the tree, the hypothesis that *C. pedunculatus* is sister to the lineage that includes Clades 8 and 9 cannot be rejected. This placement would suggest a single gain of the corky rachilla followed by one or more losses. More complete taxon sampling and more informative sequence data are needed to carry this investigation further.

Cyperus stenolepis and *C. strigosus*

Cyperus stenolepis and *C. strigosus* form a well-supported clade nested within Clade 6 (fig. 7). There is evidently little divergence in the molecular markers employed in this study between samples representing these taxa. This result supports a close relationship between these samples, rejecting the hypothesis of similarity due to morphological convergence. The phylogenies presented in fig. 7 and electronic appendix 3A support classifications that treat *Cyperus stenolepis* as a synonym of *C. strigosus* (Govaerts et al. 2016, Tucker et al. 2002). An analysis including more samples of these taxa and

employing different molecular markers may yield different results. Until a more intensive analysis is performed, *Cyperus stenolepis* is best treated as a variety of *C. strigosus* considering results of this study and morphological and ecological traits reviewed above.

Taxonomic insights in section *Umbellati*

Most of the taxa in Clade 6 (fig. 7) are members of section *Umbellati*. This group is paraphyletic since Clade 6 also includes members of sections *Laxiglumi*, *Strigosi*, and *Tetragoni* Kük. Specific goals of this research were to gain greater understanding of *Cyperus blodgettii*, *C. echinatus*, and *C. retrorsus*.

This phylogenetic analysis shows a sister relationship between *Cyperus ovatus* and *C. blodgettii* (Clade 6, fig. 7). This result supports a relationship between *Cyperus blodgettii* and *C. ovatus* as suspected by Carter (1984) based on detailed morphological study and could be cited as evidence supporting the treatment of *C. blodgettii* as a variety of *C. ovatus*. Additional support from a phylogenetic analysis including more samples of both taxa would be prudent prior to making this new combination or solidifying the decision to recognize *Cyperus blodgetti* at the rank of species.

Typical *Cyperus echinatus*, *C. echinatus* "var. *sphaericus*", and *C. lancastriensis* (fig. 4B) form a strongly supported clade (Clade 6, fig. 7). A previous analysis by Reid et al. (2014) showed a well-supported sister relationship between *Cyperus echinatus* "var. *sphaericus*" and *C. filiculmis*. The present analysis does not resolve an immediate sister relationship between these taxa, though *Cyperus filiculmis* samples do fall in a clade that is sister to the *C. echinatus*-"var. *sphaericus*"- *C. lancastriensis* clade (Clade 6, fig. 7). The "*sphaericus*" variant has no legitimate name. Therefore, *Cyperus echinatus*, as currently circumscribed (Carter 1984, Carter & Kral 1990) is paraphyletic due to its phylogenetic position relative to *C. lancastriensis*. Results of this analysis, in combination with morphological and ecological evidence (reviewed above), support the treatment of "var. *sphaericus*" as a distinct taxon at the rank of species. However, a molecular study involving multiple samples of typical *Cyperus echinatus*, *C. echinatus* "var. *sphaericus*", and *C. lancastriensis* is warranted before proceeding with formal naming of "*sphaericus*" as a new species.

Cyperus retrorsus var. *retrorsus*, *C. retrorsus* var. *nashii*, *C. plankii*, *C.* sp. ined., and *C. tetragonus* form a weakly supported sub-clade (bsp/pp = 35/0.81) nested within Clade 6 (fig. 7). With the exception of the sister relationship between two *Cyperus retrorsus* var. *retrorsus* samples, node support values within this sub-clade are weak suggesting these taxa are related but providing little confidence. The erratic placement of *Cyperus retrorsus* var. *nashii* may be due to lack of ITS sequences for both samples of this taxon (electronic appendix 2). The ITS region was the most informative molecular marker based on inspection of single-locus trees (electronic appendix 3B–F). Since DNA sequences employed thus far have provided little resolution among *Cyperus retrorsus* and often-included taxa, morphological and ecological evidence should be given more weight in taxonomic decisions involving *C. retrorsus* and its segregates and hypothetical relatives.

Relationships and biogeography of the *Cyperus rigens* group

The *Cyperus rigens* group, including *C. cephalanthus*, *C. impolitus*, *C. prolixus*, and two subspecies of *C. rigens*, is monophyletic with moderate support (bsp/pp = 75/0.99) (Clade 9, fig. 7). The two *Cyperus cephalanthus* samples have a strongly-supported sister relationship and are sister to *C. impolitus* (7901) with moderate support. The other *Cyperus impolitus* sample (7838) is distantly related, resolved as sister to *C. prolixus*. Poor resolution within Clade 9 is likely due to very little sequence variation. More informative molecular data and more intensive sampling are required to assess relationships in the *Cyperus rigens* group and to test for divergence between *C. cephalanthus* and its South American relatives.

While relationships are still unclear within the *Cyperus rigens* group, results strengthen support for an amphitropical disjunction within this group. Tucker et al. (2002) suggested that *Cyperus cephalanthus* is naturalized rather than native to North America. Since *Cyperus cephalanthus* was collected and described early in the European colonization of the southern United States and is a conservative component of coastal tall-grass prairies (Carter & McInnis 1993, Grace et al. 2000, Allain et al. 2004), it is most likely native to the northern Gulf of Mexico coast rather than a recent anthropogenic introduction. Sedges have small fruits and are known to disperse long distances (Kern 1974). Fruits embedded in mud can adhere to birds' feet, or become lodged in their feathers (Darwin 1859, Ridley 1930). Raven (1963) noted that amphitropical disjuncts between North and South America correspond closely to bird migration routes and that these species tend to occur in open habitats, such as coastal and herbaceous wetland communities. Rosen (2007) reported eleven amphitropical disjuncts, including *Cyperus cephalanthus*, in his floristic study of a high-quality coastal prairie remnant in southeastern Texas. There are many avian candidates that may have served as vectors for *Cyperus cephalanthus*. The presence of this species in North America could have resulted from one or more dispersal events, depending on the probability of successful dispersal and establishment. Since the once-extensive coastal prairie habitat of *Cyperus cephalanthus* in Louisiana and Texas has been reduced to less than 1 % of its historical extent (Smeins et al. 1991, Holcomb et al. 2015), it is likely that this species is now effectively isolated from South American populations.

General biogeographical observations

This phylogenetic analysis includes c. 10 % of the known species in *Cyperus*. Sampling intensity is inadequate to rigorously examine biogeographic patterns. However, it does present some preliminary biogeographic insights. The overwhelming majority of North American endemics sampled fall in Clade 6 (fig. 7). *Cyperus regiomontanus* and *C. manimae*, which are sister to the remainder of Clade 6, have more southerly distributions, occurring in Mexico and Central America and extending into tropical South America. *Cyperus thyrsiflorus* is also more southerly, being present in subtropical North America and extending into tropical America. From the phylogenetic hypothesis presented in fig. 7, it

seems possible that many North American endemics were derived from a more southern ancestor. Clade 6, consisting of mostly North American endemics and many species of decidedly upland habitats, may represent a natural radiation. Future studies including more taxa are needed to elucidate the origins of *Cyperus* species endemic to North America.

CONCLUSIONS

This research made several contributions to the body of knowledge regarding the systematics of *Cyperus* and Cyperaceae. The position of *Karinia* was resolved with confidence; the only species in this genus, *K. mexicana*, was transferred to *Scirpoides*. This research provides evidence that *Cyperus seslerioides* and *C. andinus* are closely related to members of section *Leucocephali*, and that the species of this section are sister to C_4 *Cyperus*. Sampling additional members of section *Leucocephali* and providing a taxonomic revision of this group that is informed by molecular evidence should be near-term priorities for the *Cyperus* research community. Several taxonomic problems were addressed with some satisfaction among North American C_4 taxa (Clade 6, fig. 7). Evidence generated by this research supports formal recognition of *Cyperus blodgettii* and *C. echinatus* "var. *sphaericus*". A greater number of samples and more informative molecular data (especially in the *Cyperus retrorsus* complex) are needed to increase confidence. This work provided strong evidence of the amphitropical disjunction within the *Cyperus rigens* group. Lack of sequence variation likely hindered the generation of a well-resolved phylogenetic hypothesis among taxa within that group. *Cyperus cephalanthus* is a species of conservation concern in North America (Holcomb et al. 2015, NatureServe 2015). Genetic data yielding meaningful variation at the population level are needed to test for divergence between North America and relatives in South America and to examine genetic diversity within *Cyperus cephalanthus* populations. Such studies may garner evidence supporting evolutionary independence and rarity of *Cyperus cephalanthus*, elevating it as a conservation priority.

SUPPLEMENTARY DATA

Supplementary data are available at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingentaconnect.com/content/botbel/plecevo/supp-data) and consist of the following: (1) scanned herbarium specimens of *Cyperus stenolepis*, *C. strigosus*, *C. echinatus* and *C. echinatus* "var. *sphaericus*" (pdf); (2) taxa studied, with voucher specimen information and GenBank accession numbers (Excel spreadsheet); (3) phylogenetic trees based on analyses of concatenated plastid sequences and all individual loci used for this research (ITS, *matK*, *ndhF*, *rpl32-trnL*[(UAG)], and *trnH-psbA*) (pdf); and (4) aligned concatenated matrix of all genic loci analysed to generate figs 6 & 7 (NEX file).

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