

## Molecular Systematics of Cyperaceae Tribe Cariceae Based on Two Chloroplast DNA Regions: *ndhF* and *trnL* Intron-intergenic Spacer

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**ABSTRACT.** A phylogenetic analysis of Cyperaceae tribe Cariceae was conducted using chloroplast DNA sequences from the gene *ndhF* and *trnL* intron and *trnL-trnF* intergenic spacer. Twenty nine taxa within Cariceae, four outgroup genera, and approximately 3,000 bp of cpDNA were included in the study. Our analysis reveals a monophyletic Cariceae with 100% bootstrap support. Within Cariceae, the South African genus *Schoenoxiphium* forms a clade that is sister to the rest of the tribe. Our results indicate that genus *Carex* is paraphyletic with respect to *Kobresia*, *Cymophyllus*, and *Uncinia*. *Cymophyllus* and *Uncinia* are nested within an assemblage containing *Kobresia*, *Cymophyllus*, and several unispicate *Carex* species. At the subgeneric level within *Carex*, only *Carex* subgenus *Vignea* appears monophyletic. Several well supported clades were identified within the Cariceae, including the *Schoenoxiphium* clade, *Uncinia* clade, *Carex* subgenus *Indocarex/Carex* clade, and subgenus *Vignea* clade; however, relationships among some clades are only moderately supported. Interpretation of the phylogenetic patterns and an account of past phylogenetic hypotheses with respect to the new data are provided.

The sedge tribe Cariceae, with over 2,000 species worldwide, is the largest tribe in the family Cyperaceae. Five genera, including the large genus *Carex* L., generally are included in this tribe. Several evolutionary schemes have been proposed for *Carex* and related genera based on inflorescence morphology and cytology (Kükenthal 1909; Heilborn 1924; Kreczetovicz 1936; Nelmes 1952; Savile and Calder 1953; Koyama 1961, 1962; Smith and Faulkner 1976; Kern and Nooteboom 1979; Reznicek 1990); however, there is substantial disagreement among various authors.

Members of tribe Cariceae are characterized by having monoecious flowers and a sac-like structure termed a perigynium, that subtends the gynoceium. Inflorescence morphology, the degree of closure of the perigynium, and the morphology of the rachilla, are the key characters used in delimiting genera. *Carex* L. (2,000 species) is found in all temperate regions of the world as well as montane areas in the tropics (Nelmes 1951). *Kobresia* Willd. (50 species), is widely distributed at high altitudes in the Himalayas, China, and central Asia, with a few species found in the high mountains of Europe and North America (Dahlgren et al. 1985). *Uncinia* Pers. (50 species), is found widely in highlands of Central and South America, Australia, New Zealand, and oceanic islands throughout the Southern hemisphere (Kukkonen 1967). *Schoenoxiphium* Nees (17 species), is found in mountains in eastern and

southern Africa and Madagascar (Kukkonen 1986). The monotypic *Cymophyllus* (Ker-Gawler) Kartesz & Gandhi occurs in the southeastern United States.

Plants in *Carex*, *Uncinia*, and *Cymophyllus* have one-flowered, unisexual spikelets with closed perigynia, whereas plants in *Kobresia* and *Schoenoxiphium* usually have several-flowered, bisexual spikelets with open perigynia. In addition, *Uncinia* species are characterized by having a hook-like structure, formed by the extension of the rachilla and a leaf, that extends beyond the opening of the perigynium. *Kobresia* has straight, terete rachillae, whereas *Schoenoxiphium* has straight, but flattened, rachillae. In both *Kobresia* and *Schoenoxiphium*, the rachillae sometimes are well developed and bear male flowers. Rachillae in *Carex* are of various forms including a reduced bud-like structure, a straight type, or a bent structure that is morphologically similar to those in *Uncinia* (Snell 1936; Reznicek 1990).

Because generic delimitation within Cariceae is based largely on the morphology of the inflorescence structure, blurring of the generic boundaries becomes a problem for some taxa having morphological characters that are interpreted as intermediate. Clarke (1908) placed a few species of *Schoenoxiphium* in *Carex*. Ivanova (1939) transferred several *Carex* species to *Kobresia*. Kern (1958) considered the recognition of *Kobresia* and *Schoenoxiphium* as two genera to be artificial. Koyama (1961)

merged *Uncinia* with *Carex*, and *Schoenoxiphium* with *Kobresia*.

At the subgeneric level in *Carex*, the present classification largely follows the system of Kükenthal (1909), who organized *Carex* into four subgenera based on inflorescence structure. Recognition of subgenera within *Carex* is based entirely on phenetic similarity in the inflorescence structure and probably does not reflect phylogenetic relationships. Subgenus *Primocarex* Kükenth. (60 species), characterized by a single terminal inflorescence, generally is considered to be artificial (Kreczetovicz 1936; Nelmes 1952; Smith and Faulkner 1976; Reznicek 1990). To reduce confusion, instead of using the designation "subgenus *Primocarex*," we refer to these taxa simply as "unispicate species" in this paper. Subgenus *Vignea* (P. Beauv.) Nees (400 to 500 species), is characterized by having bisexual spikes and two stigmas, and generally is considered a natural group (Reznicek 1990), although this hypothesis has not been verified cladistically. Subgenus *Carex* (= *Eucarex* Coss. et Germ.) (1,400 species), is a morphologically diverse group characterized by having a cladoprophyll (a tubular or utriculiform structure found at the base of the inflorescence) and usually unisexual spikes with flowers having two or three stigmas. It is unclear from the taxonomic literature whether subgenus *Carex* is a natural group or what the phylogenetic relationships between it and the other subgenera may be. Subgenus *Indocarex* Baill. (100 mostly tropical species), is characterized by the presence of a cladoprophyll and highly branched, bisexual spikes with tristigmatic flowers, and is considered the most primitive subgenus in *Carex* by many (Kreczetovicz 1936; Nelmes 1952; Koyama 1962; Smith and Faulkner 1976; Kern and Nootboom 1979), but not all authors (Kükenthal 1909; Reznicek 1990).

Many hypotheses on the evolution of *Carex* and Cariceae have been proposed. Heilborn (1924) produced the first "phylogenetic tree" of the genus based largely on chromosome numbers. Kükenthal (1909) and Heilborn (1924) considered the unispicate species and species with low chromosome numbers to be primitive in the genus. However, Kreczetovicz (1936) suggested that unispicate species are derived from other subgenera within *Carex* through the reduction in inflorescence complexity. Kreczetovicz (1936) also suggested that some unispicate species may have had their origins outside the genus. Nelmes (1952) elaborated on the hypothesis that *Carex* may be polyphyletic by suggesting that many unispicate species may be more closely

related to *Kobresia* or *Schoenoxiphium* than they are to *Carex*. Savile and Calder (1953), in their studies of smut fungi that infect *Carex*, suggested that *Carex* is a natural group. Smith and Faulkner (1976) suggested that *Kobresia* and *Schoenoxiphium* are the most primitive genera of the Cariceae and proposed an evolutionary link between *Schoenoxiphium*, *Kobresia*, and *Carex* subgenus *Indocarex*. Reznicek (1990) suggested that *Uncinia* and *Carex* may not be closely related, and disputed the importance of the rachilla as a systematic character.

Reznicek (1990) presented the most recent hypotheses on the evolution of *Carex*, which he considered to be derived from *Schoenoxiphium*- or *Kobresia*-like ancestors. He suggested that the most primitive subgenus in *Carex* is subgenus *Vignea*, which he considered to contain the most complex inflorescence structures in the genus, and that subgenus *Carex* could have evolved through a reduction in inflorescence structure and branching. However, Reznicek (1990) considered the origin and evolution of subgenus *Indocarex* to be unclear. He regarded the unispicate species to be polyphyletic and derived by reduction in inflorescence complexity on multiple occasions from the other three subgenera and possibly from other genera in the Cariceae (Nelmes 1952). Reznicek's (1990) views on the evolution and the phylogenetic position of *Carex* subgenus *Vignea* were quite different from those of most authors, who generally consider subgenus *Indocarex* to be most primitive and subgenus *Vignea* to be derived (Kükenthal 1909; Kreczetovicz 1936; Nelmes 1952; Savile and Calder 1953; Koyama 1961; Kern and Nootboom 1979).

Molecular data can provide an evaluation of classifications based on morphological characters, the interpretation of which have led to confusing and contradictory hypotheses of the phylogenetic relationships in the Cariceae. Because of the reduced floral structures, the uniform vegetative morphology, and traits unique to the Cariceae (e.g., perigynium), polarization of characters based on outgroup comparison is difficult (Crins 1990; Bruhl 1995). Recent phylogenetic studies based on morphology (Goetghebeur 1986; Bruhl 1995; Simpson 1995) and molecular data (Plunkett 1995; Muasya et al. 1998) on Cyperaceae and Cyperales (sensu Dalghren et al. 1985) indicate a monophyletic Cyperaceae and tribe Cariceae sensu Kükenthal and suggest possible sister groups of tribe Cariceae, including tribes Trilepideae (Bruhl 1995), Scirpeae (Muasya et al. 1998), or Sclerieae (Goetghebeur 1986). At present, only a few phylogenetic analyses

have been conducted on *Carex*, none of which provide a broad evolutionary framework for the whole genus (Crins and Ball 1988; Crins 1990; Starr and Ford 1995; Starr et al. 1997; Waterway et al. 1997).

Molecular techniques are useful in studying taxonomically complex groups because they offer many systematic characters independent of morphology, thereby enabling us to conduct large scale comparative studies that are necessary to reconstruct the phylogenetic history of these groups. The chloroplast gene *ndhF* has been shown to have a higher rate of base substitution than *rbcL*, making it suitable for studies at the generic level and above (Olmstead and Sweere 1994; Clark et al. 1995; Bohs and Olmstead 1997; Terry et al. 1997). For studies at the generic level and below, the non-coding region of the chloroplast DNA offers great potential. Thirty-two percent of the rice chloroplast and 42% of the maize chloroplast genome consist of non-coding DNA (Maier et al. 1995). These non-coding regions often are flanked by coding regions that have conserved sequences, which can be used to design PCR primers. These non-coding regions of the chloroplast DNA exhibit a higher level of sequence variation among closely related species than the coding region, therefore making them more useful at lower taxonomic levels (Gielly and Taberlet 1994). The introns and intergenic spacers in the cpDNA vary in length and substitution rates (Clegg et al. 1994; van Ham et al. 1994; Jordan et al. 1996; Kelchner and Clark 1997). Insertions and deletions are common in the non-coding regions of the chloroplast DNA and may result from molecular mechanisms such as slipped-strand mispairing (Takaiwa and Sugiura 1982), intra-molecular recombination (Ogihara et al. 1988; Palmer 1991), and stem-loop formation (Sears et al. 1996).

The cpDNA region bound by *trnL* 5' exon and *trnF* first was used in phylogenetic analyses by Taberlet et al. (1991). Since then, the *trnL* intron region and the intergenic spacer between *trnL* and *trnF* have been used in several studies (Gielly et al. 1996; Gielly and Taberlet 1994; van Ham et al. 1994).

We have three objectives in this study: (1) to assess the monophyly of major lineages at the generic and subgeneric levels in Cariceae, (2) to identify monophyletic groups within Cariceae and examine their phylogenetic relationships, and (3) to evaluate the utility of traditional characters for delimiting monophyletic groups and for classification.

#### MATERIALS AND METHODS

**Sampling.** Twenty nine taxa in the Cariceae and four outgroup taxa were sampled (Table 1). Sam-

pling efforts were aimed to include multiple species within each genus in the Cariceae and multiple species within each subgenus in *Carex* in order to represent a wide range of morphological and geographic diversity.

**DNA Isolation and Sequencing.** Fresh leaves, silica gel dried leaves, and herbarium samples were used in DNA isolation (Doyle and Doyle 1987). Double stranded PCR reaction mix for *ndhF* consisted of 10mM Tris-HCl, pH. 8.3, 50mM KCl, 3mM MgCl<sub>2</sub>, 2.5mM dNTP, 0.0125 units of Taq polymerase, 0.05 mM of each primer, and 1–5 ml total DNA template in a 50 ml reaction volume. Primers used for *ndhF* amplification and sequencing largely were described in Olmstead and Sweere (1994) with the following modifications to facilitate amplification and sequencing in Cyperaceae. New and modified primers include: 274Fa (CTTACCTCAATTATGT TAACACTCAT), 972Ra (GCTAGTATAATGTAAC CCAATTGAGAC), and 1006F (ATTGGTTTCGTC TCGAACTGC). Depending on taxon, double stranded *ndhF* amplification was achieved either in one step (274Fa-3') or two steps (5'-1318R, 1006F or 1318-3'). The reaction profile for *ndhF* amplification involves: 92°C for one minute; 45°C for one minute; 72°C for one minute for 40 cycles, followed by final extension at 72°C for seven minutes. PCR reaction mix for the chloroplast non-coding region consisted of 10mM Tris-HCl, pH. 8.3, 50mM KCl, 3mM MgCl<sub>2</sub>, 1.25mM dNTP, 0.0125 units of Taq polymerase, 0.05 mM of each primer (Taberlet et al. 1991), and 0.5–1 ml total DNA template in a 50 ml reaction volume. The reaction profile for the non-coding region amplification involves: 94°C for one minute; 50°C for one minute; 72°C for one minute for 35 cycles. Double stranded PCR products were cleaned using Qiaquick PCR Purification Kit (Qiagen Inc., Chatworth, CA) and quantified by spectrophotometry. DNA sequencing was accomplished using the cycle sequencing method for both strands using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (Perkin Elmer, Foster City, CA) and analyzed on an ABI 377 automated sequencer. DNA sequences were assembled using Sequencher version 3.0 (Gene Codes Corporation, Ann Arbor, MI).

**Data Analysis.** Sequence alignment was done by eye for *ndhF* and by using ClustalW (Thompson et al. 1994) and subsequently adjusted by eye for the intron-intergenic spacer region. Due to the many insertions and deletions in the intron-intergenic region and the difficulties in aligning large data matrices, sequence alignment was done in

TABLE 1. Species included in this study. Sectional designation follows Kükenthal (1909). <sup>1</sup>K.E.C. Ku ming, Edinburgh, Gothenburg Expedition.

Taxon	Subgenus	Section	Source	Location of voucher	GenBank accession number: nr/1F-trnL-F
Cariceae					
<i>Carex</i>	<i>Indocarex</i>	<i>Polystachyae</i>	Taiwan, Yen 0078	WTU	AF191806 AF191814
<i>Carex baccans</i> Nees in Wight			Ontario, Canada, A.A. Reznicek s.n.	MICH	AF163442
<i>Carex backii</i> Boott	<i>Carex</i>	<i>Phyllostachyae</i>	Sabah, Malaysia, Yen 0073	WTU	AF164927
<i>Carex capillacea</i> Boott	<i>Primocarex</i>	<i>Urciniaeformis</i>	Texas, USA, R.G. Olmstead s.n.	WTU	AF163446 AF164925
<i>Carex cephalophora</i> Muhl.	<i>Vignea</i>	<i>Muehlenbergianae</i>	Nepal	cultivated RBGE 19892590	AF163454 AF164942
<i>Carex composita</i> Boott	<i>Indocarex</i>	<i>Polystachyae</i>	Washington, USA, Yen 0094	WTU	AF191807 AF191815
<i>Carex deaxeyana</i> Schw.	<i>Vignea</i>	<i>Elongatae</i>	Scotland	cultivated RBGE 19851401	AF163455 AF164941
<i>Carex dioica</i> L.	<i>Primocarex</i>	<i>Physoglochin</i>	Mexico, A.A. Reznicek s.n.	MICH	AF191808 AF191816
<i>Carex donnell-smithii</i> L.H. Bailey	<i>Carex</i>	<i>Fecundae</i>	Kenya, A. Muasya 1051	K	AF191809 AF191817
<i>Carex echinochloë</i> Kunze	<i>Indocarex</i>	<i>Indicae</i>	Colorado, USA, Yen 0132	WTU	AF191810 AF191818
<i>Carex elymoides</i> Holm	<i>Primocarex</i>	<i>Filifoliae</i>	Virginia, USA, R.G. Olmstead s.n.	WTU	AF163447 AF164926
<i>Carex grmyi</i> Carey	<i>Carex</i>	<i>Lupulinae</i>	Mexico, A.A. Reznicek s.n.	MICH	AF163443 AF164940
<i>Carex humboltiana</i> Steud.	<i>Indocarex</i>	<i>Indicae</i>	Washington, USA, Yen 0087	WTU	AF191811 AF191819
<i>Carex mertensii</i> Prescott	<i>Carex</i>	<i>Atratae</i>	Washington, USA, Yen 0126	WTU	AF163444 AF164939
<i>Carex nigricans</i> C.A. Meyer	<i>Primocarex</i>	<i>Callistachys</i>	Washington, USA, K. Glew 96-0715PL	WTU	AF163450 AF164929
<i>Carex obtusata</i> Lili.	<i>Primocarex</i>	<i>Obtusatae</i>	Colorado, USA, Yen 0133	WTU	AF163451 AF164928
<i>Carex rupestris</i> All.	<i>Primocarex</i>	<i>Rupestris</i>		WTU	AF163453 AF164934

TABLE 1. Continued.

Taxon	Source	Location of voucher	GenBank accession number tml-F
<i>Carex scirpoides</i> Michx.	Washington, USA, Yen 0128	WTU	AF191812 AF191820
<i>Carex xerantica</i> L. H. Bailey	Colorado, USA, R. G. Olmstead 95-25	WTU	AF191813 AF191821
Other genera			
<i>Cynophyllus fraserianus</i> (Ker-Gawler) Kartesz & Gandhi	North Carolina, USA, D. Parks s.n.	WTU	AF163456 AF164930
<i>Kobresia fragilis</i> C.B. Clarke	China, K.E.G. <sup>1</sup> 1287	E	AF163457
<i>Kobresia gaminiei</i> C. B. Clarke	Sikkim, ESIK 751	E	AF164945
<i>Kobresia royleana</i> (Nee) Boeck.	China, K.E.G. <sup>1</sup> 794	E	AF163458 AF164944
<i>Kobresia simpliciuscula</i> (Wahl) Mackenzie	Colorado, USA, Weber & Cooper 1803	COLO	AF163460 AF164947
<i>Schoenoxiphium burket</i> C. B. Clarke	S. Africa, J. Browning 689	NU	AF163462 AF164948
<i>Schoenoxiphium filiforme</i> Kükenth.	S. Africa, J. Browning 699	NU	AF163463 AF164950
<i>Schoenoxiphium ludwigii</i> Hochst.	S. Africa, J. Browning 704	NU	AF163464 AF164951
<i>Uncinia filiformis</i> Boott	S. Africa, J. Browning 704	NU	AF163465 AF164949
<i>Uncinia phleoides</i> (Cav.) Pers.	New Zealand, S. Wagstaff 95-051	CHR	AF163466
<i>Uncinia uncinata</i> (L. fil.) Kükenth.	Chile, Hortus Botanicus Valdiviensis	WTU	AF163467
Outgroups			
<i>Eriophorum chamissonis</i> C. A. Mey.	Hawaii, K Millam, s.n.	WTU	AF164931 AF163468 AF164931
<i>Eriophorum polystachion</i> L.	Washington, USA, Yen 0190	WTU	AF163470 AF164954
<i>Dulichium arundinaceum</i> (L.) Britt.	Washington, USA, Yen 0185	WTU	AF163471 AF191822
<i>Scripus microcarpus</i> Presl	Washington, USA, Yen 0195	WTU	AF163472 AF164953
	Washington, USA, Yen 0085	WTU	AF163469 AF164952

three steps. First, taxa within the Cariceae and those from outside the tribe were aligned separately and those alignments were adjusted by eye. Second, selected taxa from both inside and outside the Cariceae were aligned with ClustalW and alignment adjusted by eye. The final alignment was based on all three alignments and reflects our best estimate of the positional homology.

*ndhF* and *trnL* intron-intergenic spacer data were analyzed using PAUP\* (version 4.0d60 courtesy of David Swofford). Both parsimony and maximum likelihood analyses were conducted. Regions of ambiguous alignment were excluded from analyses. Short regions of sequence at the beginning and the end of both *ndhF* and *trnL* intron and *trnL-trnF* intergenic spacer were excluded from analyses because not all sequencing reactions yielded full length fragments. Gaps were introduced in the alignment in order to optimize positional homology.

The Incongruence Length Difference (ILD) Test (Farris et al. 1995, as implemented in PAUP\*) was used to examine potential conflicts in the phylogenetic signals between *ndhF* and intron-intergenic spacer data. For the test, 100 replicates were conducted, each with ten random-order-entry heuristic searches.

For parsimony analyses, one hundred heuristic searches with random sequence addition were conducted with all characters weighted equally with TBR swapping and MULPARS in effect. To assess relative group support, one hundred bootstrap (Felsenstein 1985) replications were conducted. For each bootstrap replicate, ten heuristic searches with random sequence addition were conducted.

For maximum likelihood analyses, a substitution model where all base transformations are equally likely and a discrete gamma distribution (Yang 1994) with three rate classes to account for rate heterogeneity were used. Five heuristic searches with random sequence addition were conducted with MULPARS turned off. The tree with the maximum likelihood was retained to represent the best estimate of phylogenetic patterns.

## RESULTS

Sampling was designed to include all the genera recognized in the tribe and much of the morphological and geographic variation (Table 1). For *Uncinia* (ca. 50 spp.), *Kobresia* (ca. 50 spp.), and *Schoenoxiphium* (17 spp.), at least three species of each genus were included. For *Uncinia*, samples were in-

cluded from South America, Hawaii, and New Zealand; for *Kobresia*, samples were included from the Himalayas and North America, and for *Schoenoxiphium*, samples were included from South Africa. For *Carex*, all four subgenera were included, with more sampling of unispicate species and subgenus *Indocarex*, the two groups of taxa whose systematic positions have been controversial to researchers. Because the clades containing subgenera *Vignea* and *Carex/Indocarex* are strongly supported, these taxa were not sampled exhaustively in this study. The addition of more taxa would result in many short branches in this particular part of the tree, but probably would have no effect on the phylogenetic structure at deeper levels.

Sequences from 33 taxa included in this study (Table 1) provided a total aligned length of 2168 bp for *ndhF* and 1223 bp for *trnL* intron and *trnL-trnF* intergenic spacer. Four gaps were introduced in the *ndhF* alignment to account for unique sequences found in five taxa. Forty-two gaps, ranging from one to thirty bases long, were introduced into the *trnL* intron and *trnL-trnF* intergenic spacer. Fifteen parsimony informative gaps were included in the analyses as two-state characters.

Results of the ILD Test (Farris et al. 1995) showed that the *ndhF* dataset and the intron-intergenic spacer dataset were not significantly different from random partitions of the data ( $p = 0.53$ ). Therefore, we combined the two datasets in subsequent analyses.

In the combined dataset, 615 sites were variable, of which, 308 sites were parsimony informative. Parsimony analyses resulted in six most parsimonious trees of 885 steps (CI = 0.652 for informative characters). The strict consensus of the six trees is shown in Fig. 1. Maximum likelihood analyses yielded one tree of maximum likelihood ( $-\ln L = 9390.4936$ ). The overall topology of the maximum likelihood tree (Fig. 2) is similar to that of the parsimony strict consensus tree (Fig. 1). Differences between the maximum likelihood tree and the parsimony strict consensus tree are restricted to interspecific relationships within major lineages and in areas with weak bootstrap support and short branch lengths.

We identified several well supported lineages at the generic and subgeneric levels within the tribe Cariceae and the genus *Carex*. At the generic level, our analyses reveal a monophyletic tribe Cariceae which is supported by 100% bootstrap value, given the outgroup sampling (Fig. 1). Within Cariceae, the South African genus *Schoenoxiphium* forms a clade (100% bootstrap support) and is sister to the rest

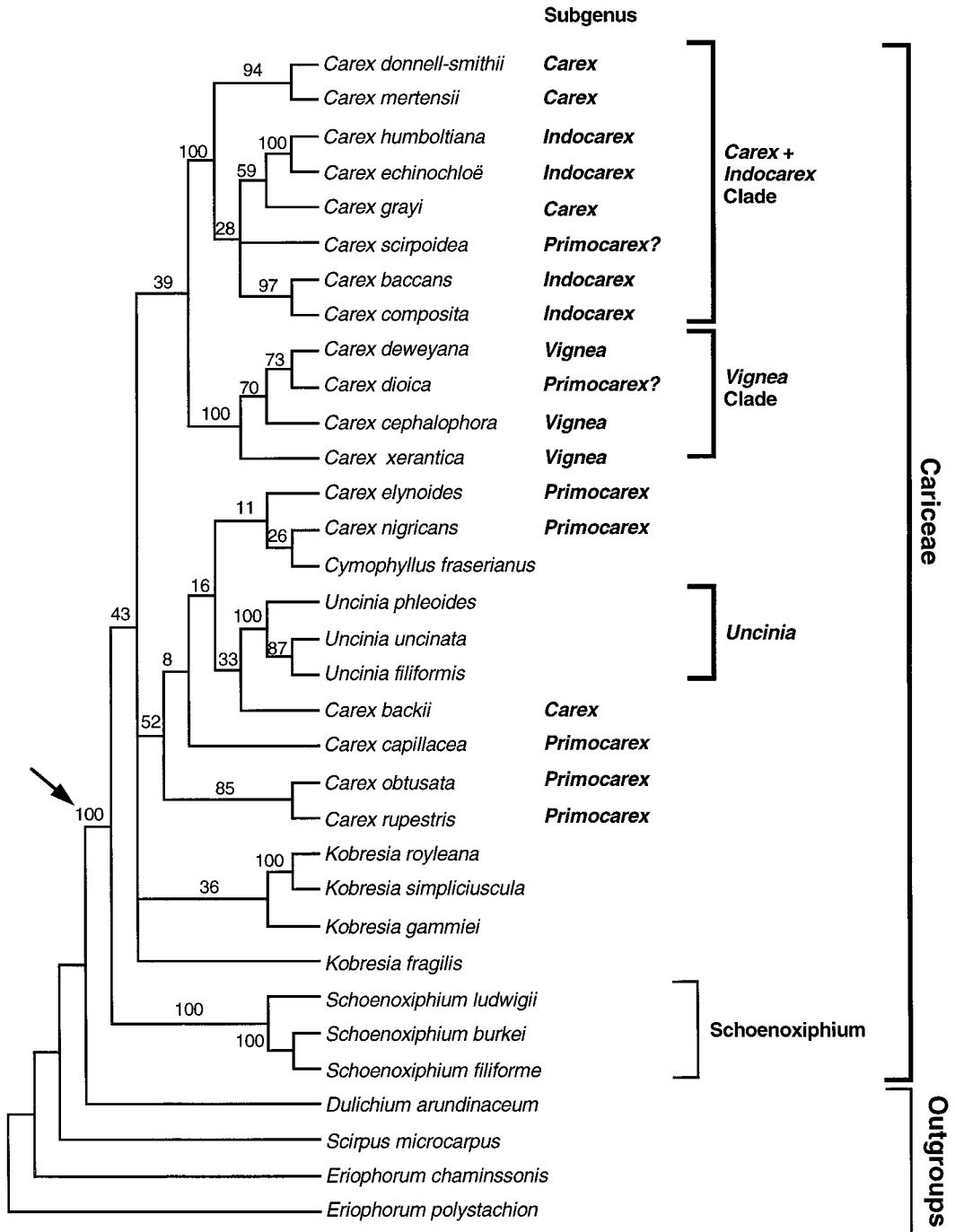


FIG. 1. Strict consensus of six most parsimonious trees. Tree length = 885; consistency index, excluding autapomorphic characters = 0.652. Bootstrap values are above the branches. Clades identified in this study are indicated by brackets to the right. Subgeneric designation follows Kükenthal (1909).

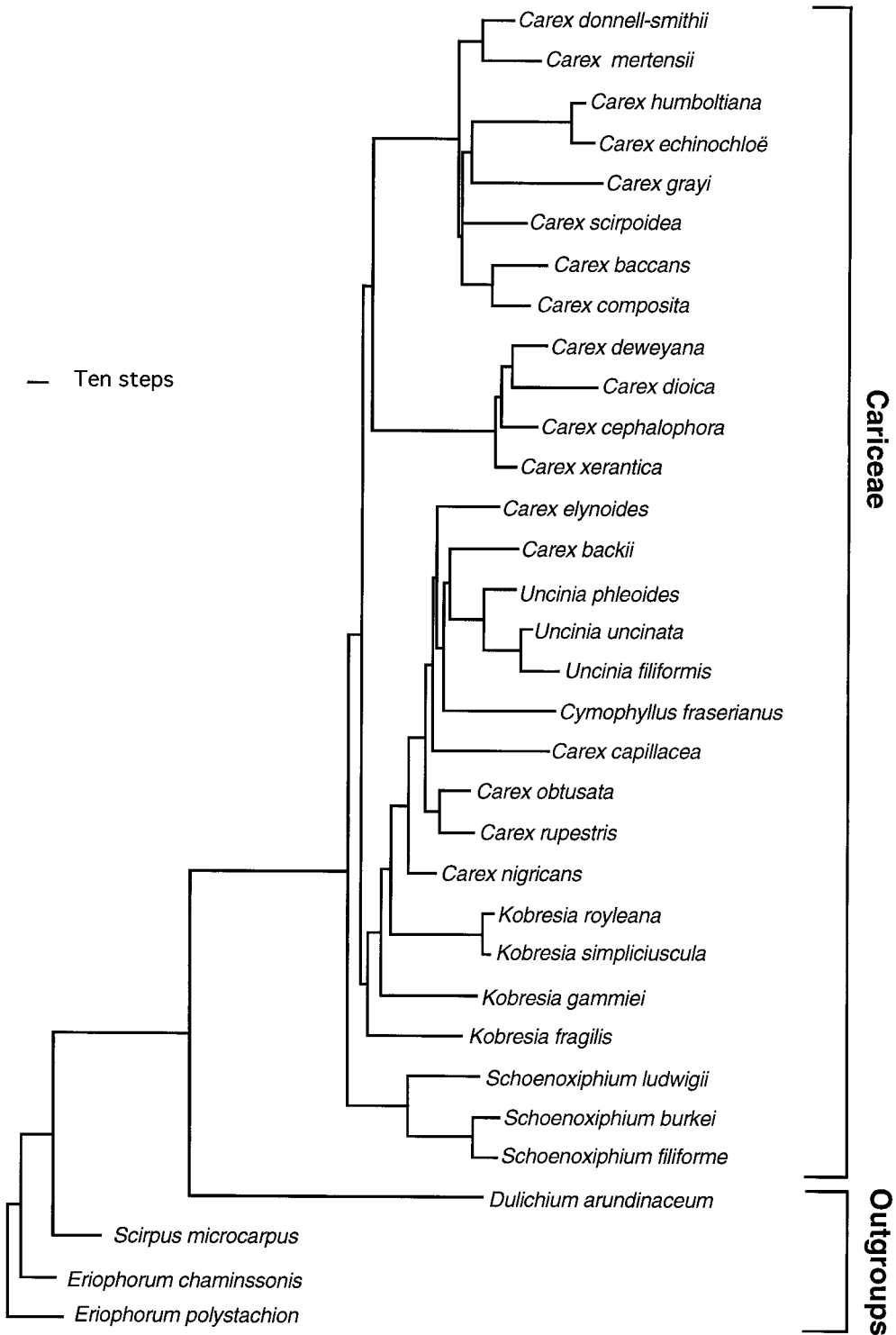


FIG. 2. Maximum likelihood tree drawn with proportional branch lengths. Subgeneric designation follows Kükenthal (1909).

of the tribe which is moderately supported as a monophyletic group (43% bootstrap support). The remainder of Cariceae forms two clades in the maximum likelihood tree (Fig. 2) and three clades plus *Kobresia fragilis* in the parsimony strict consensus tree (Fig. 1). Both analyses reveal that *Carex* is paraphyletic with respect to *Kobresia*, *Cymophyllus*, and *Uncinia*. We designated the clade containing *Carex*, *Kobresia*, *Uncinia*, and *Cymophyllus* as *Carex* sensu lato. *Uncinia* forms a derived clade (100% bootstrap support) within a paraphyletic assemblage of *Cymophyllus*, several unispicate species of *Carex*, and *Carex backii*. *Kobresia* forms a basal paraphyletic grade in the clade that includes *Carex backii*, some unispicate *Carex* species, *Uncinia*, and *Cymophyllus* in the maximum likelihood tree, and occupies an unresolved position in the parsimony strict consensus tree.

To investigate further the phylogenetic position of *Kobresia* in *Carex* s.l., we examined all six most parsimonious trees and the maximum likelihood tree for congruence. In three of the most parsimonious trees (Fig. 3A), *Kobresia* is paraphyletic to the clade containing *Carex* subgenera *Carex*, *Indocarex*, and *Vignea*, but only with 8% bootstrap support. In the other three most parsimonious trees (Fig. 3B), *Kobresia* is paraphyletic to the clade containing *Cymophyllus*, *Uncinia*, several unispicate *Carex* species, and *Carex backii*, with 32% bootstrap support. The likelihood score for trees supporting the basal position of *Kobresia* to *C. backii*, unispicate *Carex* species, *Uncinia*, and *Cymophyllus* ( $-\ln L = 9393.5657$ ) is slightly lower than that for trees supporting the basal position of *Kobresia* to subgenera *Vignea*, *Carex*, and *Indocarex* ( $-\ln L = 9392.5470$ ). However, the two tree topologies are not significantly different in a Kishino-Hasegawa test ( $p = 0.92$ ) (Kishino and Hasegawa 1989).

To investigate the hypothesized basal position of *Schoenoxiphium* and *Kobresia* in the tribe Cariceae (Reznicek 1990; Kern 1958; Koyama 1961), we performed two types of constraint analyses using the same heuristic search procedure as the unconstrained searches. In the first constrained search, we divided the Cariceae into two sisters clades, one of which comprised *Kobresia* and *Schoenoxiphium*, the other comprised the remaining genera in the tribe. In the second constraint search, we only constrained *Carex*, *Uncinia*, and *Cymophyllus* to be monophyletic, thereby allowing *Kobresia* and *Schoenoxiphium* to form a paraphyletic grade. The first constraint analysis resulted in two most parsimonious trees five steps longer and significantly worse

than the most parsimonious trees in a Kishino-Hasegawa test ( $p = 0.02$ ). The second constraint analysis resulted in nine trees that are three steps longer, but are not significantly different from the six most parsimonious trees.

At the subgeneric level, all subgenera, except subgenus *Vignea*, appear polyphyletic. *Carex backii* (subgenus *Carex*) is nested within the assemblage containing *Uncinia*, *Cymophyllus*, and several unispicate *Carex* species. *Carex backii* and associated taxa form a clade that is supported with a moderate bootstrap value (52%) in the parsimony strict consensus tree. *Carex* subgenera *Indocarex* and *Carex* (excluding *C. backii* and including *C. scirpoidea*) form a clade (100% bootstrap support) sister to subgenus *Vignea* (including *C. dioica*, 100% bootstrap support). However, the monophyletic grouping of the two clades is only moderately supported (32% bootstrap support).

#### DISCUSSION

In this study a phylogenetic reconstruction of Cyperaceae tribe Cariceae using DNA sequences is presented. This study represents a first estimate of phylogenetic relationships for tribe Cariceae. Although our sampling included only 29 taxa from a tribe with over 2,000 species, we attempted to include much of the morphological and geographical diversity of this tribe, especially at the generic level. Additional studies based on the phylogenetic framework established here are currently underway. Because patterns of phylogenetic relationships may change with additional sampling, some of our systematic conclusions may be modified as more data become available.

Our analyses reveal a monophyletic tribe Cariceae, a finding consistent with other recent cladistic analyses of Cyperaceae, based on morphology (Goetghebeur 1986; Bruhl 1995; Simpson 1995), and on DNA sequence data (Plunkett et al. 1995; Musy et al. 1998). Several morphological and anatomical characters are common to all members of the Cariceae, including the monoecious flower, the perigynium, and possessing a non-radiate form of chlorenchyma cells in the mesophyll (Metcalf 1971). Other genera, such as *Scleria* Bergius and *Bisboeckelera* Kunze, usually placed in the tribe Sclerieae Kunth ex Fenzl. (Metcalf 1971), have been suggested to belong to the tribe Cariceae on the bases of inflorescence morphology and the terminal position of the female flowers on the spikelet (Koyama 1961). Although we did not include these genera in

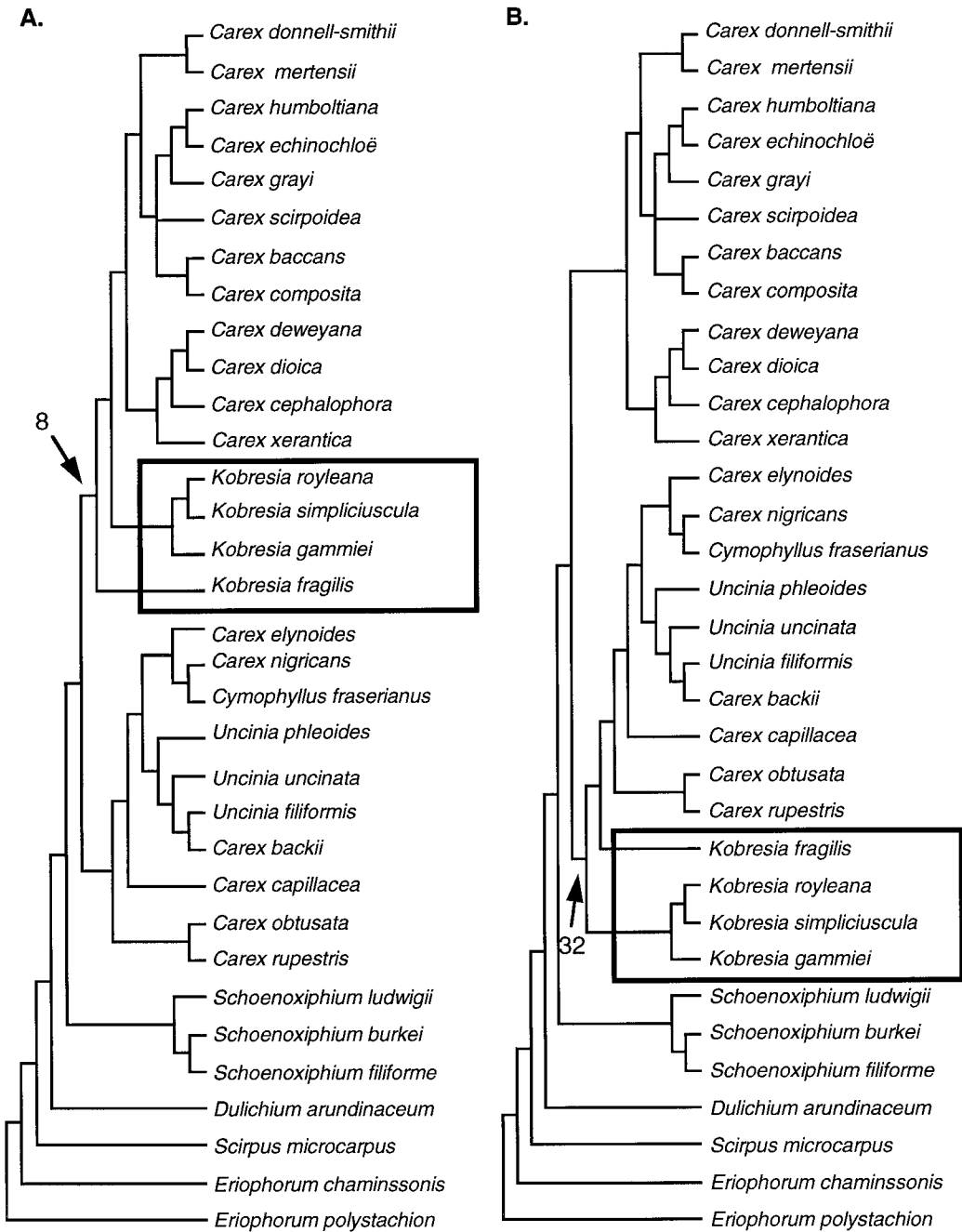


FIG. 3. A. strict consensus of three of the most parsimonious trees showing the position of *Kobresia* basal to *Carex* subgenera *Carex*, *Indocarex*, and *Vignea*. B. strict consensus of three of the most parsimonious trees showing the position of *Kobresia* basal to *Uncinia*, *Cymophyllum*, and *Carex* subgenus *Primocarex*. Bootstrap values denote the support for each topology at the first node leading to the clade containing *Kobresia*.

our study, recent studies have suggested that these taxa do not belong in the tribe Cariceae (Goetghebeur 1986; Bruhl 1995; Muasya et al. 1998).

Phylogenetic relationships within the tribe Cariceae have been postulated chiefly through studies of the inflorescence structure. An open perigynium subtending the achene and a well developed rachilla bearing male flowers have been considered to be primitive, whereas a closed perigynium with rudimentary rachilla or the absence of rachilla, have been considered to be advanced (Nelmes 1952). For these reasons, the inflorescence morphology found in *Schoenoxiphium*, which contains many species with open perigynium and well developed rachillae, has been considered to represent the most primitive form that may have given rise to inflorescence types seen in other genera within the Cariceae, through a process of reduction in inflorescence complexity (Kükenthal 1909; Smith and Faulkner 1976; Timonen 1985, 1989, 1993). The position of *Schoenoxiphium* sister to the rest of the tribe Cariceae found in our analyses provide support for this prevailing view. In addition, results from this study shows that *Schoenoxiphium* is not related closely to *Carex* subgenus *Indocarex*, as has been suggested by Smith and Faulkner (1976) and Haines and Lye (1983).

Our data indicate that *Kobresia* may not be as closely related to *Schoenoxiphium* (Kern 1958; Koyama 1961) as to other taxa within the Cariceae (Figs. 1, 2). In none of the unconstrained analyses did we observe *Schoenoxiphium* and *Kobresia* to come out together. Constrained searches forcing *Kobresia* and *Schoenoxiphium* to be monophyletic resulted in trees that are significantly worse than the most parsimonious trees. Constrained searches where *Schoenoxiphium* and *Kobresia* were paraphyletic to the remainder of the Cariceae resulted in trees that are longer and have lower likelihood than the unconstrained results, although the differences are not significant in the Kishino-Hasegawa test. While not indicating strong support, the bootstrap value supporting *Carex* s.l. (43%) is much higher than that supporting the exclusion of *Kobresia* from it (<5%).

Although Kükenthal (1909) recognized *Schoenoxiphium* and *Kobresia* as two distinct genera on the basis of rachilla morphology and for phyto-geographic reasons, others have considered this separation to be artificial (Kern 1958; Koyama 1962). Koyama (1961) formalized his hypothesis by merging *Schoenoxiphium* with *Kobresia*. Several authors (Nelmes 1952; Kern 1958; Smith and Faulkner 1976; Gordon-Gray 1995) have pointed out that although

many *Schoenoxiphium* species have well developed rachillae bearing terminal male flowers, some species, such as *S. filiforme* Kük., have very reduced rachillae and perigynia that are similar to those found in some *Kobresia* species (Kern 1958). We included *S. filiforme* in our study, the results of which support a clear phylogenetic separation between *Kobresia* and *Schoenoxiphium*. Although *Kobresia* and *Schoenoxiphium* are morphologically similar, the similarity is due mainly to the possession of plesiomorphic traits, such as the rachillae and the open perigynium. Therefore, the recognition of *Kobresia* and *Schoenoxiphium* as one taxon would result in a paraphyletic taxon based on plesiomorphic characters.

Although the position of *Kobresia* within *Carex* s.l. is unresolved in the parsimony strict consensus tree, in the maximum likelihood tree *Kobresia* came out basal to a *Carex-Uncinia-Cymophyllus* assemblage. Because our analyses provide ambiguous placement of *Kobresia* (within *Carex* s.l. (Figs. 1, 2, 3A, 3B), discussions on the evolution of this genus should be regarded as an attempt at reconciling the available data and a more definitive statement of phylogenetic relationships must wait until additional data are available. The key character in the discussion of the evolutionary position of *Kobresia* and *Carex* has been the inflorescence and perigynial morphology (Kükenthal 1909; Nelmes 1952; Timonen 1998). *Kobresia* has been considered generally to be more primitive than *Carex* due to the presence of multiflowered spikelets and open perigynia. Our analyses show that *Kobresia* is basal within *Carex* s.l. (Figs. 2, 3B).

In a study of the smut fungi (genus *Anthracoidea*) that infect members of Cariceae and by considering the cytological data of Heilborn (1924), Kukkonen (1963) hypothesized two putative lines of evolution from *Kobresia* to *Carex*. One line led to subgenus *Vignea* through some unispicate species of *Carex*, and the other led to subgenus *Carex*. Although *Kobresia* is found to be basal to *Carex* subgenera *Carex*, *Vignea*, and *Indocarex* in three of the most parsimonious trees (Fig. 3A), the bootstrap support for this grouping is weak (8%). We did not observe the two separate lines of evolution as proposed by Kukkonen (1963). In addition, because most of the unispicate *Carex* included in this study were found outside the subgenus *Vignea* clade, it seems that the link between these species and subgenus *Vignea*, as suggested by Kukkonen (1963), may require re-evaluation.

Alternatively, our finding of *Kobresia* in a position

basal to some members of unispicate *Carex* species and the polyphyly of the unispicate *Carex* as a lineage in three of the most parsimonious trees (Fig. 3B) and the maximum likelihood tree (Fig. 2), are consistent with Nelmes' (1952) hypothesis that some unispicate *Carex* species may be more closely related to *Kobresia* and *Uncinia* than they are to multispicate species of *Carex*. Nelmes (1952) considered the unispicate *Carex* lacking rachillae to be descendants from multispicate species, and those with rachillae derived from other genera such as *Uncinia*, *Kobresia*, or *Schoenoxiphium*. Although Reznicek (1990) suggested that the unispicate *Carex* taxa are relictual and derived from independent reductions from multispicate *Carex* ancestors, our analyses suggest that some reduction events leading to unispicate *Carex* species may have occurred before the divergence of subgenera in *Carex* and even genera in the Cariceae. Additional sampling of *Kobresia* and unispicate and other 'reduced' *Carex* (Starr et al. 1997) species may provide a more complete picture of the phylogenetic relationships between *Kobresia* and *Carex*.

The discovery of an evolutionarily derived *Uncinia* is contrary to most hypotheses on the evolution of Cariceae, in which *Uncinia*, with its well developed and hook-shaped rachillae, has been thought to be more primitive than *Carex* (Kükenthal 1909; Kreczetovicz 1936; Nelmes 1952; Koyama 1961). Savile and Calder (1953) and Smith and Faulkner (1976) hypothesized that because the hook-shaped rachillae in *Uncinia* are unique structures aiding seed dispersal, *Uncinia* may be a highly specialized and derived group.

Nelmes' (1952) hypothesis that the unispicate *Carex* species lacking rachillae are derived from within *Carex* with more complex morphology is consistent with that postulated by Kreczetovicz (1936), who regarded the unispicate taxa as derived from multispicate species, most of which had been thought to lack rachillae. In order to explain the presence of the rachilla, a putatively primitive trait, in what he considered to be the most derived taxa, but rarely in the more primitive (multispicate) taxa, Kreczetovicz (1936) hypothesized that the rachillae found in some unispicate taxa may not be homologous to the rachillae found in *Kobresia* or *Uncinia*. Our data showed that most unispicate *Carex* are likely to be closely related to *Kobresia*, most of which have well developed rachilla axes. Rachillae found in unispicate *Carex* may represent simply undeveloped axes (Timonen 1998). Anatomical studies (Snell 1936; Reznicek 1990) have shown that rach-

illae are present in a large number of both unispicate and multispicate *Carex* taxa and that many species which had been considered to lack rachillae actually have them. Taxa that have been considered to lack rachillae often have poorly developed rachillae that are aborted early in the ontogeny of the inflorescence (Schultz-Motel 1959; Timonen 1993 and refs. within). As with the homology issue, Schultz-Motel (1959) showed that the rachillae in *Carex* are likely to be homologous with those found in *Kobresia*. Additional studies by Timonen (1985, 1989, 1993) and Kukkonen and Timonen (1979) indicated that the rachillae found in *Carex*, *Uncinia*, *Kobresia*, and *Schoenoxiphium*, are likely to be "fundamentally similar."

Our analyses provide evidence for polyphyly of subgenus *Carex* as well as unispicate taxa as a lineage (subgenus *Primocarex* sensu Kükenthal). The phylogenetic position of *C. backii* (subgenus *Carex*, section *Phyllostachyae*) and *C. elynoides* (subgenus *Primocarex*, section *Filifoliae*) within the *Uncinia-Cymophyllus*-unispicate *Carex* assemblage is similar to recent findings based on nuclear ITS data (Starr et al. 1997).

The heterogeneity of the unispicate *Carex* taxa was first suggested by Kreczetovicz (1936). Although Kükenthal (1909) recognized unispicate taxa as a subgenus, ensuing workers have generally considered the recognition of unispicate taxa as a subgenus to be artificial. Past efforts to study the phylogenetic relationships of unispicate species tended to focus on the character of the rachilla. Because it has been shown that the rachilla is widespread throughout *Carex* and has little phylogenetic utility (Snell 1936; Reznicek 1990), a more effective approach would involve delimiting the phylogenetic positions of species within *Carex* s.l. based on data other than rachilla morphology, followed by a reassessment of the subgeneric level classification. This is a project that we currently are undertaking.

Our findings of *Carex dioica* and *C. scirpoidea* separate from the other unispicate species are consistent with recent thinking on the evolutionary relationships of these taxa. *Carex dioica* has similar chromosome morphology (Heilborn 1924) to, and has been shown to hybridize in nature with, members of the section *Heleonastes* in subgenus *Vignea* (Toivonen 1981). In addition, data from infection by the smut genus *Cintractia* also suggest a close relationship between *C. dioica* and subgenus *Vignea* (Savile and Calder 1953). In recent taxonomic treatments, *C. dioica* usually is placed in subgenus *Vignea* (Mackenzie 1931; Kreczetovicz 1936; Chater

1980). *Carex scirpoidea* has been considered to be more closely related to subgenus *Carex* based on cytological evidence (Heilborn 1924), as well as morphological evidence such as the absence of the rachilla, and occasional production of multiple female spikes (Nelmes 1952; Cronquist 1977). We observed three insertion/deletions in *C. scirpoidea* that are shared by all members of subgenus *Carex* in the *trnL* intron and *trnL-trnF* intergenic spacer.

We provide evidence for a sister relationship between the *Carex* subgenus *Vignea* clade and the subgenera *Carex* and *Indocarex* clade. Our finding of a monophyletic *Carex* subgenus *Vignea* is consistent with the mainstream belief that this is a homogeneous, natural subgenus. Members of subgenus *Vignea* lack the cladoprophyll and are characterized by sessile, bisexual spikelets, and two stigmas (Reznicek 1990). However, our data do not support the hypothesis that subgenus *Vignea* is derived with respect to subgenera *Carex* or *Indocarex* (summarized in Reznicek 1990), nor do they support the hypothesis that subgenus *Vignea* is ancestral to the other subgenera (Reznicek 1990).

In our analyses the monophyly of *Carex* subgenera *Carex* (excl. *C. backii*) and *Indocarex* together are well supported; however, limited sampling makes this conclusion tentative at this point. It is possible that some species traditionally placed in subgenus *Carex* may turn out not to belong to the subgenus *Carex/Indocarex* clade. This is most likely for the 'highly reduced' taxa such as those in *Carex* section *Phyllostachyae* (Starr et al. 1997). Both subgenera *Carex* and *Indocarex* contain the cladoprophyll, which is a tubular or utriculiform structure found at the base of the inflorescence (Koyama 1962; Kukkonen 1994). Some members of the two subgenera also have similar inflorescence branching patterns and perigynial characteristics (Nelmes 1951; Ohwi 1936; Koyama 1957, 1962). Ohwi (1936) and Koyama (1962), in their classifications of Asian *Carex* species, recognized only two subgenera within *Carex*: subgenus *Carex* that includes subgenus *Indocarex* and most of the unispicate species, and subgenus *Vignea*. Although Reznicek (1990) did not think there was "compelling evidence" to disperse subgenus *Indocarex* into the various sections in subgenus *Carex*, our data support Raymond's (1959) suggestion that *Carex* subgenus *Indocarex* is a heterogeneous assemblage. The classification schemes of Ohwi (1936) and Koyama (1962) may be a better depiction of the phylogenetic relationships in for the subgenus *Carex/Indocarex* clade.

Parsimony and maximum likelihood analyses re-

veal differences in the branching order for taxa in the assemblage containing *Uncinia*, *Cymophyllus*, and several unispicate *Carex* taxa. Although this clade is moderately supported by a 52% bootstrap, the phylogenetic relationships for taxa within it are not identical between the two types of analyses. Further down the tree we also observed differences in grouping between the maximum likelihood and parsimony analyses. Although we identified several moderately to well supported lineages in the Cariceae such as clades containing *Indocarex-Carex*, *Vignea*, and *Uncinia-Cymophyllus-unispicate Carex*, the phylogenetic relationships among these lineages are supported by low bootstrap values and few synapomorphic characters. Therefore, our data do not provide strong support for the interpretation of relationships among these lineages. The short internal branches and long terminal branches in our trees may be artifacts of sampling where sampling at the tips of the tree is too sparse to break up the long terminal branches. This is probably due to sampling at the generic and subgeneric levels to cover the taxonomic diversity at these levels, instead of focusing the sampling on closely related species. If these recognized groups represent ancient divisions within the genus, then a scheme to sample them representatively would be expected to yield a non-random distribution of branch lengths with short internal and long terminal branches. The weak internal support and short branches within each major clade could indicate a need for more data. Further sampling of progressively more closely related species and the addition of more data may help improve future phylogenetic inference.

In this study, the utility of *ndhF* and *trnL* sequence data in providing a phylogenetic hypothesis for a *Carex* and related genera is demonstrated. *Carex* is paraphyletic with *Uncinia*, *Cymophyllus*, and possibly *Kobresia* all derived from within it. Several well supported lineages within *Carex* are identified, interpretation of the phylogenetic patterns is provided, and an account of past phylogenetic hypotheses with respect to the new data is offered. This study provides a good framework for further investigation on the phylogenetic history and mechanisms of diversification in this complex genus.

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