



# Searching for the sister to sedges (*Carex*): resolving relationships in the Cariceae-Dulichieae-Scirpeae clade (Cyperaceae)

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With approximately 2000 species, tribe Cariceae (Cyperaceae) comprises a morphologically distinctive cosmopolitan clade, with holocentric chromosomes ( $N = 6$  to 56), complex biogeographical patterns, and habitat diversity ranging from rainforests to deserts. Such a remarkable combination of characteristics should make Cariceae an ideal model for studying the evolution of biodiversity, although they also obscure their relationships in Cyperaceae, complicating attempts to identify the contributing factors to diversity of Cariceae. Recent molecular studies place Cariceae in a strongly supported clade consisting of tribes Dulichieae, Scirpeae *s.s.*, and the enigmatic monotypic genus *Khaosokia*, although relationships in this clade are unresolved. Using the plastid genes *matK* and *ndhF* and a greatly improved taxonomic sampling covering 16 of 17 genera and 55% of the species outside Cariceae, our analyses firmly position Dulichieae and *Khaosokia* (79% and 85% bootstrap support) as successive sisters to a clade consisting of five major lineages (*Calliscirpus*, *Trichophorum* + *Oreobolopsis* + *Cypringlea*, Cariceae, *Scirpus* + *Eriophorum*, and *Amphiscirpus* + *Phylloscirpus* + *Zameioscirpus*), the first four of which receive good to strong support (> 80% bootstrap support). Cariceae are sister to the *Trichophorum* clade, although topological tests cannot exclude either *Calliscirpus* or a *Scirpus* clade + *Zameioscirpus* clade as sister to the tribe. *Trichophorum* appears to be paraphyletic and *Eriophorum* is firmly nested in *Scirpus*. There appears to be a trend in the increase of chromosome numbers in *Scirpus* and *Eriophorum* and a trend in the reduction and proliferation of the inflorescence throughout the major Cariceae-Dulichieae-Scirpeae clades. © 2014 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2014, 176, 1–21.

**ADDITIONAL KEYWORDS:** *Calliscirpus* – *Eriophorum* – *Khaosokia* – *matK* – *ndhF* – molecular phylogenetics – *Scirpus* – *Trichophorum*.

## INTRODUCTION

Cyperaceae (> 100 genera, approximately 5500 species) comprise the third largest family of monocotyledons and are found throughout the globe in habitats ranging from lowland marshes and alpine meadows to rainforests and deserts (Goetghebeur, 1998; Govaerts *et al.*, 2007). Approximately 36% of the family is found in a single monophyletic tribe,

Cariceae, that stands out not only for its amazing diversity (approximately 2000 species), but also for its diverse biogeography (e.g. amphiatlantic, bipolar, Gondwanan; Raymond, 1951; Croizat, 1952) and intriguing cytology (holocentric chromosomes:  $N = 6$  to 56; Davies, 1956). These characteristics should make Cariceae an ideal system for studying the evolution of biodiversity, and studies are increasingly focusing on biogeographical, ecological, and evolutionary questions (Escudero *et al.*, 2010, 2012a, b; Gehrke & Linder, 2011). However, characteristics such as their cosmopolitan distribution, high diversity, and,

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most importantly, complicated morphology mean that outgroup relationships are unresolved. This incomplete understanding of phylogenetic relationships impedes attempts to identify the contributing factors to the diversity of Cariceae.

Cariceae are morphologically isolated in Cyperaceae by the possession of unisexual flowers (Goetghebeur, 1998; but see also Smith & Faulkner, 1976; Gehrke *et al.*, 2012), by the modification of the lateral spikelet prophyll into a utriculiform structure (perigynium) bearing a female flower (Holm, 1896; Snell, 1936), and by the frequent reduction of the lateral spikelet axis (rachilla) (Snell, 1936; Smith & Faulkner, 1976). Consequently, Cariceae have sometimes been segregated into their own subfamily, Caricoideae (Koyama, 1961; Goetghebeur, 1998). Although it has been proposed that Cariceae inflorescences could be derived from a 'scirpoid' inflorescence (e.g. Abildgaardieae, Fuireneae, and Scirpeae) by lateral spikelet reduction and the modification of spikelet prophylls to fertile perigynia (Kukkonen & Timonen, 1979; Dahlgren, Clifford & Yeo, 1985), the homology of Cariceae 'spikes', 'spikelets', 'flowers', 'utricles', and 'prophylls' to those of other Cyperaceae remains unclear (Smith, 1967; Le Cohu, 1968; Smith & Faulkner, 1976; Reznicek, 1990; Vegetti, 2002; Vrijdaghs, 2006).

Before the advent of molecular phylogenetics, the most common hypotheses were that Cariceae were related to Sclerieae and Bisboeckelereae (Kern, 1958; Schultze-Motel, 1964; Goetghebeur, 1986) or to Mapanioideae (Bruhl, 1995). In each of these cases, the putative relationship was almost entirely based upon the observation that these groups shared unisexual flowers. However, inflorescence structure (Meert & Goetghebeur, 1979; Goetghebeur, 1986; Richards, Bruhl & Wilson, 2006), embryo morphology (Goetghebeur, 1986), and pollen aperture number and distinctness (Nagels *et al.*, 2009) conflict with the proposed relationships. Molecular phylogenetic analyses have since positioned Cariceae in a strongly supported and unexpected clade with Dulichieae and Scirpeae, and the genus *Khaosokia* D.A.Simpson, Chayam. & J.Parn. (clade hereafter known as CDS; Simpson *et al.*, 2005; Muasya *et al.*, 2009; Jung & Choi, 2012; Hinchliff & Roalson, 2013).

Dulichieae are a small holarctic group of three or four genera and seven species (Oteng-Yeboah, 1977; Goetghebeur, 1998). The type genus *Dulichium* Pers. was once included in Cypereae on account of its distichously arranged spikelets and glumes (Holm, 1897), although members of Dulichieae differ from Cypereae by the possession of perianth bristles (Holm, 1897; except for *Dracoscirpoides* Muasya and *Erioscirpus* Palla, see Muasya *et al.*, 2012; Yano *et al.*, 2012), a *Carex*-type embryo (Goetghebeur, 1998), and, most

importantly, by fertile spikelet prophylls (Goetghebeur, 1998). *Dulichium* has also been aligned with Rhynchosporeae and Schoeneae (Kükenthal, 1952; Schultze-Motel, 1959), although the current genera included in Dulichieae differ by the possession of many fertile flowers per spikelet and by nonwinged spikelet glumes (Kükenthal, 1952; Schultze-Motel, 1959). Goetghebeur (1986: 158) shows a cladogram placing Dulichieae sister to a clade including tribes Abildgaardieae, Cypereae, Eleocharideae, and Scirpeae, although no synapomorphies are provided to support the relationships. The association of Dulichieae with Cariceae and Scirpeae is unexpected given that they share few if any obvious morphological synapomorphies. The position of Dulichieae in CDS is also unclear, with previous molecular studies having placed it nested within Scirpeae (Muasya *et al.*, 2009), in a sister position to the remainder of CDS (Gilmour, Starr & Naczi, 2013; Hinchliff & Roalson, 2013) or in both positions depending on sampling (Jung & Choi, 2012).

With nine genera and 98 species, Scirpeae contain the majority of CDS diversity outside of Cariceae (Govaerts *et al.*, 2007), with most species divided between just three genera, *Scirpus* Tourn. ex L. (50 species), *Eriophorum* L. (21 species), and *Trichophorum* Pers. (12 species) (Novoselova, 1995; Govaerts *et al.*, 2007; Muasya *et al.*, 2012; Yano *et al.*, 2012). Almost all species of Scirpeae have at one time or another been included in the highly heterogeneous *Scirpus* Tourn. ex L. *s.l.* (Govaerts *et al.*, 2007), a taxon with the unifying characteristics of spirally inserted bisexual flowers and six bristle-like perianth parts (Koyama, 1958); these are most likely plesiomorphic in Cyperaceae (Goetghebeur, 1998). When circumscribed in this manner, *Scirpus* contains approximately 200 species (Koyama, 1958; Govaerts *et al.*, 2007). Although modern circumscriptions now recognize only 50 species (Govaerts *et al.*, 2007; Muasya *et al.*, 2012), the limits of the genus are still not entirely resolved. This is clearly demonstrated by a series of genera segregated from *Scirpus* over the past 10 years (*Calliscirpus* C.N.Gilmour, J.R.Starr & Naczi, *Cypringlea* M.T.Strong, *Dracoscirpoides* Muasya, *Zameioscirpus* Dhooge & Goetgh.) (Dhooge, Goetghebeur & Muasya, 2003; Strong, 2003; Muasya *et al.*, 2012; Gilmour *et al.*, 2013). In addition, previous studies have struggled to draw the line between *Scirpus* and *Eriophorum*. This is mainly a result of morphologically intermediate species, such as *Scirpus cyperinus* (L.) Kunth, which has long *Eriophorum*-like bristles (Fernald, 1905), and *Scirpus maximowczii* C.B.Clarke, which combines the six barbed bristles of *Scirpus* with the black bracts and glumes and few large spikelets characteristic of *Eriophorum* (Koyama, 1958). The only phylogenetic analysis to

include both of these species shows *Eriophorum* to be nested in *Scirpus*, although sampling (five species) and clade support was poor (Gilmour *et al.*, 2013). Similarly, the monophyly of Scirpeae is uncertain. Gilmour *et al.* (2013) showed, on the basis of molecular and embryological data, that the recently recognized genus *Calliscirpus* and species of *Cypringlea*, *Oreobolopsis* T.Koyama & Guagl., and *Trichophorum* may not be closely related to *Scirpus* and *Eriophorum*. Although other phylogenetic studies have also consistently indicated that Scirpeae are paraphyletic, the relationships and taxonomic composition of major clades have varied widely and clade support has always been weak. For example, Cariceae have been positioned as sister to *Calliscirpus* (< 50% bootstrap support or BS; Gilmour *et al.*, 2013), in a clade with *Scirpus*, *Eriophorum*, *Zameioscirpus*, and allies (< 50% BS; Jung & Choi, 2012), and in a large polytomy that included all genera of Scirpeae (77% BS; Hinchliff & Roalson, 2013).

*Khaosokia* is a recently discovered monotypic genus endemic to limestone cliffs of peninsular Thailand that is similar to *Dulichium* in having flowers with more than six bristles and elongate spikelets in racemes, but has spirally inserted glumes, as in Scirpeae (Simpson *et al.*, 2005). Although it has been placed in the CDS clade in molecular phylogenetic analyses, branch support has always been weak and its position has varied from sister to Cariceae + Scirpeae to sister to the whole CDS (Jung & Choi, 2012; Gilmour *et al.*, 2013; Hinchliff & Roalson, 2013). The tribal affinities of this morphologically enigmatic genus remain unresolved.

In CDS as a whole, the relationships between Cariceae and the genera of Dulichieae and Scirpeae are unresolved. Most notably, the sister group to Cariceae is unknown, relationships of Scirpeae genera are unstable and the position of Dulichieae and *Khaosokia* in the clade is ambiguous (Simpson *et al.*, 2005; Muasya *et al.*, 2009; Jung & Choi, 2012; Gilmour *et al.*, 2013). Previous phylogenetic studies have included no more than 32% of the species of CDS excluding Cariceae in a single analysis (Muasya *et al.*, 2009), and the markers used were either largely uninformative at this level of investigation (e.g. *rbcL*; Simpson *et al.*, 2005; Muasya *et al.*, 2009) or contained such high levels of variability that alignment was difficult (e.g. *trnL-F*, ITS; Simpson *et al.*, 2005; Muasya *et al.*, 2009; Jung & Choi, 2012). The present study aimed to provide a better understanding of the relationships between and among the genera and tribes of CDS using new sequences from two rapidly evolving plastid genes (*matK* and *ndhF*) and a greatly improved taxonomic sampling, covering all but one [*Sumatrosirpus* (Miq.) Oteng-Yeb.] of the currently recognized genera and more than half of the diversity

of the clade outside of Cariceae. The bearing of our data on the sister relationship to Cariceae, the phylogenetic position of *Khaosokia*, the taxonomy of Scirpeae, and the monophyly of *Scirpus* and *Trichophorum* are discussed.

## MATERIAL AND METHODS

### TAXONOMIC SAMPLING AND MARKERS

One hundred and twelve individuals from 83 taxa were included in the present study, covering all currently recognized genera of CDS (Govaerts *et al.*, 2007), except for the monotypic *Sumatrosirpus* (Dulichieae; Oteng-Yeboah, 1974) (Table 1). Most of the 224 sequences are new but 37 were already published by Gilmour *et al.* (2013). This sampling covers 55% of all species and infraspecific taxa (approximately 114) recognized for this clade outside of Cariceae (Novoselova, 1995; Govaerts *et al.*, 2007). Sampling within Cariceae aimed to represent all the major lineages currently known (Starr & Ford, 2009; Waterway, Hoshino & Masaki, 2009). Outgroup taxa were selected to represent major lineages of the Abildgaardieae-Cypereae-Eleocharideae-Fuireneae clade, which has been shown to be sister to CDS (Muasya *et al.*, 2009; Jung & Choi, 2012; Hinchliff & Roalson, 2013). Taxonomy follows Govaerts *et al.* (2007), except for *Eriophorum*, which follows the revision of the genus by Novoselova (1995).

The plastid genes *matK* and *ndhF* were used because (1) they are easy to amplify even from relatively degraded tissue (herbarium specimens); (2) pilot studies suggested they would have an appropriate level of divergence for assessing tribal and generic level relationships within CDS; and (3) because they are coding sequences, alignment is almost always unambiguous.

### MOLECULAR METHODS

Genomic DNA was extracted from herbarium specimens or from field samples dried in silica gel using the silica-column protocol of Alexander *et al.* (2007), as modified by Starr, Naczi & Chouinard (2009). Primers for the amplification of the *matK* and *ndhF* sequences are provided in Gilmour *et al.* (2013). PCR amplifications consisted of 1 × reaction Buffer (Sigma Aldrich), 2 mM MgCl<sub>2</sub> (Sigma Aldrich), 0.2 mM of each deoxynucleotide (dATP, dCTP, dTTP, and dGTP), 0.25 μM of each primer, 1.0 μL of bovine serum albumin (BioShop), 4 U of Hot Start (HS) Taq DNA polymerase (BioShop) and 1–3 μL of genomic DNA extract, adjusted to an end volume of 15 μL using nuclease-free MilliQ H<sub>2</sub>O. Amplification was conducted using an Eppendorf EPGradientS Mastercycler with 2 min of initial denaturation followed by 40 cycles of 30 s of denaturation at 94 °C, 60 s of primer

**Table 1.** Samples included in the molecular study with taxonomic name, voucher information and GenBank accession numbers

Taxonomic name	Collector(s)	Collection number	Herbarium	Origin	<i>matK</i> accession number	<i>ndhF</i> accession number
<i>Bulbostylis atrosanguinea</i> (Boeckeler) C.B. Clarke	Muasya	1037	K	Kenya	KJ513580	KJ513485
<i>Eleocharis acicularis</i> (L.) Roem. & Schult.	Fields	2583	WIS	USA, Wisconsin	KJ513595	KJ513502
<i>Erioscirpus comosus</i> (Wall.) Palla	Hing & al.	22413	A	China	KJ513619	KJ513526
<i>Fimbristylis dichotoma</i> (L.) Vahl	Muasya	1006	K	Kenya	KJ513620	KJ513527
<i>Fimbristylis ovata</i> (Burm.f.) J.Kern	Muasya & al.	684	K	Kenya	**JX065086	**JX074642
<i>Isolepis aucklandica</i> Hook.f.	McIntosh	12-II-1977	CAN	New Zealand	KJ513621	KJ513528
<i>Amphiscirpus nevadensis</i> (S.Watson) Oteng-Yeb.	Hudson	5177	CAN	Canada, Saskatchewan	**JX065075	**JX074631
<i>Blysmus compressus</i> (L.) Panz. ex Link	Kotowicz	871	CAN	Poland	KJ513577	KJ513482
<i>Blysmus compressus</i> (L.) Panz. ex Link	Shtamm	15-VIII-1962	CAN	Russia	KJ513578	KJ513483
<i>Blysmus rufus</i> (Huds.) Link	Jokela	9-VIII-1958	CAN	Finland	**JX065076	**JX074632
<i>Blysmus sinocompressus</i> Tang & F.T.Wang var. <i>sinocompressus</i>	Stangokovich	30-VII-1955	CAN	Tajikistan	KJ513579	KJ513484
<i>Calliscirpus brachythrix</i> C.N.Gilmour, J.R.Starr & Naczi	Janeway	6344	CHS	USA, California	**JX074667	KJ513486
<i>Calliscirpus brachythrix</i> C.N.Gilmour, J.R.Starr & Naczi	Ahart & Oswald	5099	CHS	USA, California	**JX065078	**JX074634
<i>Calliscirpus criniger</i> (A.Gray) C.N.Gilmour, J.R.Starr & Naczi	Tracy	9380	DAO	USA, California	**JX074654	KJ513487
<i>Calliscirpus criniger</i> (A.Gray) C.N.Gilmour, J.R.Starr & Naczi	Chambers	2973	DAO	USA, Oregon	**JX074655	KJ513488
<i>Carex acicularis</i> Boott in J.D.Hooker	Ford	29/94	CHR	New Zealand	KJ513581	KJ513489
<i>Carex aphylla</i> Kunth	Starr & Villaverde	P20-2	CAN	Argentina	KJ513582	KJ513490
<i>Carex blanda</i> Dewey	Bakowski	97–176	WIN	Canada, Ontario	KJ513583	KJ513491
<i>Carex camptoglochin</i> V.I.Krecz.	Molau & al.	2329	GB	Ecuador	KJ513584	KJ513492
<i>Carex capitata</i> Sol.	Starr & Thibeault	6016	CAN	USA, California	KJ513585	KJ513493
<i>Carex conferta</i> Hochst. ex A.Rich.	Muasya	1055	K	Kenya	KJ513586	KJ513494
<i>Carex gynocrates</i> Wormsk.	Ford & al.	02283	WIN	Canada, Manitoba	KJ513587	KJ513495
<i>Carex monostachya</i> A.Rich.	Muasya	1052	K	Kenya	KJ513588	KJ513496

Table 1. Continued

Taxonomic name	Collector(s)	Collection number	Herbarium	Origin	<i>matK</i> accession number	<i>ndhF</i> accession number
<i>Carex polystachya</i> Sw. ex Wahlenb.	Jones & Wipff	1127	MICH	Belize	KJ513589	KJ513497
<i>Carex pulicaris</i> L.	Starr & Scott	98001	FHO	UK	KJ513590	KJ513576
<i>Carex rupestris</i> All.	Starr	10S-029 P29-10	CAN	USA, Colorado	KJ513591	KJ513498
<i>Carex siderosticta</i> Hance	Léveillé-Bourret	545	CAN	Garden	KJ513592	KJ513499
<i>Carex stipata</i> Muhl. ex Willd.	Dugal & Camfield	3728	CAN	USA, Ontario	KJ513593	KJ513500
<i>Carex ursina</i> Dewey	Porsild	8828	CAN	Greenland	**JX065081	**JX074637
<i>Cypringlea analecta</i> (Beetle) M.T.Strong	Reznicek & al.	11094	MICH	Mexico	KJ513594	KJ513501
<i>Cypringlea evadens</i> (C.D.Adams) Reznicek & S.González	Rawlins & Sholes	2830	MICH	Mexico	**JX065082	**JX074638
<i>Dulichium arundinaceum</i> (L.) Britton	Ford & Punter	94233	FHO	Canada, Manitoba	**JX065083	**JX074639
<i>Eriophorum angustifolium</i> Honck. subsp. <i>angustifolium</i>	Scoggan	10947	CAN	Canada, Manitoba	KJ513597	KJ513504
<i>Eriophorum angustifolium</i> Honck. subsp. <i>angustifolium</i>	Starr & al.	10S-011	CAN	USA, New Mexico	KJ513598	KJ513505
<i>Eriophorum angustifolium</i> Honck. subsp. <i>angustifolium</i>	Judziewicz	11218	WIS	USA, Wisconsin	KJ513596	KJ513503
<i>Eriophorum angustifolium</i> Honck. subsp. <i>komarovii</i> (V.N.Vassil.) Vorosch. in A.K.Skvortsov (ed.)	Given & Soper	73466	CAN	Canada, British Columbia	KJ513599	KJ513506
<i>Eriophorum brachyantherum</i> Trautv. & C.A.Mey.	Gillett & Boudreau	17512	CAN	Canada, British Columbia	KJ513600	KJ513507
<i>Eriophorum brachyantherum</i> Trautv. & C.A.Mey.	Schofield & al.	7645	CAN	Canada, Yukon	KJ513601	KJ513508
<i>Eriophorum brachyantherum</i> Trautv. & C.A.Mey.	Roivainen	15-VII-1958	CAN	Finland	KJ513602	KJ513509
<i>Eriophorum callitrix</i> Cham. ex C.A.Mey.	Malte	126887	CAN	Canada, Nunavut	KJ513603	KJ513510
<i>Eriophorum callitrix</i> Cham. ex C.A.Mey.	Porsild & Porsild	4753	CAN	Canada, Northwest Territories	**JX074653	**JX074641
<i>Eriophorum gracile</i> Koch in A.W.Roth	Talbot	6237-4	CAN	Canada, Northwest Territories	KJ513604	KJ513511
<i>Eriophorum gracile</i> Koch in A.W.Roth	Starr & Thibeault	6014	CAN	USA, California	KJ513605	KJ513512
<i>Eriophorum latifolium</i> Hoppe	Jokela	20-VII-1965	OSC	Finland	KJ513606	KJ513513

**Table 1.** *Continued*

Taxonomic name	Collector(s)	Collection number	Herbarium	Origin	<i>matK</i> accession number	<i>ndhF</i> accession number
<i>Eriophorum russeolum</i> Fr. ex Hartm. subsp. <i>albidum</i> F.Nyl.	Pegg	19-VI-1957	CAN	Canada, Alberta	KJ513607	KJ513514
<i>Eriophorum russeolum</i> Fr. ex Hartm. subsp. <i>russeolum</i>	Gauthier	75–208	CAN	Canada, Québec	KJ513608	KJ513515
<i>Eriophorum scheuchzeri</i> Hoppe subsp. <i>scheuchzeri</i>	Pearson	67–80	CAN	Canada, Yukon	KJ513609	KJ513516
<i>Eriophorum scheuchzeri</i> Hoppe subsp. <i>scheuchzeri</i>	Jorgensen & Larsson	66–1555	CAN	Greenland	KJ513610	KJ513517
<i>Eriophorum scheuchzeri</i> Hoppe subsp. <i>scheuchzeri</i>	Argus & Chunys	5813	CAN	USA, Alaska	KJ513611	KJ513518
<i>Eriophorum tenellum</i> Nutt.	Dugal & Shchepanek	6354	CAN	Canada, Nova Scotia	KJ513612	KJ513519
<i>Eriophorum vaginatum</i> L. subsp. <i>spissum</i> (Fernald) Hultén	Porsild	12	CAN	Canada, Labrador	KJ513614	KJ513521
<i>Eriophorum vaginatum</i> L. subsp. <i>spissum</i> (Fernald) Hultén	Spalink	160	WIS	USA, Wisconsin	KJ513613	KJ513520
<i>Eriophorum vaginatum</i> L. subsp. <i>vaginatum</i>	Starr & Scott	98007	FHO	UK	KJ513615	KJ513522
<i>Eriophorum virginicum</i> L.	Shchepanek	1415	CAN	Canada, Québec	KJ513616	KJ513523
<i>Eriophorum virginicum</i> L.	Dickson & Brunton	3214	CAN	Canada, Newfoundland	KJ513617	KJ513524
<i>Eriophorum viridicarinaratum</i> (Engelm.) Fernald	Darbyshire	2532	CAN	Canada, Ontario	KJ513618	KJ513525
<i>Eriophorum viridicarinaratum</i> (Engelm.) Fernald	Shea	11351	CAN	Canada, Ontario	**JX074652	**JX074640
<i>Khaosokia caricoides</i> D.A.Simpson, Chayam. & J.Parn.	Middleton & al.	4071	MICH	Thailand	**JX065087	**JX074643
<i>Kobresia myosuroides</i> (Vill.) Fiori in A.Fiori & al.	Jones	146	UBC	Canada, British Columbia	KJ513622	KJ513529
<i>Kobresia simpliciuscula</i> (Wahlenb.) Mack.	Porsild	1825	CAN	Canada, Yukon	**JX065088	**JX074644
<i>Oreobolopsis tepalifera</i> T.Koyama & Guagl.	Salvador & al.	749	MICH	Peru	KJ513623	KJ513530
<i>Oreobolopsis tepalifera</i> T.Koyama & Guagl.	Wood	1046	NY	Bolivia	**JX065089	**JX074645
<i>Phylloscirpus deserticola</i> (Phil.) Dhooge & Goetgh.	Solomon	15819	CAS	Bolivia	KJ541072	KJ541073
<i>Phylloscirpus deserticola</i> (Phil.) Dhooge & Goetgh.	Ru	9797	US	Argentina	**JX065090	**JX074646

Table 1. Continued

Taxonomic name	Collector(s)	Collection number	Herbarium	Origin	<i>matK</i> accession number	<i>ndhF</i> accession number
<i>Schoenoxiphium lanceum</i> (Thunb.) Kük.	Dahlstrand	1302	PRE	South Africa	KJ513625	KJ513532
<i>Schoenoxiphium sparteum</i> (Wahlenb.) C.B.Clarke	Smook	6625	PRE	South Africa	KJ513626	KJ513533
<i>Scirpus ancistrochaetus</i> Schuyler	Cippolini	SA-13	Wright	USA, Pennsylvania	KJ513627	KJ513534
<i>Scirpus atrocinctus</i> Fernald	Spalink	283	WIS	USA, Massachusetts	KJ513628	KJ513535
<i>Scirpus atrovirens</i> Willd.	Spalink	180	WIS	USA, Wisconsin	KJ513629	KJ513536
<i>Scirpus atrovirens</i> Willd.	Spalink	186	WIS	USA, Ohio	KJ513630	KJ513537
<i>Scirpus cyperinus</i> (L.) Kunth	Lindsay	1025	CAN	Canada, Ontario	**JX065092	**JX074648
<i>Scirpus cyperinus</i> (L.) Kunth	Spalink	164	WIS	USA, Wisconsin	KJ513631	KJ513538
<i>Scirpus cyperinus</i> (L.) Kunth	Spalink	188	WIS	USA, Ohio	KJ513632	KJ513539
<i>Scirpus divaricatus</i> Elliott	Spalink	124	WIS	USA, Alabama	KJ513633	KJ513540
<i>Scirpus expansus</i> Fernald	Spalink	158	WIS	USA, Michigan	KJ513634	KJ513541
<i>Scirpus flaccidifolius</i> (Fernald) Schuyler	Spalink	193	WIS	USA, Virginia	KJ513635	KJ513542
<i>Scirpus georgianus</i> R.M.Harper	Hudson	409	CAN	USA, Missouri	KJ513637	KJ513544
<i>Scirpus georgianus</i> R.M.Harper	Spalink	121	WIS	USA, Alabama	KJ513636	KJ513543
<i>Scirpus hattorianus</i> Makino	Baldwin & Breitung	4196	CAN	Canada, Québec	KJ513638	KJ513545
<i>Scirpus hattorianus</i> Makino	Shchepanek & Dugal	5974	CAN	Canada, New Brunswick	KJ513639	KJ513546
<i>Scirpus hattorianus</i> Makino	Bergeron & al.	81–111	CAN	Canada, Québec	KJ513640	KJ513547
<i>Scirpus karuisawensis</i> Makino	Jung	807017	AJOU	South Korea	KJ513641	KJ513548
<i>Scirpus longii</i> Fernald	Spalink	251	WIS	USA, New Jersey	KJ513642	KJ513549
<i>Scirpus maximowiczii</i> C.B.Clarke	Petrochenko & al.	5613	CAN	Russia	KJ513643	KJ513550
<i>Scirpus maximowiczii</i> C.B.Clarke	Petrochenko	357	CAN	Russia	KJ513644	KJ513551
<i>Scirpus microcarpus</i> J.Presl & C.Presl	Dugal & Camfield	3770	CAN	Canada, Ontario	KJ513646	KJ513553
<i>Scirpus microcarpus</i> J.Presl & C.Presl	Spalink	284	WIS	USA, Massachusetts	KJ513645	KJ513552
<i>Scirpus pallidus</i> (Britton) Fernald	Hudson	5079	CAN	Canada, Saskatchewan	KJ513647	KJ513554
<i>Scirpus pedicellatus</i> Fernald	Houle	76–1185	CAN	Canada, Québec	KJ513648	KJ513555
<i>Scirpus pendulus</i> Muhl.	Cruise	1388	CAN	Canada, Ontario	KJ513649	KJ513556
<i>Scirpus polyphyllus</i> Vahl	Spalink	246	WIS	USA, Virginia	KJ513650	KJ513557
<i>Scirpus polystachyus</i> F.Muell.	Pullen	4091	A	Australia	KJ513651	KJ513558
<i>Scirpus radicans</i> Schkuhr	Samuelsson	296	CAN	Sweden	KJ513653	KJ513560
<i>Scirpus radicans</i> Schkuhr	Jung	80632	AJOU	South Korea	KJ513652	KJ513559

**Table 1.** *Continued*

Taxonomic name	Collector(s)	Collection number	Herbarium	Origin	<i>matK</i> accession number	<i>ndhF</i> accession number
<i>Scirpus sylvaticus</i> L.	Jung	806038	AJOU	South Korea	KJ513654	KJ513561
<i>Scirpus wichurae</i> Boeckeler	Jung	808322	AJOU	South Korea	KJ513655	KJ513562
<i>Trichophorum alpinum</i> (L.) Pers.	Spetzman	4941	CAN	USA, Alaska	**JX065093	**JX074649
<i>Trichophorum alpinum</i> (L.) Pers.	Cayouette & al.	75–78	CAN	Canada, Québec	KJ513656	KJ513563
<i>Trichophorum cespitosum</i> (L.) Hartm.	Saarela & Percy	1219	CAN	Canada, British Columbia	**JX065094	**JX074650
<i>Trichophorum cespitosum</i> (L.) Hartm.	Aiken & Iles	02–048	CAN	Canada, Nunavut	KJ513657	KJ513564
<i>Trichophorum clintonii</i> (A.Gray) S.G.Sm.	Pratt	128	CAN	Canada, Ontario	KJ513658	KJ513565
<i>Trichophorum pumilum</i> (Vahl) Schinz & Thell.	Bennett & al.	06–097	CAN	Canada, Yukon	KJ513659	KJ513566
<i>Trichophorum pumilum</i> (Vahl) Schinz & Thell.	Mejland	5-VII-1963	CAN	Norway	KJ513660	KJ513567
<i>Trichophorum rigidum</i> (Steud.) Goetgh., Muasya & D.A.Simpson subsp. <i>rigidum</i>	Ritter & Wood	2832	A	Bolivia	KJ513662	KJ513569
<i>Trichophorum rigidum</i> (Steud.) Goetgh., Muasya & D.A.Simpson subsp. <i>rigidum</i>	Unknown collector	1102	NY	Bolivia	KJ513661	KJ513568
<i>Trichophorum subcapitatum</i> (Thwaites & Hook.) D.A.Simpson	Luo	1903	CAS	China	KJ513663	KJ513570
<i>Trichophorum uniflorum</i> (Trautv.) Malyshev & Lukitsch.	Malishev	27-VII-1950	CAN	Russia	KJ513664	KJ513571
<i>Trichophorum uniflorum</i> (Trautv.) Malyshev & Lukitsch.	Ivanova & Moskvina	756	CAN	Russia	KJ513665	KJ513572
<i>Uncinia banksii</i> Boott in J.D.Hooker	Ogle	303	CHR	New Zealand	KJ513666	KJ513573
<i>Uncinia ecuadorensis</i> G.A.Wheeler & Goetgh.	Starr & Amigo	99020	FHO	Ecuador	KJ513667	KJ513574
<i>Zameioscirpus atacamensis</i> (Phil.) Dhooge & Goetgh.	Ru	9884	US	Argentina	**JX065095	**JX074651
<i>Zameioscirpus muticus</i> Dhooge & Goetgh.	Salvador & al.	881	MICH	Mexico	KJ513668	KJ513575

Herbarium acronyms follow Index Herbarium, except for Wright State University Herbarium (Wright). \*\*Sequence already published (Gilmour *et al.*, 2013).

annealing at 47 °C, and 90 s (*matK*) or 120 s (*ndhF*) of DNA extension at 72 °C, with a final extension step of 8 min. Minor adjustments were made to the recipe or cycling conditions for problematic samples. Successful amplifications were purified using an exonuclease I –

shrimp alkaline phosphatase protocol (MJS Biolynx Inc., Canada) and cycle sequenced using an ABI Prism Big Dye terminator kit, version 3.1 (Applied Biosystems). Sequencing termination products were purified in accordance with a sodium acetate/alcohol



protocol (Applied Biosystems) and sequenced on a 3130x1 Genetic Analyzer. Reads were corrected and assembled with SEQUENCHER, version 4.10 (Gene Codes Corporation) and all sequences were submitted to GenBank (Table 1).

#### PHYLOGENETIC ANALYSIS

Sequences of *matK* and *ndhF* were concatenated by individual and the matrix was aligned with the MUSCLE algorithm as implemented in GENEIOUS, version 4.8.5 (Biomatters). Minor adjustments to the alignment were made by hand using parsimony as an objective criterion (*sensu* Starr, Harris & Simpson, 2004). Bases 81–113 (*matK*) were excluded for 13 individuals because of indels that made alignment ambiguous only in these individuals. Excluding this region for all individuals or including the region assuming two independent indel events gave essentially the same parsimony bootstrap values (results not shown). Only results from combined analyses are reported because no well-supported (> 75%) topological incongruence was observed in independent gene analyses (results not shown), and an incongruence length-difference test (Farris, Källersjö & Kluge, 1995) was insignificant ( $P = 0.19$ ,  $N = 1999$ ). The alignment and all the most-parsimonous trees found during searches are available online on TreeBASE (<http://treebase.org/treebase-web/>).

Heuristic parsimony searches were conducted in PAUP\* 4.0 (Swofford, 2003) using 1000 random addition sequence replicates, followed by swapping with tree bisection–reconnection (TBR) and with the MULTREES and COLLAPSE options on. As a result of the length of the analysis and the large number of trees saved per replicate, a limit of 5000 saved trees and a time limit of 4 min was imposed on each replicate. Additionally, 100 parsimony ratchet searches using a random addition sequence were conducted with TNT, version 1.1 (Goloboff, Farris & Nixon, 2008). Ratchet searches used 2500 unconstrained and 2500 constrained iterations on unweighted, 5% upweighted, and 5% downweighted matrices, with a maximum of 15 TBR swaps per iteration, and keeping all optimal trees found in each replicate. A strict consensus of all the most-parsimonous trees was assembled in PAUP\* using the best trees found in the standard and ratchet searches. Branch support was assessed using 10 000 bootstrap replicates in PAUP\*, with the MULTREES option off (DeBry & Olmstead, 2000). To determine what would be the next best CDS topology to one that contains a Cariceae + *Trichophorum* clade, a search with an inverse constraint was conducted in PAUP\*.

Model-based searches were conducted using Bayesian Markov chain Monte Carlo (MCMC) methods in MrBayes, version 3.2.1 (Ronquist *et al.*, 2012) on the

CIPRES server 3.3 (Miller, Pfeiffer & Schwartz, 2010). Two partitions were enforced: the first included first and second codon positions and the second included only third codon positions for both genes. This partition scheme was selected in PARTITION-FINDER, version 1.0.1 (Lanfear *et al.*, 2012) with a greedy search using Bayesian information criterion on all possible partition schemes and all implemented models. A GTR+ $\Gamma$  model was used for both partitions (with six categories discrete gamma approximation). Topology and branch lengths were linked between the two partitions with all other model parameters unlinked and allowing for rate variation between partitions. The branch length prior was lowered to Unconstrained:Exponential(10) to decrease the probability of overestimating branch lengths (Marshall, 2010) and proposal parameters were adjusted to achieve acceptance rates of between 10% and 50% (although it was not possible to attain 10% acceptance for the TBR proposals). Two independent chains were run for 20 million generations. Each run was made with one cold and seven heated chains with a temperature parameter of 0.08 to achieve swap frequencies of 30% to 50% between adjacent chains. Convergence of model parameters was checked with TRACER, version 1.5.0 (Drummond *et al.*, 2012). Topological convergence was assessed using the mean SD of split frequencies reported by MrBayes and by visualizing tree samples with multidimensional scaling in TREESETVIS, version 3.0 (Hillis, Heath & St. John, 2005), a module of MESQUITE, version 2.75 (Maddison & Maddison, 2011).

Parsimony BS values were added to the strict consensus with SUMTREES, version 3.3.1 (Sukumaran & Holder, 2010) and posterior probabilities with TREEANNOTATOR, version 1.7.5 (Drummond *et al.*, 2012). Unambiguous changes along the branches of the strict consensus were calculated with WINCLADA, version 1.00.08 (Nixon, 2002). In the presence of polytomies, the unambiguous changes for the branches of the polytomy were calculated on the corresponding branches of a randomly chosen tree from the parsimony search. Tree figures were produced with TREEGRAPH, version 2.0.47 (Stöver & Müller, 2010). Clade support was characterized subjectively as weak (< 75% BS), moderate (75–84% BS), good or well supported (85–95% BS) and strong (95–100% BS). When two species are named to circumscribe a clade given in the Results and Discussion, it refers to the smallest monophyletic group comprising both species.

#### TOPOLOGICAL TESTS

To determine whether the data could exclude important monophyly or sister group hypotheses presented in previous taxonomic or phylogenetic studies,

**Table 2.** Sequence statistics for the separate and combined *matK* and *ndhF* data sets used in the phylogenetic analysis

	<i>matK</i>	<i>ndhF</i>	Combined
Sequence length range (bp)			
Ingroup only	697–1295	656–1209	1828–2498
Ingroup and outgroup	697–1295	656–1209	1828–2498
Aligned length			
Ingroup only	1324	1229	2553
Ingroup and outgroup	1330	1229	2559
Number of indels			
Ingroup only	6	6	12
Ingroup and outgroup	7	9	16
Gaps and missing data (%)			
Ingroup only	2.6	2.7	3.8
Ingroup and outgroup	3.1	2.7	3.9
GC content (%)			
Ingroup only	28.8	29.1	27.9
Ingroup and outgroup	28.2	29.0	29.0
Variable sites			
Ingroup only	362 (27.3%)	319 (26.0%)	681 (26.6%)
Ingroup and outgroup	484 (36.4%)	402 (32.7%)	886 (34.6%)
Potentially informative sites			
Ingroup only	218 (16.5%)	208 (16.9%)	426 (16.6%)
Ingroup and outgroup	304 (22.8%)	273 (22.2%)	557 (21.8%)

The sequence length range includes incompletely sequenced taxa.

constraint trees were used to find the optimal tree(s) under these alternative hypotheses. A Shimodaira–Hasegawa (SH) test (Shimodaira & Hasegawa, 1999; Goldman *et al.*, 2000) using the criterion of parsimony was implemented *sensu* Near, Pesavento & Cheng (2003) to be consistent with our use of the parsimony criterion for tree selection during searches. Likelihood-based SH tests gave similar results (not shown). Each constraint tree was used in a parsimony search in PAUP\* using 500 random sequence replicates followed by TBR, with MULTREES off and a maximum of 10 saved trees per replicate. The minimal length of each constrained search ( $l_x$ ) was compared with the length of the unconstrained search ( $l_{\text{best}}$ ) by computing the length difference ( $d_x = l_x - l_{\text{best}}$ ). One thousand bootstrap replicates of the whole matrix were produced with MESQUITE. Each bootstrap replicate (i) was used to calculate the parsimony score of a randomly chosen tree from the unconstrained search ( $l_{\text{best}}^{(i)}$ ) and a randomly chosen tree from each constrained search ( $l_x^{(i)}$ ) in PAUP. The mean length of each tree across all replicates ( $m_x = n^{-1} \sum l_x^{(i)}$ ) was subtracted to the score of all individual bootstrap replicates of the tree for centering ( $l_x^{(i)} - m_x$ ). The difference between each centered length and the minimum centered length of each replicate gives a distribution of tree length differences ( $d_x^{(i)}$ ) for each topology. This distribution was used to compute the

one-tailed *P*-values for each length difference ( $d_x$ ) between constrained and unconstrained trees. Significance was assessed at the  $\alpha = 0.05$  level.

## RESULTS

Alignment and character statistics are shown in Table 2. Standard parsimony TBR searches found 699 415 trees of 1822 steps (consistency index = 0.61, retention index = 0.84) in 64.5 h. The ratchet searches found 226 trees of the same length in < 10 h; 223 of which were not found by the standard searches. Despite this, the strict consensus of all the trees and of separate standard and ratchet analyses produced the same topology (Fig. 1). Results of the parsimony bootstrap searches are shown on the strict consensus (Fig. 1). The Bayesian MCMC chains quickly stabilized in model parameters and topology. The first 500 000 generations (2.5%) of each chain were discarded as burn-in, and the remaining 19 500 trees from both chains were used to compute the posterior probabilities of clades (Fig. 1).

Analyses position the strongly supported Dulichieae (100% BS) and *Khaosokia* as successive sisters (79% and 85% BS) to a strongly supported (85% BS) clade consisting of five major lineages (*Calliscirpus*, Cariceae, *Trichophorum* + *Oreobolopsis* + *Cypringlea*



**Figure 1.** Strict consensus tree of parsimony searches, with parsimony bootstrap support (bold) and unambiguous branch lengths (italics, in parentheses) indicated over branches, and Bayesian posterior probabilities of clades under branches. Tribes and major clades are indicated on the right.

Continues above



Figure 1. Continued

**Table 3.** Parsimony-based Shimodaira-Hasegawa test results for different topological hypotheses

Topology	Length	Length difference	Parsimony <i>P</i> -value
Best tree (Fig. 1)	1822	Best	
Scirpeae monophyletic (excluding <i>Khaosokia</i> )	1826	4	0.520
Scirpeae monophyletic (including <i>Khaosokia</i> )	1832	10	0.085
<i>Scirpus</i> monophyletic	1829	7	0.171
Cariceae and <i>Calliscirpus</i> monophyletic	1823	1	0.866
Cariceae and Dulichieae monophyletic	1836	*14	*0.017
Cariceae and <i>Khaosokia</i> monophyletic	1832	10	0.066
Cariceae and the <i>Scirpus</i> + <i>Zameioscirpus</i> clades monophyletic	1824	2	0.760
<i>Khaosokia</i> sister to monophyletic CDS	1827	4	0.312
<i>Khaosokia</i> and Dulichieae monophyletic	1827	4	0.347

\*Significant at  $\alpha = 0.05$ .

or 'Trichophorum clade', *Scirpus* + *Eriophorum* or 'Scirpus clade', and *Amphiscirpus* Oteng-Yeb. + *Phylloscirpus* C.B. Clarke + *Zameioscirpus* Dhooge & Goetgh. or 'Zameioscirpus clade'), the first four of which receive moderate to strong support (> 80% BS; Fig. 1). In this clade, *Calliscirpus* (100% BS) is poorly supported as sister to a monophyletic group (42% BS) composed of a *Trichophorum* clade + Cariceae (63% BS) and a *Zameioscirpus* clade + *Scirpus* clade (78% BS). Scirpeae is paraphyletic with respect to Cariceae in the strict consensus, although the monophyly of Scirpeae cannot be rejected by the SH test (Table 3). Furthermore, the SH test cannot reject any of the major lineages as a possible sister to Cariceae, except for Dulichieae (Table 3). Parsimony searches using an inverse constraint on the Cariceae + *Trichophorum* clade found trees one step longer than the best topology, with the only major difference being the position of the *Trichophorum* clade as sister to a *Calliscirpus* + Cariceae clade (not shown). Additionally, approximately 79% of bootstrap replicates that did not find the *Trichophorum* clade sister to Cariceae instead found *Calliscirpus* as sister to Cariceae. More than 90% of the bootstrap replicates thus had either *Calliscirpus* or the *Trichophorum* clade as sister to Cariceae.

In Dulichieae, *Blysmus* Panz. ex Schult. in J.J. Roemer & J.A. Schultes forms a weakly supported monophyletic group sister to *Dulichium*, with *Blysmus rufus* (Huds.) Link sister to a strongly supported *Blysmus compressus* (L.) Panz. ex Link + *Blysmus sinocompressus* Tang & F.T. Wang clade (Fig. 1). In the *Trichophorum* clade, a weakly supported *Trichophorum alpinum* (L.) Pers. + *Trichophorum subcapitatum* (Thwaites & Hook.) D.A. Simpson clade is sister to all the other species of *Cypringlea*, *Oreobolopsis*, and *Trichophorum*. The relationships in the *Trichophorum* clade are not further resolved in the strict consensus. Inside Cariceae, *Carex siderosticta* Hance

(*Siderostictae* clade) is strongly supported (100% BS) as sister to four strongly supported (> 95% BS) major clades: (1) core *Carex*; (2) *Vignea* clade; (3) *Schoenoxiphium* clade; and (4) a core unispicate clade (Fig. 1). The core *Carex* clade is sister to the *Vignea* clade, and the *Schoenoxiphium* clade is sister to the core unispicate clade, although both relationships are weakly supported (< 50% BS; Fig. 1). Relationships among the genera of the *Zameioscirpus* clade are unresolved. *Scirpus divaricatus* Elliott is sister to all other *Scirpus* clade species (95% BS), with *Scirpus pendulus* Muhl. sister (47% BS) to a polytomy involving *Scirpus maximowiczii* C.B. Clarke, *Scirpus radicans* Schkuhr, and four major clades: (1) a moderately supported (76% BS) *Scirpus expansus* Fernald + *Scirpus microcarpus* J. Presl & C. Presl clade; (2) a well-supported (89% BS) clade composed of *Scirpus wichurae* Boeckeler–*Scirpus pedicellatus* Fernald; (3) a moderately supported (79% BS) clade composed of *Scirpus ancistrochaetus* Schuyler–*Scirpus hattorianus* Makino; and (4) a well-supported (88% BS) monophyletic *Eriophorum* L. In *Eriophorum*, a strongly supported (98% BS) *Eriophorum virginicum* L.–*Eriophorum gracile* Koch in A.W. Roth clade is sister to all other sampled species of *Eriophorum*. Within the bulk of *Eriophorum* L., a weakly supported (55% BS) *Eriophorum russeolum* Fr. ex Hartm.–*Eriophorum brachyantherum* Trautv. & C.A. Mey. clade consists of all sampled unispicate species of the genus (the unispicate *Eriophorum* clade; Fig. 1).

## DISCUSSION

### MAJOR CLADE RELATIONSHIPS WITHIN CDS

The enhanced taxonomic sampling of the present study mostly confirms the relationships inferred by

Gilmour *et al.* (2013), except for the position of *Calliscirpus*, which is weakly supported as sister to Cariceae in Gilmour *et al.* (2013) but is weakly positioned in the present study (42% BS) as sister to a clade comprising Cariceae and other Scirpeae. The results are also comparable to previous phylogenetic studies in that the five major clades that received moderate to strong support in our analyses were also present in other studies including representatives of those clades (Dhooge, 2005; Muasya *et al.*, 2009; Jung & Choi, 2012; Gilmour *et al.*, 2013; Hinchliff & Roalson, 2013, although note *Zameioscirpus*). Although, in general, the backbone of our tree is weakly supported, the position of Dulichieae as sister to Cariceae + Scirpeae and the sister relationship of the *Zameioscirpus* and *Scirpus* clades are both congruent with previous molecular phylogenetic analyses, although they receive better parsimony BS with our dataset (Muasya *et al.*, 2009; Jung & Choi, 2012; Gilmour *et al.*, 2013). Our improved taxonomic sampling also gives us better insight into the relationships of *Scirpus* and *Eriophorum* and of *Trichophorum* and its allied genera.

The strongly supported monophyly of Dulichieae in our plastid dataset (Fig. 1) is consistent with previous studies (Muasya *et al.*, 2009; Jung & Choi, 2012; Gilmour *et al.*, 2013; Hinchliff & Roalson, 2013) and supports the distichous arrangement of the spikelets and the fertile prophylls as two good morphological synapomorphies for the tribe (Goetghebeur, 1998). The position of Dulichieae as sister to Cariceae and Scirpeae is also well supported and congruent with previous results (Dhooge, 2005; Gilmour *et al.*, 2013; Hinchliff & Roalson, 2013), although the position of *Khaosokia* in relation to these tribes is not clear.

Previous studies have found *Khaosokia* as either sister to the remainder of CDS (Muasya *et al.*, 2009; Jung & Choi, 2012) or in a polytomy with Cariceae and Scirpeae (Hinchliff & Roalson, 2013). Using the same plastid markers as the present study, Gilmour *et al.* (2013) found a strongly supported *Khaosokia* + Cariceae + Scirpeae clade. Our significantly increased taxonomic sampling appears to reduce the support for this relationship, highlighting the fact that the interpretation of phylogenetic results and their taxonomic significance must take limited sampling into account (Hedtke, Townsend & Hillis, 2006). Similar to *Dulichium arundinaceum* (L.) Britton, *Khaosokia caricoides* D.A.Simpson, Chayam. & J.Parn. has more than six bristles per flower (Simpson *et al.*, 2005) and long spikelets in an elongate raceme of spikes. Although *matK* and *ndhF* sequences alone cannot exclude the possibility of a sister relationship between *Khaosokia* and Dulichieae (Table 3), including *Khaosokia* in this tribe would have to allow for the antrorsely scabrous perianth bristles, spirally

inserted spikelets, and sterile prophylls of *Khaosokia* (Simpson *et al.*, 2005). This would make Dulichieae morphologically heterogeneous as currently defined. Constraining Scirpeae to be monophyletic with *Khaosokia* included resulted in a tree ten steps longer than the most-parsimonous trees, although SH tests could not reject this topology (Table 3). Our current data therefore suggest that *Khaosokia* could be treated either in Scirpeae or as a separate tribe, although only additional data will resolve this problem.

Scirpeae are characterized by what appear to be morphological plesiomorphies, and the tribe is often considered a dumping ground for genera that do not fit easily in other tribes of Cyperoideae (Goetghebeur, 1998). This is clearly reflected by the continuing trend of gradually segregating *Scirpus* spp. in other genera [*Calliscirpus*, *Fuirena* Rottb., *Isolepis* R.Br., *Schoenoplectus* (Rchb.) Palla, *Trichophorum* Pers.] and transferring traditional genera of Scirpeae to other distantly related tribes (e.g. Cyperaceae, Eleocharideae, Fuireneae; Koyama, 1958; Schultze-Motel, 1971; Goetghebeur, 1986; Gilmour *et al.*, 2013). It therefore comes as no surprise that Scirpeae are paraphyletic in our strict consensus tree (Fig. 1), although SH tests could not reject the possibility of a monophyletic Scirpeae including or excluding *Khaosokia* (Table 3).

Scirpeae form three groups that appear natural based on morphological and embryological characters. The *Trichophorum* clade is strongly supported and contains all genera of Scirpeae that possess a *Carex*-type embryo except *Calliscirpus* (i.e. *Trichophorum*, the closely allied *Oreobolopsis* and *Cypringlea*; Fig. 1) (Dhooge, 2005). This clade has also been found in most previous studies (Dhooge, 2005; Muasya *et al.*, 2009; Jung & Choi, 2012; Gilmour *et al.*, 2013) and only conflicts in a minor way with the supertree approach of Hinchliff & Roalson (2013; see discussion below on *Zameioscirpus*). Despite being consistently monophyletic in other studies, the position of the *Trichophorum* clade has varied from sister to the remainder of Scirpeae + Dulichieae (Muasya *et al.*, 2009), sister to Dulichieae or the *Scirpus* clade + Cariceae (Jung & Choi, 2012), sister to a *Scirpus*–*Zameioscirpus* clade (Hinchliff & Roalson, 2013), to sister to a *Calliscirpus* + Cariceae clade (Gilmour *et al.*, 2013). It therefore appears that, with the addition of our results, the *Trichophorum* clade has been associated with almost all other major lineages of the CDS.

The *Zameioscirpus* clade is an almost entirely South American group consisting of species with capitate to unispicate inflorescences of sessile spikelets, *Schoenus*-type embryos, and distally ascending rhizomes (Oteng-Yeboah, 1974; Dhooge *et al.*, 2003; Dhooge & Goetghebeur, 2004; Dhooge, 2005). *Amphis-*

*cirpus* has been treated as synonymous with *Phylloscirpus* on the basis of their minutely alveolate fruit epidermis and *Schoenus*-type embryo (Goetghebeur, 1986). However, *Phylloscirpus* and *Zameioscirpus* share a series of characters such as a loosely tufted habit (colonial in *Amphiscirpus*), short, arched rhizomes (long, mostly horizontal rhizomes in *Amphiscirpus*), spreading leaves in a basal rosette (stiffly erect in *Amphiscirpus*), and clearly terminal inflorescences (pseudolateral in *Amphiscirpus*), suggesting that *Phylloscirpus* is not only distinct from *Amphiscirpus* (Goetghebeur & Simpson, 1991; Dhooge, 2005) but may be closer to *Zameioscirpus*. However, the current analysis does not resolve the position of *Amphiscirpus* as a result of a lack of statistical support for the *Zameioscirpus* clade and poor resolution in this group. The *Zameioscirpus* clade had already been found on some occasions (Dhooge, 2005; Gilmour *et al.*, 2013), although the position of *Amphiscirpus* is highly unstable, having been recovered as sister to the *Scirpus* clade (Dhooge, 2005), or in a polytomy with the *Scirpus* clade and the *Zameioscirpus* clade *p.p.* (Muasya *et al.*, 2009). *Zameioscirpus* has also been found in the *Trichophorum* clade with the supermatrix approach of Hinchliff & Roalson (2013), although this position conflicts with our analysis, embryological data, and all previous analyses (Dhooge, 2005; Muasya *et al.*, 2009; Jung & Choi, 2012; Gilmour *et al.*, 2013). This incongruity might be a result of the fact that the only locus sequenced across most members of the *Zameioscirpus* and *Trichophorum* clades was the largely uninformative *rbcL*, suggesting that this topology may be a result of noise rather than a phylogenetic signal.

The *Scirpus* clade contains the type genus of Scirpeae and it is characterized by often pedicellate spikelets in open anthelae, leafy culms with long internodes, and *Fimbristylis*-type embryos (Schuyler, 1963; Van der Veken, 1965; Ball & Wujek, 2002). It has consistently been seen in previous studies, although sampling within or support for the clade was generally poor (Dhooge, 2005; Muasya *et al.*, 2009; Jung & Choi, 2012; Gilmour *et al.*, 2013; Hinchliff & Roalson, 2013). Our analyses place it as sister to the *Zameioscirpus* clade, a relationship that has been found in most previous molecular phylogenetic studies (Dhooge, 2005; Muasya *et al.*, 2009; Jung & Choi, 2012; Gilmour *et al.*, 2013; Hinchliff & Roalson, 2013), although it has never received more than approximately 65% parsimony BS. Although molecular support is weak, these two clades are united by morphologically similar embryos of the *Schoenus*-type and *Fimbristylis*-type, with the root cap (sub-)laterally displaced (Van der Veken, 1965; Goetghebeur, 1986). By contrast, species of *Calliscirpus*, Cariceae, Dulichieae, and the *Trichophorum*

clade possess embryos of the *Carex*-type (Van der Veken, 1965; Goetghebeur, 1986; Gilmour *et al.*, 2013), a state that is probably ancestral for CDS, if not for Cyperaceae as a whole (Goetghebeur, 1986).

Cariceae are nested in a paraphyletic Scirpeae and sister to the *Trichophorum* clade in the strict consensus (Fig. 1). This relationship is novel, with previous authors having variably found Cariceae to be sister to a monophyletic Scirpeae (Muasya *et al.*, 2009), to a *Scirpus*–*Zameioscirpus* clade (Jung & Choi, 2012) or to *Calliscirpus* (Gilmour *et al.*, 2013). An association between Cariceae and *Trichophorum* was originally proposed by Kukkonen & Timonen (1979) based on the infection of *Trichophorum cespitosum* (L.) Hartm. by a species of *Anthracoidea*, a genus of smut fungi once considered to be an exclusive parasite on Cariceae, although now known to infect several distantly related sedge genera such as *Cyperus* Banks & Sol. ex R.Br., *Fuirena* and *Schoenus* (Fuireneae and Schoeneae; Vánky, 2002). A sister relationship of the *Trichophorum* clade and Cariceae would be interesting because the *Trichophorum* clade contains mostly unispicate species (Crins, 2002; Liang & Tucker, 2010c), whereas Starr & Ford (2009) have found that multispicate inflorescences are probably ancestral to Cariceae. However, the sister relationship of the *Trichophorum* clade and Cariceae receives low support and only Dulichieae could be excluded as sister to Cariceae in the topological test (Table 3). Based on our parsimony bootstrap results and constrained searches, we suggest that the most probable sister group to Cariceae is either *Calliscirpus* or the *Trichophorum* clade.

#### DULICHIEAE

In Dulichieae, *B. rufus* (Huds.) Link is weakly supported as sister to the rest of the genus. Its branch is long (26 unambiguous changes) as is the branch leading to *B. compressus* (L.) Panz. ex Link and *B. sinocompressus* Tang & F.T.Wang (11 unambiguous changes) but, as a genus, *Blysmus* Panz. ex Schult. in J.J.Roemer & J.A.Schultes is supported by only two unambiguous changes. On account of its channeled, subterete leaves (flat in other *Blysmus* spp.), obscure antrorse barbs on whitish caducous bristles (retro-sely barbed, yellowish persistent bristles in other *Blysmus* spp.), its smooth anther crest (scabrous in other *Blysmus* spp.), and anatomical differences, such as the absence of adaxial bulliform cells in the leaf (present in other *Blysmus* spp.) and the presence of large air spaces in the stem (absent in other *Blysmus* spp.), Oteng-Yeboah (1974) erected the monotypic genus *Blysmopsis* Oteng-Yeb. The high molecular divergence between *Blysmus rufus* (Huds.) Link and its congeners would appear to support the recognition

of *Blysmopsis*. Our analysis also appears to support the recognition of *B. sinocompressus* Tang & F.T.Wang (Tang & Wang, 1961), a species recently segregated from *B. compressus* (L.) Panz. ex Link mostly based on perianth bristle and anther length (Liang & Tucker, 2010a).

#### THE *TRICHOPHORUM* CLADE

Until the inclusion of *Cypringlea* (Gilmour *et al.*, 2013), with its simple or compound anthelae and well developed leaves (Strong, 2003; Reznicek & González Elizondo, 2008), the *Trichophorum* clade could be characterized by spikelets solitary or in paucispicate racemes and by the frequent reduction of leaves to mucronate sheaths (Beetle, 1946; Crins, 2002; Dhooge & Goetghebeur, 2002). *Cypringlea* was segregated from *Scirpus* largely on the basis of its *Carex*-type embryo and aligned with *Trichophorum* for this reason (Strong, 2003). It can be further linked to *Trichophorum* by its mostly basal leaves and patent bristle barbs (Strong, 2003), whereas the leaves are cauline and the barbs most often retrorse in *Scirpus* (Schuyler, 1963). Despite its distinct morphology in the *Trichophorum* clade, it is not clear whether *Cypringlea* is nested in *Trichophorum* or if the genera are reciprocally monophyletic (Fig. 1). Although the position of *Cypringlea* in a clade of species with reduced vegetative and reproductive features may appear incongruent, close relationships among multi-plicate, paucispicate, and unispicate species are seen in all the major CDS clades (apart from *Calliscirpus*, two species), suggesting that reduction and proliferation are common throughout CDS. *Oreobolopsis* differs from *Trichophorum* mostly on the basis of its tepaloid perianth, which is bristle-like or absent in *Trichophorum* (Koyama & Guaglianone, 1987; Dhooge & Goetghebeur, 2002). However, the taxonomic value of this perianth character is not clear because some *Oreobolopsis* spp. appear to be most closely related to *Trichophorum* spp. [e.g. *Oreobolopsis tepalifera* T.Koyama & Guagl. and *Trichophorum rigidum* (Steud.) Goetgh., Muasya & D. A. Simpson; Dhooge & Goetghebeur, 2002]. A tepaloid perianth has also been observed in a specimen of *T. subcapitatum* (Thwaites & Hook.) D.A.Simpson, a species that normally possesses long bristles (noted by T. Koyama and confirmed by ÉLB on a 1972 collection from China by Shiu Ying Hu, no. 11812, MICH). Pending more studies, *Oreobolopsis* may need to be synonymized with *Trichophorum*, as previously noted by Dhooge (2005).

#### THE *SCIRPUS* CLADE

In the *Scirpus* clade, *S. divaricatus* Elliott is sister to all other sampled species and is unique as a result of

features such as spikelets in open terminal anthelae, glumes with broad green midribs and concavely trigonous nutlets (Schuyler, 1963). It also has the lowest chromosome number known for this clade ( $N = 14$ ; Schuyler, 1963). Although the backbone relationships in *Scirpus* are largely unresolved, there is a series of clades that appears natural on the basis of morphological and molecular characters. In the bulk of *Scirpus*, a moderately supported *S. expansus* Fernald–*S. microcarpus* J.Presl & C.Presl clade is characterized by culms growing singly from rhizomes, red-coloured base of leaf sheaths, spikelets in dense glomerules and short stout bristles with sharp retrorse barbs (Schuyler, 1963). Two representatives of this clade were also monophyletic in the study of Jung & Choi (2012), and Hinchliff & Roalson (2013) found weak support for a monophyletic *S. microcarpus* J.Presl & C.Presl + *Scirpus sylvaticus* L. clade, although *S. expansus* Fernald was in an unresolved *Scirpus* polytomy. The well-supported *S. wichuriae* Boeckeler–*S. pedicellatus* Fernald clade is characterized by caespitose growth (except for *Scirpus longii* Fernald) and by bristles that are smooth or antrorsely barbed at the tip and many times longer than the glumes (Koyama, 1958; Schuyler, 1963; Liang & Tucker, 2010b). Representatives of this clade have appeared as a monophyletic group in several previous analyses (Muasya *et al.*, 2009; Jung & Choi, 2012); however, in the analysis of Hinchliff & Roalson (2013), they form separate East Asian and North American clades that are part of a large *Scirpus* polytomy. *Scirpus pendulus* Muhl. appears transitional in its possession of glumes with conspicuous green midribs similar to *S. divaricatus* Elliott but with smooth and contorted bristles as in the *S. wichuriae* Boeckeler–*S. pedicellatus* Fernald clade, whereas its bristle length is intermediate between the two (Schuyler, 1963; Whittmore & Schuyler, 2002). This morphological situation is congruent with its phylogenetic placement in our analyses (Fig. 1). A moderately supported *S. ancistrochaetus* Schuyler–*S. hattorianus* Makino clade is morphologically characterized by a caespitose habit, spikelets in dense glomerules and retrorse barbs (Schuyler, 1963). This clade is also supported by a 12-bp deletion in *matK* (see matrix in TREEBASE), and it is monophyletic but weakly supported in all previous analyses incorporating representatives of the clade (Muasya *et al.*, 2009; Hinchliff & Roalson, 2013). In this clade, a strongly supported *Scirpus flaccidifolius* (Fernald) Schuyler–*S. hattorianus* Makino subclade can be characterized by weak and blunt bristle barbs (Schuyler, 1963). Finally, the genus *Eriophorum* forms a well-supported clade in the *Scirpus* clade that is natural based on inflorescences reduced to a simple anthela or a single spikelet, large spikelets, and a



high number (> 10) of long, smooth, and contorted perianth bristles (Koyama, 1958) that emerge from a ring primordium (Mora-Osejo, 1987; Vrijdaghs *et al.*, 2005). The genus has also been found to be monophyletic with weak support in Jung & Choi (2012) and strong support in Hinchliff & Roalson (2013) and Gilmour *et al.* (2013), although sampling in all these studies was limited to no more than four species.

Inside *Eriophorum*, the resolution is surprisingly good with many morphologically recognizable clades. The *E. virginicum* L.–*E. gracile* Koch in A.W.Roth clade is sister to the rest of *Eriophorum* and it can be morphologically characterized by obtuse glumes with many prominent nerves and a rhizomatous habit (Novoselova, 1995; Ball & Wujek, 2002). The bulk of *Eriophorum* comprises both rhizomatous and caespitose taxa, and both multispicate and/or unispicate species, with all of them possessing a single prominent midnerve on glumes (Novoselova, 1995; Ball & Wujek, 2002). Within a weakly supported unispicate clade (Fig. 1), the *E. russeolum* Fr. ex Hartm.–*E. scheuchzeri* Hoppe complex is strongly supported as monophyletic and characterized by a rhizomatous habit, one to seven sterile proximal glumes, and by glumes with well-defined hyaline margins (Novoselova, 1995). The sister clade to this complex comprises species with a caespitose habit and more than 12 sterile glumes that generally lack clear hyaline margins (Novoselova, 1995). Overall, there appears to be a reductive trend in inflorescence complexity within the *Scirpus* clade, with *Scirpus* spp. possessing compound anthelae, followed by a reduction to a simple anthela in multispicate *Eriophorum* and to a solitary terminal spikelet in the nested unispicate *Eriophorum* clade. Another trend is that of ascending chromosome counts, with *S. divaricatus* Elliott having  $N = 14$ , *S. pendulus* Muhl.  $N = 20$ , the bulk of *Scirpus* having  $N = 25–34$ , and *Eriophorum* L. having  $N = 29–30$  meiotic units, respectively (Schuyler, 1963; Ball & Wujek, 2002). Such a trend appears to further support the strict consensus tree, although a more resolved topology would be necessary to objectively study chromosome evolution.

#### ERIPHORUM NESTED IN SCIRPUS

Our analysis supports the position of *Eriophorum* as nested in a grade of *Scirpus* spp. in the *Scirpus* clade. The evolution of *Eriophorum* from within *Scirpus* has already been hypothesized, mostly on the basis of transitional species such as *S. maximowiczii* C.B.Clarke (Koyama, 1958; Gilmour *et al.*, 2013). Nonetheless, only Koyama (1958) has gone so far as to include all *Eriophorum* spp. in *Scirpus*, although he also included many other species now known to belong to distant lineages (e.g. *Fuirena*, *Isolepis*,

*Schoenoplectus*; Muasya *et al.*, 2009). Previous molecular phylogenetic analyses have not been able to discriminate between the possibility of reciprocally monophyletic genera or a nested *Eriophorum* (Muasya *et al.*, 2009; Jung & Choi, 2012; Gilmour *et al.*, 2013; Hinchliff & Roalson, 2013) as a result of insufficient taxonomic sampling and low branch support. Our analysis, which includes over half of *Eriophorum* spp. and > 40% of *Scirpus* spp., uncovers two levels of branching before the *Scirpus* + *Eriophorum* polytomy, making *Scirpus* clearly paraphyletic (Fig. 1). Assuming our relationships hold, Koyama's lumping of *Eriophorum* in *Scirpus* will be necessary if paraphyletic genera are to be avoided. However, the SH test could not reject the hypothesis of a monophyletic *Scirpus* (Table 3), indicating that more data are needed before taxonomic changes should be considered. We are currently developing low copy nuclear genes and plastid markers to resolve the phylogeny of the *Scirpus* clade, with emphasis on the position of *Eriophorum*.

#### CARICEAE

All five major clades previously discovered in wide analyses of Cariceae (Starr & Ford, 2009; Waterway *et al.*, 2009) are also present in our tree, although our analysis generally differs in the level of clade support. Unlike previous analyses, all clades receive strong BS, including the *Schoenoxiphium* and core unispicate clades, which have never received strong support in the same analysis (Waterway & Starr, 2007; Starr & Ford, 2009; Gehrke *et al.*, 2010) with the exception of Hinchliff & Roalson (2013) who used a 23-locus supermatrix of 16 016 aligned base pairs. We achieved comparable support with just *matK* and *ndhF*, highlighting the phylogenetic utility of these two genes for exploring relationships above and below the tribal level.

Among the major CDS clades, Cariceae have by far the greatest number of unambiguous molecular synapomorphies, with 11 more changes than are seen in the next longest branch to a major clade (Cariceae 28 versus Dulichieae 17; Fig. 1). This long branch parallels the morphological distinctiveness of Cariceae, the inflorescence morphology of which is so derived that it is difficult to determine the homology of its unusual structures with other Cyperaceae. This is also confounded by the fact that some of the most important characters of Cariceae appear to have been independently derived in other lineages; for example, fertile prophylls are also found in Dulichieae and unisexual flowers in *Khaosokia* and *Cryptangia*, *Trilepidae*, *Sclerieae*, and *Bisboeckelereae* (Goetghebeur, 1998; Simpson *et al.*, 2005). The origins and homologies of other highly derived groups such as the

Podostemaceae (Gustafsson, Bittrich & Stevens, 2002), Ceratophyllaceae (Les, 1988; Soltis *et al.*, 2011) and aquatic genera such as *Callitriche* L and *Hippuris* L. (Olmstead & Reeves, 1995) have been equally difficult to resolve on the basis of morphology, although their relationships are now being successfully addressed using plastid markers. However, the origin of Cariceae may prove harder to determine, given the fact that *matK* and *ndhF* are among the fastest evolving genes in the angiosperm plastome (Moore *et al.*, 2010; Liu *et al.*, 2012) but the backbone of our tree consists of short and poorly supported branches. The use of more rapidly evolving noncoding regions (e.g. ITS, *trnL-F*) is problematic at this level largely as a result of numerous insertion-deletion events among taxa, which can make alignment (= homology assessment) ambiguous. This may explain the high levels of homoplasy seen in some studies employing such markers at this taxonomic level and above (e.g. consistency index = 0.34, Starr & Ford, 2009; consistency index = 0.27, Jung & Choi, 2012). This suggests that further investigations on the origins of Cariceae should focus on the development of new, rapidly evolving coding markers to avoid alignment ambiguities.

#### CONCLUSIONS

Our results strongly support four major clades in CDS: Dulichieae, *Calliscirpus*, the *Trichophorum* clade, and Cariceae. Dulichieae are moderately supported as sister to all other lineages, with *Khaosokia* sister to a well-supported clade consisting of Cariceae and a paraphyletic Scirpeae. The *Scirpus* clade receives good support and is sister to a weakly supported *Zameioscirpus* clade. Our study further indicates that the genus *Khaosokia* could warrant tribal status and that the sister group to Cariceae will most likely be found in Scirpeae as currently circumscribed. It is also probable that *Eriophorum* is nested in *Scirpus*, and that *Trichophorum* and tribe Scirpeae as a whole are paraphyletic. There appears to be a trend in the increase of chromosome numbers in *Scirpus* and *Eriophorum* and a trend in the reduction and proliferation of the inflorescence throughout CDS. Future work needs to concentrate on the development of new coding markers to resolve relationships among the major lineages of CDS.

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