Aliso: A Journal of Systematic and Floristic Botany

Volume 23 | Issue 1 Article 13

2007

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Aliso 23, pp. 165–192

PHYLOGENETIC RELATIONSHIPS IN TRIBE CARICEAE (CYPERACEAE) BASED ON NESTED ANALYSES OF FOUR MOLECULAR DATA SETS

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ABSTRACT

Phylogenetic reconstruction for *Carex* and relatives in tribe Cariceae is complicated by species richness and nearly cosmopolitan distribution. In this investigation, our main objective was to estimate evolutionary relationships in tribe Cariceae using DNA sequence data from two spacer regions in nuclear ribosomal genes (ITS and ETS-1f) combined with noncoding chloroplast DNA (*trn*L intron, *trn*L–*trn*F intergenic spacer, and *trn*E–*trn*D intergenic spacers). Parsimony analyses of separate and combined data and Bayesian analysis of the combined data matrix revealed strong support for monophyly of tribe Cariceae and for monophyly of two major lineages, one comprising principally *Carex* subgen. *Carex* and *Vigneastra,* and the other representing subgen. *Vignea.* A third clade with representatives from *Kobresia* and *Uncinia,* along with *Cymophyllus fraserianus, Carex curvula,* and several unispicate *Carex* received weak-to-moderate support. A small clade comprising *Schoenoxiphium* and two unispicate carices was placed as sister to the clades comprising multispicate *Carex* species in the parsimony analysis, but sister to the clade of *Kobresia, Uncinia,* and unispicate *Carex* in the Bayesian analysis. Two large widespread groups within subgen. *Carex,* sect. *Hymenochlaenae* and sect. *Physocarpae* s.l. (''bladder sedges''), were highly polyphyletic, while ten clades that grouped species from two or more sections were each strongly supported as monophyletic. Within subgen. *Vignea,* three sections were strongly supported as monophyletic while sects. *Phaestoglochin* and *Vulpinae* were polyphyletic. Adding the variable ETS-1f region improved resolution and bootstrap support values over previous studies, but many of the characters supporting major branches came from the *trn*L region.

Key words: *Carex,* Cariceae, Cyperaceae, external transcribed spacer, internal transcribed spacer, *Kobresia,* phylogeny, *Schoenoxiphium, trn*L intron, *Uncinia.*

INTRODUCTION

Species-rich genera like *Carex* (Cyperaceae), which occupy a wide variety of habitats across a broad geographical distribution (Ball 1990; Catling et al. 1990), provide unparalleled opportunities to test ecological and evolutionary hypotheses related to adaptive radiation, diversification rates, niche differentiation, and the relative roles of stochastic vs. adaptive processes in community assembly (cf. Losos 1996; Sanderson and Wojciechowski 1996; Warheit et al. 1999; Losos and Miles 2002; Webb et al. 2002; Ackerly 2003). A prerequisite to such studies is a robust hypothesis of phylogenetic relationships that leads to a stable and predictable classification system (Silvertown et al. 2001).

The same features that make *Carex* so appealing for ecological studies within a phylogenetic framework also make it difficult to produce the necessary robust phylogeny. With the exception of Kükenthal's (1909) global monograph, floristic treatments of the genus are generally regional in scope (e.g., Mackenzie 1931–35; Ohwi 1936; Nelmes 1951; Koyama 1962; Chater 1980; Egorova 1999; Kukkonen 2001; Ball and Reznicek 2002) and recent published systematic studies are usually restricted to regional treatments within single sections or species complexes (e.g., Reznicek and Ball 1980; Standley 1985; Reznicek 1986; Crins and Ball 1989; Dunlop and Crow 1999) even though many sections are circumscribed to include species from more than one continent. Although Kükenthal presented the 793 species he recognized within a phylogenetic framework, the basis for this framework has been frequently called into question (Kreczetovich 1936; Nelmes 1952; Egorova 1999), resulting in inconsistent infrageneric classification and nomenclature.

It has been clear from the earliest attempts at phylogenetic analysis of *Carex* using DNA sequence comparisons (Starr et al. 1999; Yen and Olmstead 2000*a*, *b*) that the genus *Carex* could not be considered in isolation. While the monophyly of tribe Cariceae has long been recognized and has been supported by recent molecular analyses of Cyperaceae (Muasya et al. 1998, 2000), the monophyly of all currently recognized genera within the tribe (except monotypic *Cymophyllus*) has been questioned (Kreczetovicz 1936; Nelmes 1952; Hamlin 1959; Reznicek 1990). The variety of geographic patterns in genera of tribe Cariceae, from narrow endemics to species that can be found on four continents, increases the difficulty of appropriate sampling but adds interesting biogeographic questions to those that require a robust phylogeny to be answered.

Previous attempts to reconstruct the phylogeny of Cariceae using molecular data have concentrated on either single sections of *Carex* and their potential relatives (*Limosae,* Wa-

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terway et al. 1997; *Phyllostachyae,* Starr et al. 1999; *Acrocystis,* Roalson et al. 2001; Roalson and Friar 2004) or genera (*Uncinia,* Starr et al. 2003, in press) or have focused on the relationship of *Carex* to other genera of Cariceae. There has been a particular emphasis on subgen. *Psyllophora,* the small and controversial group of unispicate *Carex* species that Kükenthal (1909) treated as subgen. *Primocarex,* reflecting his view that they were primitive within the genus (Starr et al. 2003, 2004, in press).

None of these studies have used more than two different gene regions and, with the exception of recent work by Starr et al. (2003, 2004, in press), resolution has been poor and few clades have had significant statistical support. Different researchers have used different genes or combinations of genes for different sets of species. Yen and Olmstead (2000*a*, *b*) used one coding and one noncoding chloroplast DNA region (*ndh*F and *trn*L–*trn*F region including the *trn*L intron) but found that variability in *ndh*F was too low within Cariceae to allow much resolution. Starr et al. (1999) initially used only ITS data in a small study of sect. *Phyllostachyae* and potential relatives, but Starr et al. (2003) recently developed primers for part of the external transcribed spacer (ETS-1f) of the ribosomal gene repeat and found levels of variability high enough to give good resolution when combined with ITS data in their studies of *Uncinia* and *Carex* subgen. *Psyllophora* (Starr et al. 2004, in press). Roalson et al. (2001) combined ITS data with the noncoding chloroplast *trn*T–*trn*L–*trn*F region in their study of *Carex* sect. *Acrocystis* and a diverse array of other species of Cariceae (100 in total), but had low resolution and poor support for most clades. Using ITS data only, Heindrichs et al. (2004*a*, *b*) used Bayesian and distance methods to estimate phylogenetic trees for several species of European *Carex,* noting that parsimony analysis gave poor resolution and statistical support. Combining data from ITS, ETS-1f, and one or more of the noncoding chloroplast DNA regions has the potential to provide better resolution and increase statistical support for both interior branches and terminal clades. However, with previous studies using different sets of species and different DNA regions, sequences from more than two different DNA regions are currently available in GenBank for only a few species of Cariceae.

The objective of this investigation is to propose a phylogenetic hypothesis for Cariceae that is based on both ITS and ETS-1f sequences along with at least one chloroplast DNA region using a sampling that is approximately proportional to the relative number of species of each genus or subgenus within Cariceae. The analyses presented here are the first to be based on more than two different DNA data sets and incorporate the broadest sampling to date from among *Carex* subgen. *Carex* and *Vignea,* which together comprise almost 90% of Cariceae. Our sampling is also designed to test the monophyly of some commonly recognized sections or groups of sections within subgen. *Carex* and to evaluate the relative molecular divergence for each gene region among species thought to be closely related based on morphology. We also compare the results of model-based Bayesian analysis to parsimony analysis of these data.

MATERIALS AND METHODS

Source of Material and Choice of Taxa

The sequence data were assembled to represent a diverse range of taxa within tribe Cariceae, while including at least three species of each genus (except monotypic *Cymophyllus*), at least five from each subgenus of *Carex,* and at least two from each of several sections within subgen. *Carex* and *Vignea* (Table 1). Within these constraints, sampling of each major group was roughly proportional to its frequency within tribe Cariceae, with three species of *Schoenoxiphium* (ca. 20 spp.), three of *Uncinia* (ca. 65 spp.), six of *Kobresia* (ca. 79 spp.), ten of *Carex* subgen. *Primocarex* sensu Kükenthal (ca. 70 spp.), five of *Carex* subgen. *Vigneastra* (= subgen. *Indocarex* sensu Kükenthal, ca. 100 spp.), 29 of *Carex* subgen. *Vignea* (ca. 450 spp.), 60 of *Carex* subgen. *Carex* (ca. 1400 spp.), and *Cymophyllus fraserianus* (Table 1). We also sampled ten pairs of taxa assumed to be quite closely related based on morphology to allow comparison of relative sequence divergence at this level. Within subgen. *Carex,* we sampled several species from sect. *Hymenochlaenae* (sensu Reznicek 1986) and from a group of apparently closely related sections, collectively known as the ''bladder sedges,'' to test the monophyly of these groups. The sample for subgen. *Carex* and *Vignea* is over-weighted in North American species for logistic reasons but still represents a wide range of structural diversity within these subgenera.

Extraction, Amplification, and Sequencing

For new sequences reported in this paper, a modified 2% CTAB protocol (Doyle and Doyle 1987) was used to extract DNA from fresh (95% of samples) or silica-dried leaf material. Fresh samples were ground directly in CTAB buffer, while dried leaf tissue was ground in liquid nitrogen to which hot CTAB buffer was added immediately after grinding. Amplifications for ITS and ETS-1f followed the protocols given in Starr et al. (1999, 2003) except that 10% DMSO (replacing betaine) was used in the reaction to avoid amplification of divergent paralogues (Buckler et al. 1997). Amplification primers for ETS-1f were those of Starr et al. (2003) and for ITS were ITS N18L18 (Yokota et al. 1989) and ITS-4 (White et al. 1990). The *trn*L–*trn*F region was amplified using primers ''c'' and ''f'' of Taberlet et al. (1991) under reaction conditions given by Yen and Olmstead (2000*a*). Primers designed by Alan Yen (University of Washington, Seattle, USA) were used to amplify the *trn*E–*trn*D region of chloroplast DNA, which includes the *trn*E–*trn*Y intergenic spacer, the tRNA-tyrosine (*trn*Y) gene (84 base pairs [bp]), and the *trn*Y–*trn*D intergenic spacer. The forward primer was NE: 5′-CACCTCTCTTTTCAAGGAGGCA-3′ while the reverse primer was ND: 5′-CGCAGCTTCCGCCTTGACAG-3′. Reaction conditions for amplifying the *trn*E–*trn*D region were identical to those for the *trn*L–*trn*F region. Three to five microliters of each reaction product were electrophoresed on 1.2% agarose gels to verify amplification of single fragments. The amplification reaction products were then purified using QiaQuick™ PCR purification columns (QIAGEN, Inc., Valencia, California, USA) and quantified by spectrophotometry prior to use in the sequencing reactions. DyenamicET™ (Amersham Biosciences, Inc., Piscataway, New Jersey, USA) was used for

Table 1. Classification and accession data for voucher specimens for DNA sequences used in this study. Species classified in tribe Cariceae are arranged alphabetically within generic, subgeneric, and sectional groups, followed by the three outgroup species in alphabetical order. Generic delimitation follows Kükenthal (1909) and Ball et al. (2002), while subgenera follow the circumscriptions of Kükenthal (1909) and Zhang (2001), except where modified by Egorova (1999). Sectional placement follows Ball et al. (2002) for North American species, Wheeler (1989*a, b*) for South American species, Egorova (1999) for Eurasian species, Dai and Liang (2000) for East Asian species, and Zhang (2001) for *Kobresia.* GenBank numbers representing sequences from Starr et al. (1999, 2003, 2004, in press) or from Yen and Olmstead (2000*a, b*) or Roalson et al. (2001) are given in parentheses. Locality, collector with number, and herbarium acronym (Holmgren et al. 1990) are reported for all new DNA sequences. For new sequences, the GenBank accession numbers are ordered as ITS, ETS-1f, *trn*L–*trn*F, and *trn*E–*trn*D (where applicable). Individuals of the same species, sampled from different localities are numbered (1) and (2).

Taxon	Source/voucher	GenBank numbers
Carex L.		
subgen. *Carex*		
sect. *Abditispicae* G. A. Wheeler		
C. collumanthus (Steyerm.) Mora	COLOMBIA: Arauca, Sierra Nevada del Cocuy, *Cleef 8875* (NY)	(AY241987, AY241988) AY757548
sect. *Acrocystis* Dumort.		
C. pensylvanica Lam.	CANADA: Quebec, Mont St. Hilaire, *Waterway 99.013* (MTMG)	AY757622, AY57682, AY57550
sect. *Anomalae* J. Carey		
C. amplifolia Boott	USA: California, Plumas Co., *Waterway 99.076* (MTMG)	AY757584, AY75645, AY757511
C. scabrata Schwein.	CANADA: Quebec, Mont St. Hilaire, *Waterway 99.001* (MTMG)	AY757585, AY757646, AY757512
sect. *Aulocystis* Dumort.		
C. fissuricola Mack.	USA: California, Mono Co., *Waterway 2000.156* (MTMG)	AY757617, AY757678, AY757544
C. luzulifolia W. Boott	USA: California, Lassen Co., *Waterway 2000.164* (MTMG)	AY757618, AY757679, AY757545
sect. *Carex*		
C. trichocarpa Willd.	USA: Virginia, Montgomery Co., *Waterway 2000.092* (MTMG)	AY757570, AY757632, AY757497
sect. *Careyanae* Kük.		
C. plantaginea Lam.	CANADA: Quebec, Hudson, *Waterway 2000.002* (MTMG)	AY757613, AY757674, AY757540
sect. *Ceratocystis* Dumort.		
C. flava L.	CANADA: Quebec, Mont Rigaud, *Waterway 2001.086* (MTMG)	AY757596, AY757657, AY757523
C. viridula Michx.	CANADA: Quebec, Bristol, *Waterway 99.082* (MTMG)	AY757597, AY757658, AY757524
sect. *Clandestinae* G. Don		
C. digitata L.	UK: Lancashire, Silverdale, Eaves Wood, *Blackstock s. n.* (MTMG)	AY757624, AY757684, AY757552
C. pedunculata Willd.	CANADA: Quebec, Hudson, *Waterway 2000.001* (MTMG)	AY757623, AY757683, AY757551
sect. *Collinsiae* (Mack.) Mack.		
C. collinsii Nutt.	USA: New Jersey: Bass River State Forest, *Waterway 98.086* (MTMG)	AY757616, AY757677, AY757543
sect. *Depauperatae* Meinsh.		
C. depauperata Curtis ex With.	(1) UK: ex hort Godalming, Surrey, UK garden, ex hort Edge Hill, *Phillips s. n.* (MTMG)	AY757621, AY757549
	(2) UK: *Rich 01* (OXF)	(AY241985)
sect. *Griseae* (L. H. Bailey) Kük.		
C. hitchcockiana Dewey	CANADA: Quebec, Ile Perrot, *Waterway 2001.082* (MTMG)	AY757614, AY757675, AY757541
C. oligocarpa Willd.	USA: Illinois, Union Co., *Waterway 98.030* (MTMG)	AY757615, AY757676, AY757542
sect. *Hallerianae* (Asch. & Graebn.) Rouy		
C. tenax Dewey	USA: Louisiana, Natchitoches Co., *Hyatt 10401* (MTMG)	AY757610, AY757671, AY757537
sect. *Hirtifoliae* Reznicek		
C. hirtifolia Mack.	USA: Illinois, Union Co., *Waterway 98.029* (MTMG)	AY757611, AY757672, AY757538

Table 1. Continued.

Taxon	Source/voucher	GenBank numbers
sect. *Hymenochlaenae* (Drejer) L. H. Bailey		
C. cherokeensis Schwein.	USA: Florida, Holmes Co., *Waterway 2000.004* (MTMG)	AY757619, AY757680, AY757546
C. debilis Michx.	USA: Florida, Holmes Co., *Waterway 2002.043* (MTMG)	AY757608, AY757669, AY757535
C. mendocinensis W. Boott	USA: California, Mendocino Co., *Waterway 99.036* (MTMG)	AY757609, AY757670, AY757536
C. misera Buckley	USA: North Carolina, Jackson Co., *Waterway 2002.021* (MTMG)	AY757607, AY757668, AY757534
C. obispoensis Stacey	USA: California, San Luis Obispo Co., *Roalson 1413* (RSA)	AY757620, AY757681, AY757547
C. prasina Wahlenb.	USA: Virginia, Rockbridge Co., *Waterway 2000.080* (MTMG)	AY757593, AY757654, AY757520
C. sylvatica Huds.	SWITZERLAND: forest near Basal, *Lechowicz s. n.* (MTMG)	AY757599, AY757660, AY757526
sect. *Lamprochlaenae* (Drejer) L. H. Bailey		
C. glacialis Mack.	CANADA: Quebec, 25 km N of Schefferville, *Waterway 2001.114* (MTMG)	AY757625, AY757685, AY757553
sect. *Laxiflorae* (Kunth) Mack.		
C. albursina E. Sheld.	USA: Illinois, Jackson Co., *Waterway 98.050* (MTMG)	AY757626, AY757686, AY757554
C. blanda Dewey	USA: Illinois, Jackson Co., *Waterway 98.044* (MTMG)	AY757627, AY757687, AY757555
sect. *Limosae* (Heuff.) Meinsh.		
C. limosa L.	CANADA: Quebec, Chisasibi, *Novo 1.15* (MTMG)	AY757595, AY757656, AY757522
C. magellanica Lam. subsp. *irrigua* (Wahlenb.) Hiitonen	CANADA: Quebec, Schefferville region, *Waterway 97.090* (MTMG)	AY757594, AY757655, AY757521
sect. *Longicaules* Mack. ex Reznicek		
C. whitneyi Olney	USA: California, Shasta Co., *Waterway 99.065* (MTMG)	AY757605, AY757666, AY757532
sect. *Lupulinae* J. Carey		
C. grayi J. Carey	USA: Illinois, Jackson Co., *Waterway 98.036* (MTMG)	AY757580, AY757642, AY757507
C. intumescens Rudge	USA: South Carolina, Monticello, *Waterway 2000.014* (MTMG)	AY757579,AY757641, AY757506
C. lupulina Willd.	CANADA: Quebec, Hull, Lac Leamy, *Waterway 97.127* (MTMG)	AY757576, AY757638, AY757503
sect. *Paludosae* G. Don		
C. acutiformis Ehrh.	UK: ex hort Edge Hill, source: Lancashire, Silverdale, *Blackstock s. n.* (MTMG)	AY757583, AY757644, AY757510
C. riparia Curtis	UK: Warwickshire, Kenilworth Castle, *Blackstock s. n.* (MTMG)	AY757571, AY757633, AY757498
C. vestita Willd.	USA: New Hampshire, Rockingham Co., *Waterway 2001.088* (MTMG)	AY757581, AY757643, AY757508
sect. *Paniceae* G. Don		
C. livida (Wahlenb.) Willd.	USA: New Jersey, Burlington Co., *Waterway 98.078* (MTMG)	AY757628, AY757688, AY757556
C. vaginata Tausch	CANADA: Labrador, ca. 12 km E of Schefferville, *Waterway 97.085* (MTMG)	AY757629, AY757689, AY757557
sect. *Phacocystis* Dumort.		
C. aquatilis Lam.	CANADA: Quebec, Lac St. François, *Bérubé 99.009* (MTMG)	AY757590, AY757651, AY757517
C. crinita Wahlenb.	CANADA: Quebec, Vaudreuil, *Waterway 99.002* (MTMG)	AY757589, AY757650, AY757516
sect. *Phyllostachyae* Tuck. ex Kük.		
C. backii Boot	CANADA: Quebec, Mont St. Hilaire, *Waterway 98.003* (MTMG)	AY757402, AY757398, AY757494, AY757462
sect. *Porocystis* Dumort.		
C. complanata Torr. & Hook.	USA: South Carolina, Lancaster Co., *Waterway 2000.010* (MTMG)	AY757604, AY757665, AY757531

Table 1. Continued.

Taxon	Source/voucher	GenBank numbers
C. pallescens L.	CANADA: Quebec, Lac Memphremagog, *Bérubé 99.019* (MTMG)	AY757612, AY757673, AY757539
C. swanii (Fernald) Mack.	(1) USA: Illinois, Pope Co., *Waterway 98.024* (MTMG)	AY757603, AY757530
	(2) USA: Virginia, Floyd Co., *Waterway 2000.134* (MTMG)	AY757664
C. virescens Willd.	USA: Illinois, Saline Co., *Waterway 98.012* (MTMG)	AY757606, AY757667, AY757533
sect. *Racemosae* G. Don		
C. mertensii J. D. Prescott	USA: Washington, Chelan Co., *Waterway 97.054* (MTMG)	AY757592, AY757653, AY757519
C. stylosa C. A. Mey.	CANADA: Quebec, Schefferville region, *Waterway 97.095* (MTMG)	AY757591, AY757652, AY757518
sect. *Rhynchocystis* Dumort.		
C. pendula Huds.	UK: Devon, Slapton Ley Field Centre, *Watson-Jones s. n.* (MTMG)	AY757600, AY757661, AY757527
sect. *Rostrales* Meinsh.		
C. folliculata L.	USA: New Jersey, Burlington Co., *Waterway 98.094* (MTMG)	AY757601, AY757662, AY757528
C. michauxiana Boeck.	CANADA: Quebec, Mont Tremblant, *Gold & Pushkar 22* (MTMG)	AY757602, AY757663, AY757529
sect. *Scirpinae* (Tuck.) Kük.		
C. scirpoidea Michx.	(1) CANADA: Alberta, Jasper National Park, *Bayer AB-96010 et al.* (WIN)	(AY241991)
	(2) CANADA: Quebec, 25 km N of Schefferville, *Waterway 2001.113* (MTMG)	AY757582, AY757509
sect. *Shortianae* (L. H. Bailey) Mack.		
C. shortiana Dewey	USA: Illinois, Pope, Co., *Waterway 98.023* (MTMG)	AY757586, AY757647, AY757513
sect. *Spirostachyae* (Drejer) L. H. Bailey		
C. punctata Gaudin	UK: ex hort Sandy Hills Bay, Dumfries & Galloway, ex hort Edge Hill, *Smith s. n.* (MTMG)	AY757598, AY757659, AY757525
sect. *Squarrosae* J. Carey		
C. squarrosa L.	USA: Illinois, Pope Co., *Waterway 98.020* (MTMG)	AY757587, AY757648, AY757514
C. typhina Michx.	USA: South Carolina, Manchester State Forest, *Waterway 2000.016* (MTMG)	AY757588, AY757649, AY757515
sect. *Vesicariae* (Heuff.) J. Carey		
C. comosa Boott	CANADA: Quebec, Iberville, St. Armand, *Bérubé 99.035* (MTMG)	AY757575, AY757637, AY757502
C. hystericina Willd.	USA: Virginia: Montgomery Co., *Waterway 2000.096* (MTMG)	AY757574, AY757636, AY757501
C. oligosperma Michx.	CANADA: Quebec, Schefferville region, *Waterway 2002.091* (MTMG)	AY757578, AY757640, AY757505
C. retrorsa Schwein.	CANADA: Quebec, Hull, Lac Leamy, *Waterway 97.125* (MTMG)	AY757577, AY757639, AY757504
C. schweinitzii Dewey	USA: Virginia, Montgomery Co., *Waterway 2000.101* (MTMG)	AY757572, AY757634, AY757499
C. tuckermanii Dewey	CANADA: Quebec, Hull, Lac Leamy, *Waterway 97.128* (MTMG)	AY757573, AY757635, AY757500
subgen. *Psyllophora* (Degl.) Peterm. (= subgen. *Primocarex* Kük.)		
sect. *Aciculares* (Kük.) G. A. Wheeler		
C. acicularis Boott	NEW ZEALAND: Fiordland, Southland Land District, *Ford 113/98* (FHO)	(AY242012, AY242013) AY757562
sect. *Dornera* Heuff.		
C. nigricans C. A. Mey.	(1) CANADA: British Columbia, Mount Revelstoke National Park, *Ford 9720* (WIN)	(AY242042, AY242043)
	(2) USA: Washington, *Yen 0126* (WTU)	(AF164929)

Table 1. Continued.

Taxon	Source/voucher	GenBank numbers
sect. *Leptocephalae* L. H. Bailey		
C. leptalea Wahlenb.	USA: Maine, Oxford Co., *Waterway 20001.099* (MTMG)	AY757630, AY757690, AY757559
sect. *Leucoglochin* Dumort.		
C. parva Nees	(1) CHINA: Yunnan, Diqing Prefecture, *Aldén et al. s. n., K.E.G. No. 1252* (E)	(AY244523, AY244524)
	(2) CHINA: *K.E.G. 174* (E)	AF164935
C. pauciflora Lightf.	CANADA: Quebec, 20 km N of Schefferville, *Waterway 2002.098* (MTMG)	AY757631, AY757691, AY757569
sect. *Obtusatae* (Tuck.) Mack.		
C. obtusata Lilj.	(1) CANADA: Manitoba, Portage Sand Hills, *Ford 9601 et al.* (WIN)	(AY241981, AY241982)
	(2) USA: Washington, *Glew 96-0715PL* (WTU)	(AF164298)
sect. *Psyllophora* (Degl.) Koch		
C. pulicaris L.	UK: Yorkshire Dales National Park, *Starr 98001 & Scott* (FHO)	(AY242018, AY242019) AY757563
sect. *Rupestres* (Tuck.) Meinsch.		
C. rupestris All.	(1) FRANC: Col de Galibier, *Playford 9801* (FHO)	(AY244521, AY244522)
	(2) USA: Colorado, *Yen 0133* (WTU)	(AF164934)
subgen. *Vignea* (P. Bauv. ex Lestib. f.) Peterm.		
sect. *Chordorrhizae* (Heuff.) Meinsh.		
C. chordorrhiza Ehrh. ex L. f.	CANADA: Quebec, Schefferville region, *Waterway 2001.107* (MTMG)	AY757409, AY757389, AY757485, AY757453
sect. *Curvulae* Tuck. ex Kük.		
C. curvula All.	FRANCE: Col du Galibier, *Playford 9803 et al.* (FHO)	(AY242030, AY242031) AY757564
sect. *Deweyanae* (Tuck. ex Mack.) Mack.		
C. bromoides Schkuhr ex Willd.	CANADA: Quebec, Mont St. Hilaire, *Waterway 98.004* (MTMG)	AY757404, AY757378, AY757474, AY757442
C. deweyana Schwein.	CANADA: Quebec, Mont St. Hilaire, *Waterway 98.005* (MTMG)	AY757412, AY757379, AY757475, AY757443
sect. *Dispermae* Ohwi		
C. disperma Dewey	CANADA: Quebec, Mont Tremblant, *Bond s. n.* (MTMG)	AY757414, AY757393, AY757489, AY757457
sect. *Foetidae* (Tuck. ex L. H. Bailey) Kük.		
C. maritima Gunnerus	CANADA: Yukon, Kluane Lake, *Waterway 96.098* (MTMG)	AY757421, AY757397, AY757493, AY757461
sect. *Glareosae* G. Don		
C. brunnescens (Pers.) Poir.	USA: Maine, Oxford Co., *Waterway 2001.100* (MTMG)	AY757405, AY757385, AY757481, AY757449
C. canescens L.	CANADA: Quebec, Mont Tremblant, *Bond s. n.* (MTMG)	AY757406, AY757384, AY757480, AY757448
C. heleonastes L. f.	CANADA: Quebec, Schefferville region, *Waterway 97.078* (MTMG)	AY757418, AY757388, AY757484, AY757452
C. tenuiflora Wahlenb.	CANADA: Quebec, Schefferville region, *Waterway 97.079* (MTMG)	AY757427, AY757386, AY757482, AY757450
C. trisperma Dewey	CANADA: Quebec, Duhamel near Lac Doré, *Bérubé 99.032* (MTMG)	AY757429, AY757387, AY757483, AY757451
sect. *Heleoglochin* Dumort.		
C. decomposita Muhl.	USA: South Carolina, Manchester State Forest, *Waterway 2000.011* (MTMG)	AY757411, AY757376, AY757472, AY757440
C. diandra Schrank	JAPAN: Hokkaido, Kushiro Mire, *Waterway 4009* (MTMG)	AY757413, AY757377, AY757473, AY757441
sect. *Multiflorae* (J. Carey) Kük.		
C. vulpinoidea Michx.	USA: California, Mendocino Co., *Waterway 99.033* (MTMG)	AY757430, AY757372, AY757468, AY757436
sect. *Ovales* Kunth		
C. bicknellii Britton	CANADA: Quebec, cultivated in greenhouse, *Waterway s. n.* (MTMG)	AY757403, AY757392, AY757488, AY757456
C. projecta Mack.	USA: Maine, Oxford Co., *Waterway 2001.097* (MTMG)	AY757423, AY757391, AY757487, AY757455

Table 1. Continued.

Taxon	Source/voucher	GenBank numbers
C. tribuloides Wahlenb.	CANADA: Quebec, St. Lazare, *Waterway 2001.143* (MTMG)	AY757428, AY757390, AY757486, AY757454
sect. *Phaestoglochin* Dumort.		
C. appalachica J. M. Webber & P. W. Ball	CANADA: Quebec, Mont St. Hilaire, *Bérubé 99.008* (MTMG)	AY757401, AY757394, AY757490, AY757458
C. cephaloidea (Dewey) Dewey	USA: Maine, Essex Co., *Waterway 2001.087* (MTMG)	AY757407, AY757369, AY757465, AY757433
C. cephalophora Muhl. ex Willd.	USA: Maine, Essex Co., *Waterway 2001.092* (MTMG)	AY757408, AY757370, AY757466, AY757434
C. muehlenbergii Schkuhr ex Willd.	USA: Arkansas, Izard Co., *Waterway 2001.023* (MTMG)	AY757422, AY757371, AY757467, AY757435
C. radiata (Wahlenb.) Small	CANADA: Quebec, Baie d'Urfé, Montreal, *Waterway 99.006* (MTMG)	AY757424, AY757396, AY757492, AY757460
C. rosea Schkuhr ex Willd.	CANADA: Quebec, Mont St. Hilaire, *Lechowicz s. n.* (MTMG)	AY757425, AY757395, AY757491, AY757459
sect. *Physoglochin* Dumort.		
C. gynocrates Wormsk. ex Drejer	CANADA: Quebec, Schefferville region, *Dabros s. n.* (MTMG)	AY757417, AY757383, AY757479, AY757447
sect. *Stellulatae* Kunth		
C. echinata Murray	USA: Maine, Oxford Co., *Waterway 2001.101* (MTMG)	AY757415, AY757381, AY757477, AY757445
C. exilis Dewey	CANADA: Quebec, Schefferville region, *Waterway 2001.103* (MTMG)	AY757416, AY757382, AY757478, AY757446
C. interior L. H. Bailey	CANADA: Quebec, Schefferville region, *Waterway 97.087* (MTMG)	AY757419, AY757380, AY757476, AY757444
sect. *Vulpinae* (Heuff.) H. Christ		
C. crus-corvi Shuttlew.	USA: Illinois, Jackson Co., *Waterway 98.037* (MTMG)	AY757410, AY757373, AY757469, AY757437
C. laevivaginata (Kük.) Mack.	USA: Virginia, *Waterway 2000.082* (MTMG)	AY757420, AY757374, AY757470, AY757438
C. stipata Muhl. ex Willd.	USA: California, Plumas Co., *Waterway 99.072* (MTMG)	AY757426, AY757375, AY757471, AY757439
subgen. *Vigneastra* (Tuck.) Kük. (= subgen. *Indocarex* (Baill.) Kük.)		
sect. *Baccantes* (T. Koyama) P. C. Li		
C. baccans Nees	TAIWAN: Wu Lai, Taipei, *Yen 078* (WTU)	(AF027449, AF027488, AY241994, AF191814)
sect. *Indicae* Tuck.		
C. cruciata Wahlenb.	MALAYSIA: Mulu National Park, Sarawak, *Yen 075* (WTU)	(AF027450, AF027489, AY241995) AY757558
C. filicina Nees	(1) TAIWAN: Yang Ming Shan National Park, Da Tun Shan, *Yen 076* (WTU)	(AY241996, AY241997)
	(2) CHINA: Sichuan, *Boufford & Bartholomew 24364* (RSA)	(AF284879)
C. echinochloe Kunze	KENYA: *Muasya 1051* (K)	(AY241992, AY241993, AF191818)
sect. *Polystachyae* Tuck.		
C. polystachya Sw.	(1) BELIZE: Cayo District, *Jones 11275 & Wipff* (MICH)	(AF027448, AF027487, AY241998)
	(2) BRAZIL: Federal District, *Irwin et al. 26503* (RSA)	(AF284912)
Cymophyllus Mack.		
C. fraserianus (Ker Gawl.) Kartesz & Gandhi	(1) USA: Tennessee, Blount Co., along road to Cades Cove, *Sharp s. n.* (cultivated at K), *Starr 98024* ex RBG Kew (FHO)	(AY241969, AY241970)
	(2) USA: Tennessee, Carter Co., *Waterway 2000.113* (MTMG)	AY757431, AY757399, AY757495, AY757463
Kobresia Willd.		
subgen. *Compositae* (C. B. Clarke) Kukkonen		
K. laxa Nees	(1) INDIA: Sikkim, North District, *Long & Noltie s. n., E.E.N.S. No. 211* (E)	(AY241975, AY241976)
	(2) INDIA: *McBeach 2325* (E)	(AF164943)

Table 1. Continued.

Taxon	Source/voucher	GenBank numbers
subgen. *Kobresia*		
sect. *Hemicarex* (Benth.) C. B. Clarke		
K. esenbeckii (Kunth) Noltie	INDIA: Sikkim, West District, Bikbari, *Long et al. s. n., E.S.I.K. No. 335* (E)	(AY242032, AY242033) AY757565
K. nepalensis (Nees) Kük.	INDIA: Sikkim, North District, *Long & Noltie, E.E.N.S. No. 291* (E)	(AY242034, AY242035) AY757567
sect. *Kobresia*		
K. myosuroides (Vill.) Fiori	(1) FRANCE: Col du Galibier, *Playford 9804 et al.* (FHO)	(AY242036, AY242037) AY757566
	(2) USSR: Siberia, *Elias et al. 7337* (RSA)	(AF284985)
K. schoenoides (C. A. Mey.) Steud.	INDIA: Sikkim, West District, Chhoptha, *E.S.I.K. No. 647* (E)	(AY242038, AY242039) AY757568
K. simpliciuscula (Wahlenb.) Mack.	(1) CANADA: British Columbia, Yoho National Park, *Ford 9710* (FHO)	(AY241971, AY241972)
	(2) USA: Colorado, *Weber & Cooper 1803* (COLO)	(AF164948)
Schoenoxiphium Nees		
S. filiforme Kük.	(1) SOUTH AFRICA: Eastern Cape, Drakensbergs, *Phillipson 666* (PRE)	(AY242020, AY242021)
	(2) SOUTH AFRICA: *Browning 699* (NU)	(AF164951)
S. lehmannii (Nees) Steud.	SOUTH AFRICA: Natal Province, Ngoye Forest Reserve, *Williams 1007* (PRE)	(AY242026, AY242027) AY757560
S. sparteum (Wahlenb.) C. B. Clarke	SOUTH AFRICA: Orange Free State, Ladybrand, *de Lange FA 57* (PRE)	(AY242022, AY242023), AY757561
Uncinia Pers.		
subgen. *Eu-Uncinia* Kük.		
sect. *Platyandrae* C. B. Clarke		
U. phleoides (Cav.) Pers.	(1) CHILE: Isla Grande de Chiloé, P. N. de Chiloé, *Vann 9801* (FHO)	(AY012670, AY012671)
	(2) CHILE: Hortus Botanicus Valdivensis (WTU)	(AF164931)
U. uncinata Kük.	(1) NEW ZEALAND: North Island, Auckland Ecological Region, *de Lange s. n., AK226837* (AK)	(AY242054, AY244543)
	(2) USA: Hawaii, *Millam s. n.* (WTU)	(AF164932)
U. brevicaulis Thouars	(1) ST. HELENA: Tristan da Cunha, Inaccessible Island, *Christophersen 2473* (BM)	(AY244533)
	(2) ST. HELENA: Tristan da Cunha, above Burntwood, *Dickson 25* (AAS)	(AY244534)
	(3) CHILE: San Fernandez Island, *Solbrig 3647* (RSA)	(AF284885)
	Outgroups	
Eriophorum vaginatum L.	(1) UK: *Starr 98007 & Scott* (FHO)	(AY242008, AY242009)
	(2) CANADA: Quebec, Schefferville region, *Waterway 2002.094* (MTMG)	AY757692
Scirpus polystachyus F. Muell.	AUSTRALIA: New South Wales, *Wilson s. n. (MWC 5927)* (K)	(AY242010, AY242011)
	AUSTRALIA: *Pullen 4091* (K)	(AJ295813)
Trichophorum alpinum (L.) Pers.	CANADA: Quebec, Schefferville region, *Waterway 2001.110* (MTMG)	AY757432, AY757400, AY757496, AY757464

most ITS and all ETS-1f sequencing reactions. Either the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase FS (Perkin-Elmer Applied Biosystems, Wellesley, Massachusetts, USA) or the ABI PRISM Big Dye™ Cycle Sequencing Kits, vers. 1.0, 1.1, 2.0, or 3.1 (Applied Biosystems, Foster City, California, USA) were used for the chloroplast DNA (cpDNA) sequencing reactions and some ITS sequencing. The double-stranded amplification products were sequenced in both directions using the amplification primers and internal primers ''d'' and ''e'' (Taberlet et al. 1991) for the *trn*L–*trn*F region, using the ITS amplification primers and internal primers ITS-2 and ITS-3 (White et al. 1990) for ITS, and using amplification primers only for ETS-1f. Dye terminators were removed us-

ing the ethanol precipitation methods recommended by the manufacturers. Sequencing reactions were run on ABI 373, ABI 377, or ABI 310 automated DNA sequencers (Applied Biosystems). Sequenced fragments were edited and assembled using Sequencher™ vers. 3.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA). All sequences reported for the first time in this paper (ITS, ETS-1f, *trn*L–*trn*F region, and *trn*E–*trn*D region for 93, 91, 104, and 31 species of Cariceae, respectively, plus all four gene regions for *Trichophorum alpinum* and only *trn*L–*trn*F region for *Eriophorum vaginatum*) are deposited in GenBank (Table 1). Sequence data from GenBank was used for 15 species for the *trn*L–*trn*F region and for the previously published ETS-1f and ITS sequences of Starr et al. (1999, 2003, 2004, in press) (Table 1).

Data Analysis

Alignment and indel coding.—Sequences were assembled into two data matrices for analysis: one incorporating ITS, ETS-1f, and *trn*L–*trn*F data for 97 taxa of Cariceae, along with *Eriophorum vaginatum* and *Scirpus polystachyus* as outgroups; and the second with sequences of the *trn*E–*trn*D intergenic spacer added to the other gene regions for 29 taxa of subgen. *Vignea,* plus *Carex backii, Cymophyllus fraserianus,* and *Trichophorum alpinum* as outgroups. Sequences were aligned using CLUSTAL_X (Thompson et al. 1997), with subsequent minor adjustments. Small areas where alignments could not be unambiguously chosen were removed from the data set before analysis (45 total aligned bases from the three gene regions for the 99-taxon data matrix and 13 total aligned bases from the four gene regions for the *Vignea* data matrix). Gaps in the alignment representing putative insertion or deletion events (indels) were coded using the ''simple gap coding'' method of Simmons and Ochoterena (2000) as implemented in the computer program GapCoder (Young and Healy 2003). We included the coded indels in all parsimony analyses.

Parsimony analysis.—For the 99-taxon data matrix, heuristic searches under the criterion of maximum parsimony were conducted in PAUP* vers. 4.0b10 (Swofford 2002) using 100 random addition-sequence replicates with tree-bisection-reconnection (TBR) branch-swapping and multiple trees saved from each replication (MULTREES = yes). We used two methods to estimate bootstrap support (BS) (Felsenstein 1985) for the tree topology: 100 bootstrap replicates with the ''MULTREES = yes'' setting, TBR branch-swapping, and 30-min time restrictions on each replicate and 5000 bootstrap replicates with the ''MULTREES = no'' option, TBR branch-swapping, and no time restrictions. The two methods supported exactly the same clades at percentages that were within 5% of each other, confirming the claim of DeBry and Olmstead (2000) about the reliability of the second, much faster method. For the *Vignea* data matrix, heuristic searches were conducted in the same way but with 1000 random addition-sequence replicates. One hundred bootstrapping replications, each with 100 addition-sequence replicates, saving multiple trees from each replicate, and without time restriction were used to evaluate clade support. Based on simulation studies of Hillis and Bull (1993) and Huelsenbeck et al. (1996), we use the following terms to describe the strength of clade support: strong (95–100% BS), very good or very well (85–94% BS), good or well (75–84% BS), moderate (65–74% BS), poor or weak (55–64% BS), and very poor or very weak (<55% BS).

We also analyzed the 99-taxon data matrix independently for each gene region, including the associated indels, using the maximum parsimony criterion. In each case, the smaller number of informative characters made it difficult to thoroughly analyze a matrix with 99 species. After preliminary trials with searches constrained by limits on time spent branch-swapping each replicate or on the maximum number of trees saved, heuristic searches using ten time-limited, random addition-sequence replicates were used to determine the topology of the strict consensus tree when each gene region was analyzed alone. Statistical support for branches on these trees was assessed using 5000 bootstrap replicates with TBR branch-swapping but with the ''MULTREES = no'' option in effect (cf. DeBry and Olmstead 2000).

Bayesian analysis.—We also used Bayesian analysis (Huelsenbeck and Ronquist 2001) to estimate the topology of the phylogenetic trees calculated from each data matrix using the computer program MrBayes vers. 3.06 (Ronquist and Huelsenbeck 2003). We used four incrementally heated Metropolis-coupled Monte Carlo Markov chains with prior probabilities set using a general time-reversible evolutionary model (GTR + I + G) incorporating fixed values for the proportion of invariant sites and the shape of the gamma distribution to estimate variation in substitution rate across sites as chosen by MODELTEST vers. 3.06 (Posada and Crandall 1998). We ran the analysis twice for 600,000 generations each time, sampling one tree each 100 generations. For each analysis, we used the last 5000 of these trees to compute a consensus tree to estimate posterior probabilities of the clades. For one of these consensus trees, PAUP* vers. 4.0b10 (Swofford 2002) was used to calculate a likelihood ratio test of the null hypothesis that branch lengths were not significantly different from zero (setting ZEROLENTEST = full), using the model parameters from the Bayesian analysis as the likelihood settings. We also ran the Bayesian analysis twice using the general time reversible model with rates set to the gamma distribution but without specific fixed parameters. The topologies of all four consensus trees were nearly identical and clade posterior probabilities were no more than 7% different at any node.

Maximum likelihood analysis.—We used the same model parameters for the GTR + I + G model as chosen by MODELTEST vers. 3.06 (Posada and Crandall 1998) for the *Vignea* data matrix to calculate a maximum likelihood analysis with TBR branch-swapping using PAUP* vers. 4.0b10 (Swofford 2002). The 99-taxon data matrix was too large to make such an analysis feasible within the constraints of time and computer hardware available.

Partition homogeneity test.—For the *Vignea* data set, an incongruence-length difference (ILD) test (Farris et al. 1994, as implemented in PAUP* vers. 4.0b10) using 100 random partitions was conducted with the data partitioned into three gene regions: ITS, ETS-1f, and cpDNA (including both *trn*L–*trn*F and *trn*E–*trn*D regions). Attempts to conduct the same test for the 99-taxon data matrix were unsuccessful due

Table 2. Description of data matrix used for the phylogenetic analyses of 97 taxa of Cariceae plus *Eriophorum vaginatum* and *Scirpus polystachyus* as outgroups. The ITS-1 + ITS-2 region includes 5 bp at the 5′-end and 17 bp at the 3′-end of the 5.8S ribosomal gene. The *trn*L–*trn*F region includes the *trn*L intron, part of the *trn*L exon (51 bp), and the *trn*L–*trn*F intergenic spacer.

Feature/gene region	ITS-1 + ITS-2	ETS-1f	*trn*L–*trn*F region	All
Aligned length (bp)	569	637	1292	2498
Length range (bp)				
Including outgroups	447–534	528–602	957–1052	
Within Cariceae	447–480	584–601	957–1052	
Base frequencies (%)				
A:C:G:T	16:35:33:16	14:27:30:28	39:13:13:35	27:22:22:29
Autapomorphies, # (%)				
Including outgroups	67 (12.2)	124 (19.9)	150 (11.7)	341 (13.9)
Within Cariceae	63 (11.4)	120 (19.3)	137 (10.7)	320 (13.0)
Parsimony-informative sites, # (%)				
Including outgroups	188 (34.2)	294 (47.3)	163 (12.7)	645 (26.3)
Within Cariceae	183 (33.3)	281 (45.2)	148 (11.6)	612 (24.9)
Indels (#)				
Including outgroups	69	70	96	235
Within Cariceae	58	63	85	206
Parsimony-informative indels, # (%)				
Including outgroups	32 (46.4)	45 (64.3)	39 (40.6)	116 (49.4)
Within Cariceae	25 (43.1)	36 (57.1)	35 (41.2)	96 (46.6)
Excluded (ambiguous sites)	19	15	11	45

to the time required for branch-swapping, given the small number of informative characters when the *trn*L–*trn*F data set was used alone (cf. Table 2).

RESULTS

Descriptive Statistics

A summary of the characteristics of both individual and combined sequence data from each gene region for the 99-taxon data set is given in Table 2. Note that the base composition varies dramatically between noncoding regions of cpDNA, with mean G:C content of 26% (percentage of guanine : cytosine paired bases) and the spacer regions of the nuclear ribosomal genes, with mean G:C content 68% in ITS and 57% in ETS-1f). Although more nucleotides of cpDNA are sampled, there are fewer parsimony-informative sites than for either ITS or ETS-1f but a comparable number of informative indels from each region. A large proportion of the polymorphism is found within tribe Cariceae, making this combined data matrix very suitable for estimating phylogenetic structure within the tribe. Mean pairwise differences between species are comparable for ITS and ETS-1f and about five-fold greater than for the *trn*L–*trn*F region (Table 3). Two pairs of closely related species (*C. comosa*/*C. hystericina* and *C. squarrosa*/*C. shortiana*) had identical sequences for both the *trn*L intron and the *trn*L–*trn*F intergenic spacer.

Characteristics of individual and combined sequence data for the *Vignea* data matrix are summarized in Table 4. The mean G:C content for the *trn*E–*trn*D intergenic spacer is 31%, somewhat higher than the 26% estimate for the *trn*L–*trn*F region, while base frequencies for ITS and ETS-1f are comparable but with slightly lower G:C content compared to the larger data matrix. The relative proportions of parsimony-informative sites across the three gene regions are comparable to those in the 99-taxon data set, but the absolute percentages of informative sites are substantially lower in the smaller data matrix, reflecting the lower level of morphological variability represented within subgen. *Vignea* when compared with the entire tribe Cariceae. Note, however, that the proportions of informative indels are comparable (*trn*L–*trn*F) or higher (ITS and ETS-1f) for the *Vignea* data than for the Cariceae matrix.

Variability Between Closely Related Species

Levels of variability between closely related species differed among clades and among gene regions. The four pairs of presumed close relatives in subgen. *Vignea* had very low levels of variation within each species pair, but few differences among gene regions. Three of these pairs (*C. brunnescens/C. canescens, C. interior/C. echinata,* and *C. laevivaginata/C. stipata*) had identical ITS sequences and only one mutation each for ETS and the *trn*L–*trn*F region. The fourth pair in subgen. *Vignea* (*C. projecta/C. tribuloides*) had identical ETS sequences, one mutation for ITS and two mutations in the *trn*L–*trn*F region. Only the *C. brunnescens*/*C. canescens* pair had a mutation in the *trn*E–*trn*D gene region.

Variation between species within presumed closely related

Table 3. Comparison of variability among the three gene regions used for phylogenetic analyses of 97 taxa of tribe Cariceae using *Eriophorum vaginatum* and *Scirpus polystachyus* as outgroups. The ITS-1 + ITS-2 region includes 5 bp at the 5′-end and 17 bp at the 3′-end of the 5.8S ribosomal gene. The *trn*L–*trn*F region includes the *trn*L intron, part of the *trn*L exon (51 bp), and the *trn*L–*trn*F intergenic spacer. Clade names follow Fig. 2.

Feature/gene region	ITS-1 + ITS-2	ETS-1f	*trn*L–*trn*F region	All
Pairwise distances				
Outgroups included				
Mean (%)	9.0	10.4	2.3	6.1
Range (%)	0.4–17.1	0.5–21.6	0–8.5	0.4–3.8
Cariceae only				
Mean (%)	8.8	10.1	2.1	5.9
Range (%)	0.4–16.3	0.5–21.3	0–6.1	0.4–11.6
Core *Carex* clade only				
Mean (%)	6.6	6.6	1.4	4.0
Range (%)	0.4–12.3	0.5–13.2	0–4.0	0.5–7.3
Vignea clade only				
Mean (%)	6.5	6.5	1.2	3.4
Range (%)	3.5–9.8	3.1–10.9	0.3–3.2	2.7–5.6
Caricoid clade only				
Mean (%)	8.5	9.8	1.9	5.7
Range (%)	0.9–14.2	0.7–14.8	0–0.4	0.4–9.2

pairs was higher in subgen. *Carex,* with more variability among gene regions. For the *trn*L–*trn*F region, all six of the pairs had 1–4 mutations. Three of the pairs (*C. flava/C. viridula, C. fissuricola/C. luzulifolia,* and *C. swanii/C. virescens*) also had low levels of variation for ITS (2–7 mutations) and for ETS (4–12 mutations). Other species pairs within the clade that included *C. swanii* and *C. virescens* also had very low levels of variation with pairwise distances between species in this clade ranging from 0.5 to 1.7% with a mean of only 1.04%. The other three pairs of close relatives were more variable with 16–24 ITS mutations and 13–28 ETS mutations each. The *C. squarrosa/C. typhina* pair was most variable, followed by *C. folliculata/C. michauxiana,* and *C. albursina/C. blanda.*

Phylogenetic Estimates Using the 99-Taxon Data Matrix

After removal of a few small areas where alignment was ambiguous, the combined 99-taxon data matrix with ITS, ETS-1f, *trn*L intron, and the *trn*L–*trn*F intergenic spacer sequences had 2453 nucleotide characters and 235 indels with 761 of the total being parsimony informative (Table 2). Parsimony analysis of this combined data set using 100 random addition-sequence replicates yielded 187 most-parsimonious trees in 36 islands, 14 of which had only one tree. Each tree had a length of 4105, with a consistency index (CI) of 0.410, and a retention index (RI) of 0.652. One of the most-parsimonious trees, corresponding closely to the 50% majority-rule consensus tree, is shown in Fig. 1. With the exception of clade D3, lettered clades were supported by bootstrap values greater than 55% (Fig. 1) in both the time-restricted analysis where multiple trees were saved and the faster bootstrap method where only one tree was saved from each replicate. Variation among these trees was principally in two regions: minor rearrangements in clade D4b where branch lengths were generally very short and average *p*-distance between taxa was only one percent, and several rearrangements in clade A, particularly with respect to placement of *Carex leptalea, Kobresia laxa,* and *K. simpliciuscula.*

Bayesian analysis of the same combined data matrix using a general time-reversible model (GTR + I + G) as chosen by running MODELTEST vers. 3.06 (Posada and Crandall 1998) resulted in a fully resolved tree (Fig. 2: ln likelihood = −24037.92562) quite similar to the 50% majority-rule consensus tree resulting from parsimony analysis. Each of the lettered clades had Bayesian posterior probabilities of 98% or greater. Additional clades with bootstrap support less than 70% in the parsimony analysis also had high posterior probabilities in the Bayesian analysis (>80%) (Fig. 2). Likelihood ratio tests could not reject the null hypothesis that a branch was of length zero for nine branches of the tree (shown as dotted lines in Fig. 2), including the branch supporting the monophyly of clade C + D, the deepest branch within clade A, six interior branches of clade D, and one interior branch of clade C.

Relationships Among Major Clades in the Combined Analysis

Clade A (the Core Unispicate Clade), comprising representatives of *Kobresia, Uncinia, Cymophyllus fraserianus,* and several unispicate *Carex* as well as *C. curvula* is weakly supported in the parsimony analysis (BS = 59%) but strongly supported (100%) in the Bayesian analysis. Three subclades within this clade received strong support in both analyses: *Cymophyllus fraserianus/Carex backii,* four *Kobresia* species, and three *Uncinia* species.

Clade B (the *Schoenoxiphium* Clade), with three representatives of *Schoenoxiphium* and two unispicate carices, receives moderate support in parsimony analysis and strong support in Bayesian analysis. The Bayesian analysis further supports a sister-group relationship between clades A and B

Table 4. Comparison of gene regions used for the phylogenetic analyses of 29 taxa of *Carex* subgen. *Vignea* using *Carex backii, Cymophyllus fraserianus,* and *Trichophorum alpinum* as outgroups. Percentages of autapomorphies and parsimony-informative sites are calculated based on the number of nucleotides included in the analysis from each gene region; percentages of parsimony-informative indels are calculated based on the total number of indels for each gene region. The *trn*L–*trn*F region includes the *trn*L intron, part of the *trn*L exon (51 bp), and the *trn*L–*trn*F intergenic spacer. The *trn*E–*trn*D region includes the tRNA-Tyrosine gene (84 bp), which has only a single nucleotide substitution in the data set.

Feature/gene region	ITS-1 + ITS-2	5.8S	ETS-1f	*trn*L–*trn*F region	*trn*E–*trn*D region	All
Aligned length (bp)	476	166	613	1089	678	3022
Length range (bp)						
Outgroups included	441–461	166	551–598	1004–1039	626–637	
Within subgen. *Vignea*	441–446	166	592–598	1004–1034	626–637	
Autapomorphies # (%)						
Including outgroups	74 (15.5)	3	96 (15.7)	82 (7.5)	55 (8.1)	310 (10.3)
Within subgen. *Vignea*	64 (13.4)	2	65 (10.6)	37 (3.4)	28 (4.2)	196 (6.5)
Parsimony-informative sites, # (%)						
Including outgroups	112 (23.5)	1	166 (27.1)	50 (4.6)	36 (5.3)	365 (12.1)
Within subgen. *Vignea*	91 (19.1)	1	129 (21.0)	36 (3.3)	27 (4.1)	284 (9.4)
Indels #						
Including outgroups	22	0	32	31	15	100
Within subgen. *Vignea*	17	0	14	20	11	62
Parsimony-informative indels, # (%)						
Including outgroups	11 (50.0)	0	17 (53.1)	13 (41.9)	5 (33.3)	46 (46.0)
Within subgen. *Vignea*	7 (41.2)	0	8 (57.1)	9 (45.0)	5 (45.5)	29 (46.8)
Excluded (ambiguous sites)	0	0	0	0	13	13
Base frequencies (%)						
A:C:G:T	19:32:32:17	24:27:29:20	15:24:30:31	39:13:13:36	34:16:15:36	29:19:21:31
Pairwise distances						
Outgroup included						
Mean (%)	8.2	N/A	8.4	1.5	1.8	4.0
Range (%)	0–18.7	N/A	0–20.4	0–5.8	0–7.9	0.1–10.3
Within subgen. *Vignea*						
Mean (%)	7.1	N/A	6.8	1.0	1.2	3.2
Range (%)	0–12.0	N/A	0–11.4	0–2.3	0–2.8	0.1–5.2

with strong support for their monophyly (to form the Caricoid Clade). In contrast, clade B is placed as sister to clades C + D in the 50% majority-rule consensus tree and in the parsimony tree shown in Fig. 1, but this relationship has very weak bootstrap support.

Clade C (the *Vignea* Clade) recovers all species of subgen. *Vignea* included in the analysis except for *C. curvula.* It also confirms the inclusion of sect. *Physoglochin* within subgen. *Vignea,* with *C. gynocrates* well nested within this clade, as *C. dioica* L. has been in other studies (Yen and Olmstead 2000*a*; Heindrichs et al. 2004*b*; Starr et al. 2004). Clade C receives very strong support in both parsimony and Bayesian analyses. Support for some of the relationships within the *Vignea* Clade is also strong-to-moderate in both analyses, suggesting the polyphyly of sect. *Phaestoglochin* and a closer relationship of the gynecandrous sect. *Glareosae* to androgynous species than to the other gynecandrous groups represented (sects. *Ovales* and *Stellulatae*).

Clade D (the Core *Carex* Clade), including all representatives of subgen. *Carex* and *Vigneastra,* as well as the unispicate *C. scirpoidea,* receives strong support in both parsimony and Bayesian analyses. The five species of subgen. *Vigneastra* are placed into four different subclades, illustrating the polyphyly of this subgenus. *Carex cruciata* is very well to strongly supported as sister to the rest of clade D, suggesting that at least one group of *Vigneastra* may be basal to the remaining *Carex*/*Vigneastra* clade.

Although clades C and D appear as sister groups in both analyses, this relationship has bootstrap support of only 50% (Fig. 1) and Bayesian posterior probability of only 67% (Fig. 2). A likelihood ratio test could not reject the null hypothesis ($P = 0.083$) that the branch supporting this grouping has a length of zero. Similarly, no clear relationships among any of the four major clades are supported by either analysis, with the exception of support for the monophyly of clades A and B in the Bayesian analysis.

Relationships Within the Core Carex *Clade*

Within clade D, four major clades are supported in the Bayesian analyses (Fig. 2), two with very strong bootstrap

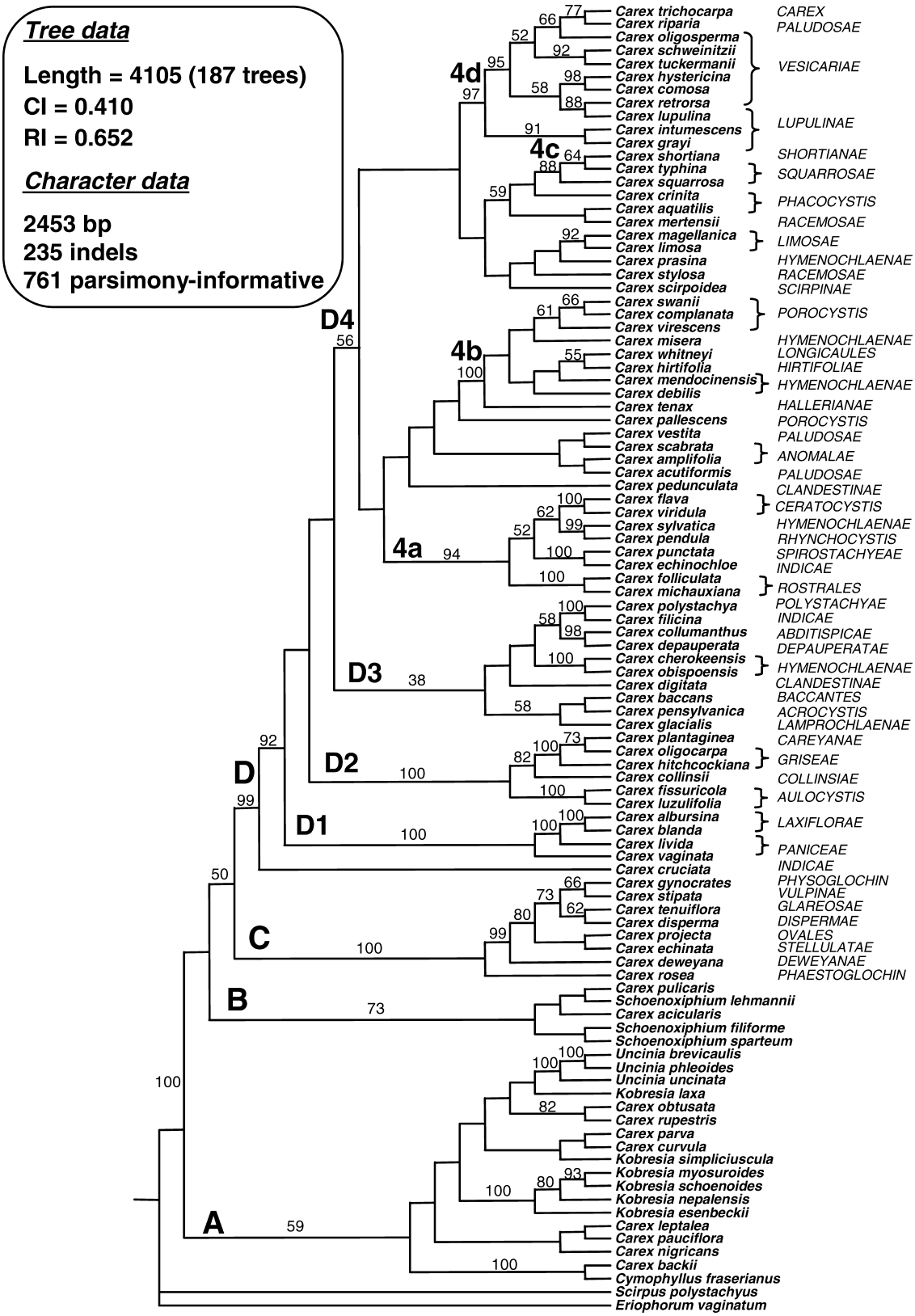

Fig. 1.—One of 187 shortest trees based on parsimony analysis with 100 random addition-sequence replicates of a combined matrix of ITS, ETS-1f, and *trn*L–*trn*F gene regions for 99 taxa including two outgroup species. Numbers above branches indicate bootstrap values based on 100 replicates. Current sectional placement for *Carex* species in clades C and D is indicated on the right side of the diagram with nomenclature following Ball and Reznicek (2002) for North American species and Jermy et al. (1982) or Egorova (1999) for Eurasian species. Clades coded with letters and numbers in bold and having bootstrap support >55% (with the exception of D3) are discussed in the text.

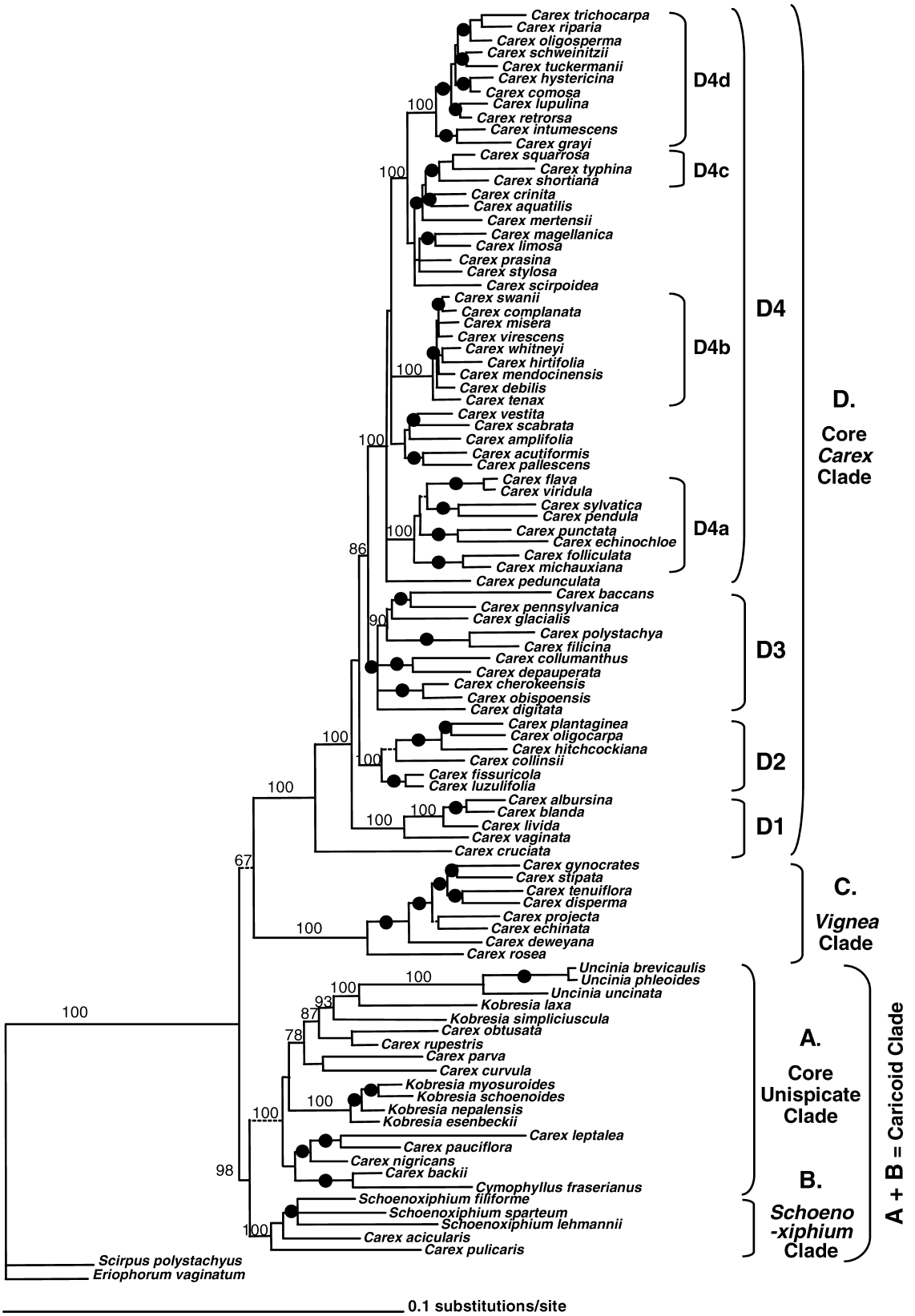

Fig. 2.—Phylogram based on consensus of 5000 trees sampled in a Bayesian analysis of ITS, ETS-1f, and *trn*L–*trn*F sequence data for 99 taxa using a GTR + I + G model with parameters as chosen by MODELTEST vers. 3.06. Numbers above the branches represent clade Bayesian posterior probabilities. Closed circles in crowded areas of the tree represent posterior probabilities greater than 95%; dotted lines indicate branches with lengths not statistically different from zero. Clades are numbered and lettered to match Fig. 1 and names for the major clades as used in the text are given on the right.

support, one with weak bootstrap support, and one with very weak support in parsimony analysis (Fig. 1). Clade D1 comprises members of sects. *Laxiflorae* and *Paniceae* with species of *Paniceae* forming a grade basal to a monophyletic *Laxiflorae*. This clade has 100% bootstrap support, as well as a Bayesian posterior probability of 100%. Clade D2 receives equally strong support in both analyses. It includes a rather surprising assemblage of species with members of sects. *Careyanae* and *Griseae* supported as monophyletic and sister to *Carex collinsii* in the monotypic sect. *Collinsiae*. The sister-group relationship between this assemblage and a strongly supported monophyletic sect. *Aulocystis* is also strongly supported.

Clade D3 is strongly supported in the Bayesian analysis (98%) and is frequently recovered in the parsimony analysis but with very weak bootstrap support (BS = 38%). Three subclades within this clade are strongly supported by both analyses: *Carex cherokeensis* plus *C. obispoensis* from sect. *Hymenochlaenae, C. polystachya* plus *C. filicina* (sects. *Polystachyae* and *Indicae,* both from subgen. *Vigneastra*), and *C. depauperata* (sect. *Depauperatae*) plus *C. collumanthus* (sect. *Abditispicae*). A fourth grouping within clade D3 has moderate Bayesian support and weak bootstrap support, indicating a possible relationship between *C. pensylvanica* (sect. *Acrocystis*), *C. baccans* (sect. *Baccantes* subgen. *Vigneastra*), and *C. glacialis* (sect. *Lamprochlaenae*). *Carex digitata* (sect. *Clandestinae*) is also part of clade D3.

Clade D4 includes the largest set of species in the sample and has four well-supported subclades. Bootstrap support is weak for this large clade, but it has a posterior probability of 100% in the Bayesian analysis. Within this clade there is very good to strong support for subclade D4a, with representatives from five different sections. Four strongly supported monophyletic pairs form this group: *Carex flava* and *C. viridula* (sect. *Ceratocystis*), *C. sylvatica* (sect. *Hymenochlaenae*) with *C. pendula* (sect. *Rhynchocystis*), *C. folliculata* plus *C. michauxiana* (sect. *Rostrales* [= *Folliculatae* Mack.]) and *C. punctata* (sect. *Spirostachyae*) plus *C. echinochloe* (subgen. *Vigneastra* sect. *Indicae*). No clear relationships among any of these pairs are apparent.

Clade D4b has 100% support in both analyses and includes North American species from sects. *Hymenochlaenae, Porocystis, Longicaules, Hallerianae,* and *Hirtifoliae. Carex pallescens* (sect. *Porocystis*), with a broad temperate distribution in eastern North America and Eurasia, is sister to this group in the parsimony tree, but without bootstrap support. In the Bayesian tree, *C. pallescens* groups instead with *C. acutiformis* (sect. *Paludosae*) with strong support.

The larger clade that includes clade D4c is strongly supported in the Bayesian analysis and frequently recovered in the parsimony analysis but with bootstrap support of less than 50%. It includes one well-supported clade (D4d) described below, which is sister to a clade with poor support in both analyses. The latter group includes two well-supported subclades: a monophyletic species pair from sect. *Limosae,* and clade D4c comprising *C. squarrosa, C. typhina* (both sect. *Squarrosae*), and *C. shortiana* (sect. *Shortianae*). Two species of the large sect. *Phacocystis, C. aquatilis,* and *C. crinita,* are supported as a monophyletic group in the Bayesian analysis but have very weak bootstrap support in the parsimony analysis. In the latter analysis, C. *aquatilis* forms a clade with *C. mertensii* (sect. *Racemosae*) more often than with *C. crinita* but with very weak bootstrap support. Clade D4d is strongly supported in both analyses and includes species from sects. *Carex, Lupulinae, Paludosae,* and *Vesicariae*. Relationships within this clade also receive strong-to-moderate support in both analyses suggesting that sects. *Lupulinae* and *Vesicariae* are not monophyletic.

Comparison of Analyses Based on Each Gene Region Separately

Parsimony analysis of the 99-taxon data matrix using each gene region individually resulted in much lower resolution, but topologies were generally, although not completely, consistent with the combined analysis. For ITS, a strict consensus of the 15,436 trees found from ten time-limited, random addition-sequence replicates, recovered the major clades C (BS = 96%) and D (BS = 80%), representing the *Vignea* and Core *Carex* groups, respectively, but species from clades A and B in the combined analysis were grouped differently. One group comprised the four unispicate *Kobresia* species plus *Cymophyllus, Carex backii, C. nigricans,* and *C. pauciflora,* while the other included the remaining unispicate *Carex* and multispicate *Kobresia* species, plus the three *Uncinia* species and the three *Schoenoxiphium* species. Neither of these two larger groups had significant bootstrap support, but the subclade grouping the four unispicate *Kobresia* species together had 94% bootstrap support, that grouping *Cymophyllus* and *Carex backii* had 97% bootstrap support, and the clade comprising the three *Uncinia* species was strongly supported at 95%. Within clade C, none of the relationships had bootstrap support greater than 65% except the grouping of *Carex tenuiflora* with *C. projecta* and *C. disperma,* which was supported at 85%. Ten of the same species pairs that were strongly supported in the combined analysis were also moderately to strongly supported in the ITS analysis, but only three groupings of more than two species were supported: (1) clade D1, (2) a subclade grouping sects. *Careyanae* and *Griseae* within clade D2, and (3) a subclade of D4b comprising *C. swanii, C. virescens,* and *C. complanata* (all sect. *Porocystis*). The remaining species in clade D formed a polytomy with these groups and the 13 species pairs. Bootstrap support for the three species pairs not supported in the combined analysis was very weak to moderate using ITS data alone. Overall, resolution within the ITS strict consensus tree was very poor, especially within clade D.

Resolution within the tree representing the strict consensus of the 12,847 trees produced from parsimony analysis of the ETS data matrix, including indels, from ten time-limited, random addition-sequence replicates was greater than in the ITS consensus tree. Major clades C and D from the combined analysis were also recovered in the ETS consensus tree, with bootstrap support of 100% and 94%, respectively. *Carex cruciata* was moderately supported as sister to the rest of clade D (BS = 74%); clades D1 and D2 also received strong bootstrap support in the ETS analysis. Within clade D, subclade D4b, as well as the *Careyanae/Griseae* subclade and the grouping of sect. *Laxiflorae* with *C. livida,* were all strongly supported. Moderately supported subclades included the core group of ''bladder sedges'' (without *C. grayi* and *C. intumescens*) and a group compris-

ing three of the four sampled species of sect. *Porocystis,* as in the analysis using only ITS data. Fourteen species pairs with bootstrap support above 50% in the combined analysis also had moderate-to-high support in the analysis using only ETS. Five of these pairs did not receive significant bootstrap support in either the ITS analysis or the *trn*L analysis.

The strict consensus of 282,243 most-parsimonious trees, found in the parsimony analysis using only *trn*L–*trn*F data, had a surprising degree of resolution given that there were only 204 parsimony-informative characters among the 99 taxa. Thirteen species pairs received significant bootstrap support with the seven pairs that were also supported by at least one other data set receiving moderate-to-high levels of bootstrap support in the *trn*L analysis, while four of the six pairs that did not receive significant support on the ITS or the ETS tree had only weak-to-moderate support in the *trn*L analysis, and the other two had support between 82 and 90%. Among the major clades in the combined analysis, clades C and D were both strongly supported, as were clade D1 and the sister-group relationship of *C. cruciata* with the rest of clade D. Within clade D, subclades D4b and D4c had moderate bootstrap support while clade D4d was strongly supported. Several of the smaller subclades within D1, D2, and D4 were also moderately supported in the *trn*L analysis. Among the three analyses based on individual gene regions, the *trn*L analysis recovered many more of the larger clades found by the combined analysis than either of the other two data sets, despite the fact that it contributed fewer informative characters to the combined data matrix. The position of *C. echinochloe* was different in the *trn*L tree than in either the ITS, ETS-1f, or the combined tree. In the *trn*L tree, it formed a strongly supported clade (BS = 100%) with *C. polystachya* and *C. filicina,* whereas it was strongly supported as sister to *C. punctata* in the ETS-1f and ITS trees, as well as in the combined analysis.

Analyses of Subgenus Vignea

Compilation of sequence data from four gene regions—nuclear ITS, ETS-1f, chloroplast *trn*L–*trn*F, and *trn*E–*trn*D—resulted in a matrix of 3009 nucleotides and 106 indels for 29 taxa of subgen. *Vignea,* plus the three outgroup taxa. Both ITS and ETS-1f were more variable than the cpDNA sequences, with 112 and 166 parsimony-informative characters, respectively, compared to 86 parsimony-informative characters for the two cpDNA regions combined (Table 4). Pairwise sequence divergence values were also six- or sevenfold greater for the ribosomal gene spacers than for the cpDNA intron and spacers (Table 4).

Parsimony analyses of these data using 1000 random addition sequences and TBR branch swapping, saving multiple trees, resulted in ten shortest trees of length 1334 (CI = 0.687, RI = 0.715). The ten trees differed principally in the positions of *C. maritima, C. disperma,* and *C. chordorrhiza,* with *C. maritima* either basal to most of the other species or placed within the clade that includes *C. disperma* and *C. chordorrhiza,* and in various relationships with them. Bootstrap support values based on 100 replications with 100 random addition-sequence replicates each are shown in Fig. 3, plotted onto one of the ten most-parsimonious trees. Posterior clade probabilities are shown on the same tree, which were nearly identical to the tree produced by both Bayesian analysis (ln likelihood = −11,102.97282) and by maximum-likelihood analysis (ln likelihood = −10,992.50718) implemented in PAUP* vers. 4.0b10. The only differences in tree topology were the positions of C. *maritima* and *C. deweyana* and whether or not *C. diandra* and *C. decomposita* formed a clade. Likelihood ratio tests could not reject the null hypothesis that the branch supporting the monophyly of *C. decomposita* and *C. diandra* had zero length ($P = 0.118$). This null hypothesis was rejected for all other branches ($P < 0.01$)

Five strongly supported clades are apparent in all analyses (Fig. 3). A basal clade comprising the *Carex rosea* complex (sect. *Phaestoglochin*) is strongly supported as sister to all other *Vignea* taxa, with the possible exception of *C. maritima,* which has a basal position in the maximum-likelihood and Bayesian trees. Other representatives of sect. *Phaestoglochin* form a moderately to strongly supported clade with *C. vulpinoidea* (sect. *Multiflorae*), *C. diandra* and *C. decomposita* (both sect. *Heleoglochin*), making sect. *Phaestoglochin* polyphyletic in all analyses. Within this larger clade, all analyses strongly support the monophyly of the three *Phaestoglochin* representatives with *C. vulpinoidea,* with the two species in sect. *Heleoglochin* well supported as a basal grade within it. The three remaining well-supported major clades each comprise representatives of a single currently accepted section—one supporting a monophyletic sect. *Ovales,* another a monophyletic sect. *Stellulatae,* and the third demonstrating the monophyly of sect. *Glareosae*—within the limits of sampling for this study.

Two additional clades receive weak support. The two representatives of sect. *Deweyanae* are moderately supported as a monophyletic group in the parsimony analysis (BS = 68%) and the maximum-likelihood analysis but not in the Bayesian analysis. *Carex gynocrates* (sect. *Physoglochin*) forms a weakly supported clade with two closely related species of sect. *Vulpinae,* while the remaining representative of sect. *Vulpinae, C. crus-corvi,* is weakly supported in all analyses as sister to the *Phaestoglochin*/*C. vulpinoidea* clade described above, rather than having a close relationship with *C. stipata* and *C. laevivaginata.*

Although an ILD test (Farris et al. 1994, as implemented in PAUP*) rejected the hypothesis that the trees supported by each individual gene region were congruent, there was considerable similarity among the trees based on each of the three gene regions independently. Nearly all clades that received strong support in the combined analysis were also supported in the independent parsimony analyses. The only exception was the sister-group relationship between the *C. canescens* plus *C. brunnescens* clade with the other three species of *Glareosae,* which was not supported by the ITS analysis but was strongly supported by all three other gene regions and by the combined analysis. Two clades receiving only moderate-to-weak support in the combined analysis were each supported by only one of the three individual analyses: *C. deweyana* plus *C. bromoides* only by ITS data, and the clade formed by species of sect. *Heleoglochin* with those from sects. *Phaestoglochin* and *Multiflorae* (see Fig. 3), supported only by ETS data. The major differences among the three data sets concerned the relative placements of *C. maritima, C. disperma,* and *C. chordorrhiza,* none of

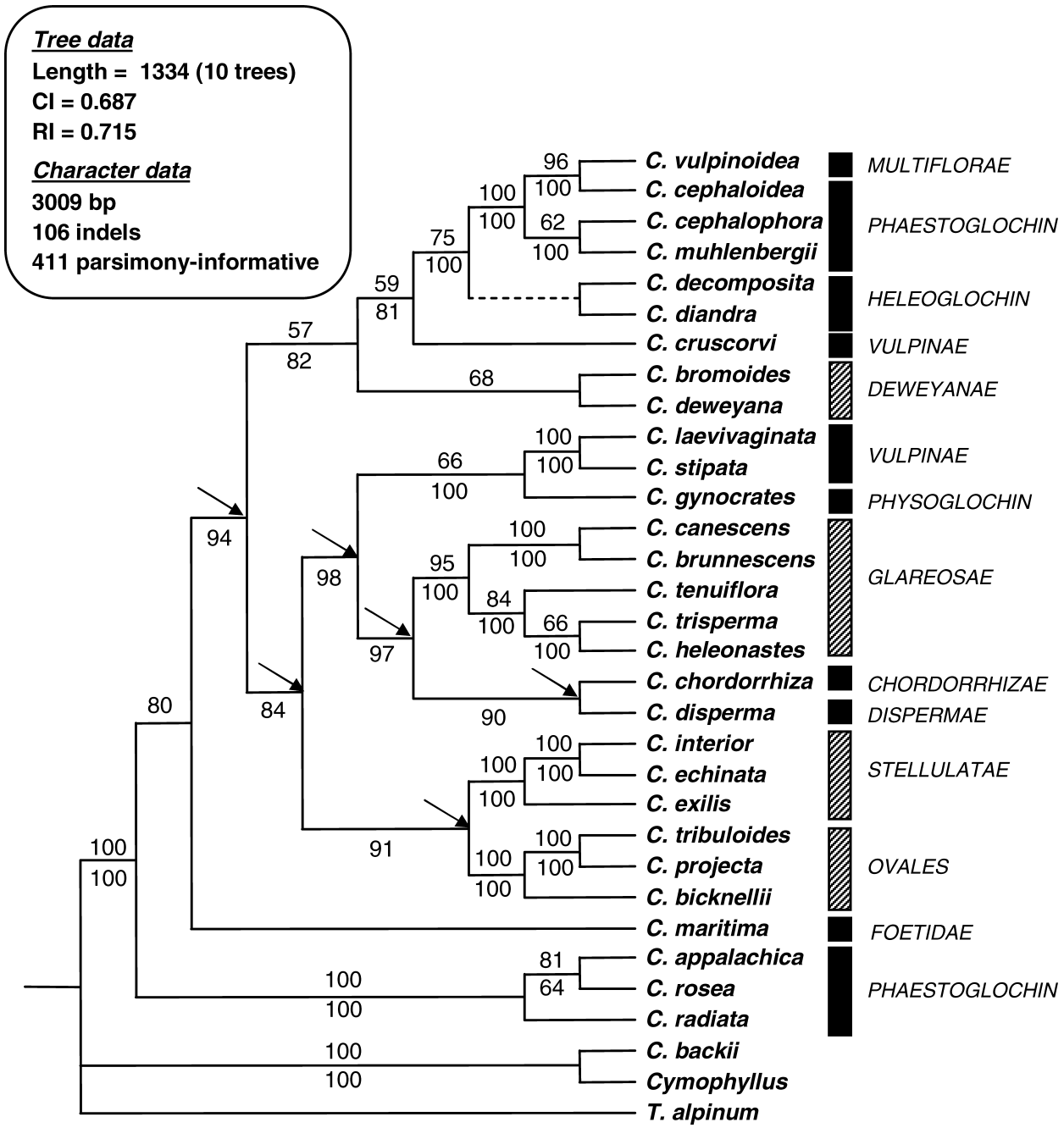

Fig. 3.—One of 10 shortest trees based on parsimony analysis with 1000 random addition-sequence replicates of a combined matrix of ITS, ETS-1f, *trn*L–*trn*F, and *trn*E–*trn*D sequence data for 29 taxa of subgen. *Vignea* and three outgroup species. Numbers above the branches indicate bootstrap support based on 100 replications each with 100 random addition-sequence replicates. Numbers below branches are clade posterior probabilities estimated by Bayesian analysis using a GTR + I + G model with parameters as chosen by MODELTEST vers. 3.06. Arrows indicate clades that collapse in the semi-strict consensus tree. Species having androgynous spikes are indicated with solid rectangles to the right of the species names while those with gynecandrous spikes are denoted by diagonally slashed rectangles.

which were consistently placed in the combined analysis either.

DISCUSSION

The analyses presented here are compatible with previous molecular analyses of tribe Cariceae (Starr et al. 1999, 2004; Yen and Olmstead 2000*a*, *b*; Roalson et al. 2001) but the use of three gene regions in the 99-taxon analysis and four in the analysis of subgen. *Vignea* has resulted in better resolution and better statistical support for the tree topology. These analyses also provide new insights into relationships among species and sections in *Carex* subgen. *Carex* and its relationship to subgen. *Vigneastra,* as well as suggesting monophyly or polyphyly of particular sections in subgen. *Vignea.* Before elaborating on these relationships, we will compare levels of variation among the gene regions and

evaluate the relative potential of each for future studies. We will also compare results from the Bayesian and parsimony analyses.

Evaluation of the Four Gene Regions

The choice of gene regions to use in a phylogenetic study is an important one, with the goal being to select regions that are variable among taxa in the analysis, but not so variable that mutations would have occurred multiple times at the same site. When trying to infer phylogeny of a genus or a group of closely related genera, as in this study, one is faced with the challenge of finding DNA regions that vary between species that may have diverged only a few hundred or a few thousand years ago, yet also give reliable information about relationships among lineages that may have diverged several million years ago. Coding regions, like the *ndh*F gene used by Yen and Olmstead (2000*a*, *b*), have low variability among species (less than 4% of the *ndh*F sites were parsimony informative), but should be more reliable to reconstruct the deeper branching patterns within the tree than much more variable, noncoding spacer regions. On the other hand, noncoding regions, such as ITS or *trn*L–*trn*F, can provide many more characters for resolving relationships within the major clades and among closely related species, and have given results consistent with those of Yen and Olmstead (2000*a*, *b*) when used alone with a small set of taxa (Starr et al. 1999), or together with more extensive sampling (Roalson et al. 2001). From the data on relative variation levels among the three gene regions used in this study (Table 2–4), it is apparent that ETS-1f is most variable and adds the most potentially informative characters to the data matrix. However, the number of informative characters is only one consideration. Levels of homoplasy are also important. Examination of the trees and bootstrap support levels that can be achieved using single-gene regions in comparison to combined analysis can provide additional information about the utility of each gene region in reconstructing tree topology at different branching levels within a tree.

As expected, trees based on analysis of a single-gene region were less highly resolved but rarely in conflict with those from the combined analysis presented here (Fig. 1, 2). Clades C (*Vignea* clade) and D (Core *Carex* clade) were strongly supported by the combined analysis and received, at worst, very good support in each single-gene analysis. Similarly, clade D1 uniting species of sects. *Laxiflorae* and *Paniceae* was strongly supported in all analyses, while the position of *C. cruciata* as sister to all other members of the Core *Carex* clade received moderate-to-strong support in all but the ITS analysis. Other groups receiving at least good bootstrap support in all analyses were the *Careyanae/Griseae* clade and four small clades, each with a pair of closely related species: *C. flava/C. viridula* representing sect. *Ceratocystis, C. folliculata/C. michauxiana* representing sect. *Rostrales, C. fissuricola/C. luzulifolia* representing sect. *Aulocystis,* and *C. cherokeensis/C. obispoensis* from sect. *Hymenochlaenae.*

More of the species pairs supported in the combined analysis were united by the ETS data alone or by the *trn*L data alone than when using just the ITS data. Furthermore, many of the relationships that appear to be least likely based on morphology are recovered only in analyses using ETS data alone and the combined analysis in which about half of the informative characters come from ETS. For example, the ETS data provide most of the support for the clade uniting sect. *Aulocystis* with sect. *Collinsiae* and with sects. *Careyanae* and *Griseae*. Similarly, most of the characters uniting the highly reduced South American species, *C. collumanthus,* with the European species, *C. depauperata,* with which it shares little morphological similarity, come from ETS data. On the other hand, *trn*L data alone not only support some of the most likely species pairs recovered in the combined analysis, but they also support relationships that appear to be more likely than those supported by ETS and combined data. For example, *C. aquatilis* and *C. crinita,* sharing numerous morphological features that characterize sect. *Phacocystis,* are well supported as monophyletic by *trn*L data but not supported by ITS, ETS-1f, or the combined parsimony analysis. The ETS-1f region has a much higher level of variability among species than the *trn*L–*trn*F region and would be more prone to cause long-branch attraction problems for species whose closest relatives were not included in the data set. This may explain some of the unusual groupings in our analyses and indicates that while ITS and ETS-1f are useful in resolving relationships among close relatives, they can be misleading when used with a set of exemplars representing a broader phylogenetic range.

The major clades are generally supported either by each of the data sets independently or only by the combined data set, suggesting the need to use multiple-gene regions to better resolve relationships among major groups. Both ETS and *trn*L data sets recover many of the subclades that unite smaller sets of species, but *trn*L appears to do better despite having fewer informative characters. ITS data provide the least information about higher-level relationships. These results suggest that using single-gene regions for resolving relationships within the Cariceae are not nearly as effective as using multiple data sets and that combined data from the ETS-1f and *trn*L–*trn*F region are most useful.

Comparison of Parsimony and Bayesian Analyses

Although the topologies of trees produced from parsimony and Bayesian analysis of the combined data for both the 99-taxa matrix and the *Vignea* matrix were quite similar, the levels of support for particular clades were often quite different, especially for clades with Bayesian posterior probabilities less than 95%. In particular, the Bayesian analysis often showed high posterior probabilities for clades that had very weak bootstrap support in the parsimony analysis. In some cases, the posterior probabilities were 80–90% for clades supported by branches that likelihood ratio tests revealed were not significantly different from zero. In all such cases, these clades had no significant bootstrap support in parsimony analysis. These results suggest that it is important to compare the two analyses, and to carefully assess the characters supporting short branches before drawing strong conclusions about clades with Bayesian posterior probabilities less than 95%.

General Cariceae Relationships

The present analysis is highly compatible with previous molecular analyses of the Cariceae (Starr et al. 1999, 2004;

Yen and Olmstead 2000*a*, *b*; Roalson et al. 2001). Three (Core *Carex, Vignea,* and Core Unispicate) of the four major clades in our trees are also common to the analyses of Yen and Olmstead (2000*a*, *b*) and Roalson et al. (2001), whereas all four major clades are also present in the analyses of Starr et al. (2004). As in previous studies (Yen and Olmstead 2000*a*, *b*; Roalson et al. 2001; Starr et al. 2004) there is strong support for a monophyletic Core *Carex* clade (subgen. *Carex*/*Vigneastra*/*Psyllophora* p. p. min. [in small part]), a subgen. *Vignea* clade, and a clade consisting of *Uncinia* species. Analyses also indicate that *Carex* subgen. *Psyllophora, Vigneastra,* and *Carex* are unnatural groups (Yen and Olmstead 2000*a*, *b*; Starr et al. 2004); that *Carex* itself is artificial (all major clades contain *Carex* species; Yen and Olmstead 2000*a*, *b*; Roalson et al. 2001; Starr et al. 2004); and that the genera *Kobresia* and *Schoenoxiphium* may be unnatural but should not be merged (Starr et al. 2004). As in the analyses of Starr et al. (2004, in press), a fundamental split between unispicate Cariceae species is evident in our trees, with androgynous taxa (the Core Unispicates + *Schoenoxiphium* clades) related to multispicate species of either *Kobresia* or *Schoenoxiphium,* while dioecious species are related to either multispicate species of *Carex* subgen. *Vignea* or *Carex*. In addition, the positions of the monotypic genus *Cymophyllus* within the Core Unispicate clade (Yen and Olmstead 2000*a*, *b*; Roalson et al. 2001; Starr et al. 2004) and of the diminutive *Carex collumanthus* (= *Vesicarex collumanthus* Steyerm.) within the Core *Carex* clade (Starr et al. 2004) are consistent with the general morphology of their respective clades (i.e., Core Unispicate = predominately unispicate, androgynous species; Core *Carex* = predominately multispicate species with terminally staminate and laterally pistillate inflorescence units). It is clear that on the basis of molecular and morphological data the monotypic genera *Cymophyllus* and *Vesicarex* Steyerm. do not warrant generic status, as previously noted for *Vesicarex* (Mora Osejo 1982; Wheeler 1989*a*). These taxa are typical examples of numerous species in Cariceae whose relationships are obscured by inflorescence reduction and specialization (Starr et al. 2004).

A major difference between this analysis and previous studies lies in the arrangement of the major clades (Core Unispicate, *Schoenoxiphium, Vignea,* Core *Carex*). Previous analyses have placed either the genus *Schoenoxiphium* (Yen and Olmstead 2000*a*, *b*; (((Core *Carex, Vignea*), Core Unispicates), *Schoenoxiphium*), maximum-likelihood analysis), the *Vignea* (Roalson at al. 2001; ((Core *Carex,* Caricoid), *Vignea*)), or the Core *Carex* (Starr et al. 2004; (((Core Unispicate, *Schoenoxiphium*), *Vignea*), Core *Carex*)) clade at the base of Cariceae. In contrast, this study places either a Core Unispicate (parsimony) clade as basal (i.e., (((Core *Carex* + *Vignea*), *Schoenoxiphium*), Core Unispicate)), or a Caricoid (i.e., Core Unispicate + *Schoenoxiphium*) clade as sister to a monophyletic Core *Carex* + *Vignea* group. Although there is general congruence among molecular analyses over the number of major clades in Cariceae and their general composition, relationships among these clades remain obscure. No analysis has yet found strong statistical support for any particular topology of these clades despite considerable increases in characters and taxa over time. Resolving the relationships of these four principal clades should be a major goal for future research since their position in the phylogeny has a strong impact on conclusions that may be drawn regarding inflorescence evolution and homology in the tribe (see Starr et al. 2004).

Phylogeny of the Core Unispicate and Schoenoxiphium *Clades*

The Core Unispicate and *Schoenoxiphium* clades are highly diverse groups comprising approximately 230 species distributed in the temperate and mountainous habitats of the Northern and Southern hemispheres (Starr et al. 2004). These two clades together contain all five genera currently recognized in the tribe, and they exhibit a considerable amount of structural variation in their inflorescence, ranging from the unispicate androgynous condition of the genera *Uncinia, Kobresia* p. p., *Cymophyllus* and *Carex* subgen. *Psyllophora* to the multispicate species of *Kobresia* and *Schoenoxiphium.* In common with the analyses of Starr et al. (2004, in press), this analysis provides strong statistical support (i.e., >94% BS) for a monophyletic *Uncinia,* a close relationship between *Cymophyllus* and *Carex* sect. *Phyllostachyae,* and for a clade in *Kobresia* that comprises all unispicate androgynous species. Moreover, Bayesian analyses are consistent with Starr et al. (2004) in suggesting that the Core Unispicates and *Schoenoxiphium* clades are sisters (i.e., a Caricoid clade), which is also consistent with the results of Roalson et al. (2001), even though their sampling included only one *Schoenoxiphium* clade member. However, the position of the *Schoenoxiphium* clade as sister to a monophyletic *Vignea* + Core *Carex* clade in parsimony trees is unique to this analysis. Even though the position of the genus *Schoenoxiphium* and its allies has differed among studies (cf. Yen and Olmstead 2000*a*, *b* with Roalson et al. 2001 and Starr et al. 2004), the very poor (<50% BS) support for this topology suggests that this relationship is unlikely. The difficulty in resolving relationships among the major clades of Cariceae might be due to rapid radiation in the early evolution of the group (Stebbins 1981; Starr et al. 1999). Alternately, the failure to resolve these relationships may result from using gene regions that evolve too rapidly. Additional data from more slowly evolving DNA regions for a carefully chosen subset of species from all major clades may be needed. (cf. Graham and Olmstead 2000 for a comparable example at a different taxonomic level).

Phylogeny of the Vignea *Clade*

As in previous studies (Yen and Olmstead 2000*a*, *b*; Roalson et al. 2001; Starr et al. 2004), support for a monophyletic subgen. *Vignea,* excluding *C. curvula,* but including sect. *Physoglochin,* is very strong in all analyses (Fig. 1–3). *Carex curvula* has also been removed from subgen. *Vignea* on morphological grounds by Egorova (1999), but she placed it as an ''archaic'' member of subgen. *Carex,* while the present and previous (Starr et al. 2004) molecular analyses position it within the Core Unispicate clade along with several unispicate carices. On the other hand, several authors (e.g., Heilborn 1924; Savile and Calder 1953; Chater 1980; Egorova 1999; Ball and Reznicek 2002) have treated the unispicate sect. *Physoglochin* (represented by *C. gynocrates* in the present analysis) within subgen. *Vignea* based on chromosome data, patterns of infestation with smut fungi, overall

similarity in morphology, and the formation of hybrids with species of sects. *Foetidae* and *Glareosae* (Toivonen 1981). The present analysis confirms this placement.

For the most part, subgen. *Vignea* has been considered a distinctive and coherent group since its original circumscription in 1819, but its relationship with the rest of the genus or with other genera of Cariceae has never been clear (Smith and Faulkner 1976; Nannfeldt 1977). The suggestion that primitive species of Cariceae might have had compound inflorescence structure similar to those in some extant groups of subgen. *Vignea* (Reznicek 1990) is not supported by the analyses presented here. In fact, two of the three species mentioned as examples of such species (*C. crus-corvi,* sect. *Vulpinae;* and *C. decomposita,* sect. *Heleoglochin*) appear in one of the most advanced lineages in our analyses along with species from two other sections (*Phaestoglochin* and *Multiflorae*) that also have compound inflorescences (Fig. 3). Other species of *Vulpinae* with compound inflorescences have an equally advanced position within a different major clade of *Vignea* (Fig. 3). Since species with congested, compound inflorescences did not form a monophyletic group in our analyses or in a previous analysis (Roalson et al. 2001), it is quite possible that other species with such inflorescences may be basal within *Vignea.* One obvious candidate is sect. *Macrocephalae* Kük., which has species with paniculate inflorescences and three stigmas, a presumably plesiomorphic state within the subgenus. *Carex macrocephala* Willd. ex Kunth is moderately supported as sister to the rest of the *Vignea* species in the combined analysis of Roalson et al. (2001), but it is not the basal group in the Bayesian analysis of subgen. *Vignea* using only ITS data (Heindrichs et al. 2004*b*). In both cases, other species with compound inflorescences assume more advanced positions, as in the present analysis.

Most identification keys for species of subgen. *Vignea* rely on the distinction between androgynous (pistillate flowers proximal in the inflorescence and staminate flowers distal) and gynecandrous (staminate flowers proximal, pistillate distal) spikes. Egorova (1999) considered those with gynecandrous spikes to be more advanced. Species representing four different sections with gynecandrous spikes were included in the *Vignea* data matrix. Three of those sections (*Glareosae, Ovales, Stellulatae*) are each strongly supported as monophyletic with moderate support for the fourth (*Deweyanae*), but there is no support for the hypothesis that they form a single lineage. In fact, sect. *Deweyanae* is firmly nested within a clade of androgynous species, rather than in a sister-group relationship to sect. *Ovales,* as might be predicted from shared features such as gynecandrous spikes and the presence of very narrow wings on the perigynia similar to those found in sect. *Ovales.*

In contrast to the gynecandrous sections, there is little or no support for the monophyly of any of the recognized sections of androgynous species, but one weakly (parsimony analysis) to moderately (Bayesian analysis) supported clade combines androgynous species with crowded, condensed simple or compound inflorescences from four sections (*Heleoglochin, Multiflorae, Phaestoglochin,* and *Vulpinae*) that have implicitly (Mackenzie 1931–35) or explicitly (Koyama 1962; Standley 2002) been considered to be closely related. Sections *Phaestoglochin* and *Vulpinae* are both clearly polyphyletic and support for the monophyly of the two *Heleoglochin* species is very weak in all analyses. While the species of sect. *Phaestoglochin* with crowded, sometimes condensed, paniculate spikes are part of the androgynous clade mentioned above, the *C. rosea* group, with simple, sessile, few-flowered androgynous spikes that are separated from one another on the culm, are strongly supported as the basal clade in most of our analyses. While the basal position is probably an artifact of the sampling scheme, as discussed above, it is quite clear that this group, long recognized as a distinctive species complex (Webber and Ball 1984), diverged much earlier than the other species of sect. *Phaestoglochin* and may warrant recognition as a distinct section.

Sampling within the large subgen. *Vignea* is too limited in this study to allow more than a few comments on the relationships of species in monotypic sections. *Carex disperma,* allied with sect. *Glareosae* in some classification schemes (Mackenzie 1931–35; Fernald 1950), but more often segregated as a monotypic section (Ohwi 1936; Chater 1980; Jermy et al. 1982; Egorova 1999), is strongly supported as part of the clade that includes sect. *Glareosae* in the Bayesian analysis. However, the Bayesian analysis also strongly supports a close relationship between *C. chordorrhiza* and *C. disperma,* which form a clade that is sister to the *Glareosae* clade. It is interesting to note that the only shared mutation in the highly conserved 5.8S ribosomal gene was shared by *C. chordorrhiza* and *C. disperma* together with all representatives of sects. *Glareosae* and *Ovales.* While sometimes treated as the monotypic sect. *Chordorrhizae* (e.g., Ball and Reznicek 2002), *C. chordorrhiza* has more often been considered part of sect. *Divisae* (Chater 1980; Egorova 1999), with which it shares many inflorescence and perigynia features but from which it differs in growth form. Neither the sister-group relationship of *C. chordorrhiza* and *C. disperma,* nor the sister-group relationship of this clade to sect. *Glareosae* receives even weak statistical support in the parsimony analysis. With broader sampling among *Vignea* species, it is quite possible that other species will emerge as closer relatives to either or both of these species.

Phylogeny of the Core Carex *Clade*

Overall relationships and the inclusion of subgenus Vigneastra.—Clade D, representing the Core *Carex* clade that groups species classified in subgen. *Carex, Indocarex* (= *Vigneastra*), and *Primocarex* (= *Psyllophora*) in Kükenthal's (1909) monograph, is well supported in all individual gene analyses and strongly supported by the combined analysis. Two species (*C. scirpoidea,* sect. *Scirpinae;* and *C. pauciflora,* sect. *Leucoglochin*) that were placed by Kükenthal in subgen. *Primocarex* (= *Psyllophora*) but allied with subgen. *Carex* by others (Bailey 1886; Mackenzie 1931–35; Ohwi 1936; Koyama 1962) were included in our analyses. The placement of *C. scirpoidea* in subgen. *Carex* is confirmed by this study and previous ones (Roalson et al. 2001; Starr et al. 1999, 2004), but as in these previous analyses, *C. pauciflora* is unambiguously nested within the Core Unispicate clade along with other unispicate carices rather than in the Core *Carex* clade.

In line with previous molecular analyses (Yen and Olm-

stead 2000*a*, *b*; Roalson et al. 2001; Starr et al. 2004), the five species of subgen. *Vigneastra* included in this study are all well supported as part of the Core *Carex* clade. Only two of the five species of *Vigneastra* are supported as a monophyletic group, providing some support for the idea that this is a heterogeneous assemblage better classified within subgen. *Carex* (Ohwi 1936; Raymond 1959; Koyama 1962). With the broader sampling among subgen. *Carex* in this study, *C. baccans* (subgen. *Vigneastra*) is weakly supported as sister to *C. pensylvanica* (sect. *Acrocystis*), rather than as sister to *C. cruciata,* as in a previous analysis of Starr et al. (1999), in which subgen. *Carex* was represented by only a few species and only ITS data were used. Most intriguing is the well-supported (92%) basal position of *C. cruciata.* Although previous molecular analyses (Starr et al. 1999, 2004) have also placed this species in a basal position (either alone or in a clade with *C. baccans*), support for this hypothesis has always been weak (i.e., <64% BS). The very good support for *C. cruciata* as separate from other *Vigneastra* lends further support to previous molecular (Starr et al. 1999, 2004; Yen and Olmstead 2000*a*, *b*) and morphological studies (Ohwi 1936; Koyama 1962) suggesting that subgen. *Vigneastra* should be merged with subgen. *Carex.* Moreover, this basal position is also compatible with many previous arguments based on phytogeography (Kreczetovicz 1936; Nelmes 1951; Ball 1990) and inflorescence structure (Kreczetovicz 1936; Koyama 1962; Smith and Faulkner 1976), which suggested that subgen. *Vigneastra* has plesiomorphic morphology and is a possible progenitor to the larger subgen. *Carex/Vigneastra/Psyllophorra* p. p. min. lineage. Nevertheless, because of the poor sampling of subgen. *Vigneastra* in this and previous studies (less than six of ca. 100 species worldwide), any decision regarding its taxonomic status or evolutionary significance can only be considered as preliminary and tentative. Most puzzling in the current analyses is the position of *C. echinochloe,* which is strongly supported in both combined analyses as sister to *C. punctata,* a species from which it differs markedly in overall morphology, inflorescence structure, and geographic distribution. The fact that this relationship is not supported by *trn*L–*trn*F data, which places *C. echinochloe* in the same clade with other *Vigneastra* species, *C. filicina,* and *C. polystachya,* is intriguing and suggests that another sample of *C. echinochloe* should be studied before any conclusions can be drawn.

Polyphyly of sect. Hymenochlaenae.—Thirteen species that have been classified in sect. *Hymenochlaenae* (Drejer) L. H. Bailey were included in these analyses. Section *Hymenochlaenae,* using any of the many different circumscriptions (e.g., Bailey 1886; Kükenthal 1909; Koyama 1962 [as a subsection]; Reznicek 1986) is polyphyletic, with the 13 sampled species found in five different lineages in our analyses. The different lineages are not congruent with Kükenthal's (1909) subsections of *Hymenochlaenae,* nor with the sections that were segregated from it by Mackenzie (1931–35).

Based on Reznicek's (1986) lectotypification of sect. *Hymenochlaenae* with *C. cherokeensis,* the only sampled species that can be placed into a monophyletic sect. *Hymenochlaenae* are *C. cherokeensis* and *C. obispoensis. Carex obispoensis* is a tall, robust sedge endemic to chaparral and open *Cupressus sargentii* Jeps. woodlands in southern California, and its affinities have never been clear. Although placed in sect. *Sylvaticae* Rouy (the sectional name used by Mackenzie [1931–35] for North American species in Kükenthal's subsect. *Debiles* (Carey) Kük. of sect. *Hymenochlaenae*) when originally described (Stacey 1936), it was anomalous there due to its numerous staminate spikes, androgynous lateral spikes, and the tendency to have fascicles of spikes at one or more nodes of the inflorescence. Sequence data place it quite clearly as a close relative of the much smaller *C. cherokeensis* from the southeastern USA, which also tends to have more than one staminate spike and frequently has two, sometimes androgynous, lateral spikes at a node. Egorova (1999) considered fascicled, androgynous lateral spikes to be primitive and suggested they might be an early stage in evolution from the paniculate inflorescences with androgynous secondary spikes of subgen. *Vigneastra* to the racemiform inflorescences with pistillate lateral spikes found in many species of subgen. *Carex.* It is interesting to note that *C. cherokeensis* and *C. obispoensis* are part of clade D3, which includes three of the five species of subgen. *Vigneastra* sampled for these analyses.

Eight of the remaining sampled species that have been classified, by one author or another, into sect. *Hymenochlaenae* form a strongly supported clade that also includes *C. tenax* (sect. *Hallerianae*). The most recent floristic treatment of North American *Carex* (Ball and Reznicek 2002) places these nine species into four different sections: *C. debilis, C. mendocinensis,* and *C. misera* into an admittedly unnatural sect. *Hymenochlaenae* (Waterway 2002); *C. complanata, C. swanii,* and *C. virescens* into sect. *Porocystis* (= *Virescentes* (Kunth) Mackenzie); *C. whitneyi* into sect. *Longicaules*; *C. tenax* into sect. *Hallerianae*; and *C. hirtifolia* into a newly created monotypic sect. *Hirtifoliae* (Reznicek 2001). These species were also dispersed among sects. *Sylvaticae, Gracillimae* J. Carey, *Virescentes, Triquetrae* (L. H. Bailey) Mack, and *Longicaules* in Mackenzie's previous treatment of North American species (Mackenzie 1931–35). In his modified circumscription of sect. *Hymenochlaenae,* Reznicek (1986) included the species that Mackenzie (1931–35) had placed in sects. *Sylvaticae, Gracillimae, Longirostres* (Kük.) Mack., and *Viridiflorae* Mack., but specifically excluded sect. *Longicaules,* citing its ''short-cylindric, erect spikes, and sheathless or nearly sheathless inflorescence bracts'' as differences from sect. *Hymenochlaenae.* Pubescence on foliage or perigynia and the length of the sheath on the proximal inflorescence bract determined whether species were previously included in or excluded from sect. *Hymenochlaenae.* Bailey (1886) following Drejer (1844) included the *Virescentes* group (= sect. *Porocystis*) but excluded *C. hirtifolia, C. whitneyi,* and *C. dasycarpa* Muhl. (from which *C. tenax* was later segregated), while Kükenthal (1909) included *C. hirtifolia* and *C. whitneyi* but excluded *C. dasycarpa* and species now placed in sect. *Porocystis.*

The apparently natural group that forms clade D4b all have membranous perigynia, maroon coloring on the basal sheaths, and an inflorescence in which staminate flowers are restricted to the terminal spike and lateral spikes are elongate or cylindrical, peduncled, and usually pistillate. They have been classified into different sections based on differences in pubescence of foliage or perigynia, whether the perigynium is obviously beaked or not, whether the proximal inflo-

rescence bract has an obvious sheath, and whether the terminal spike is staminate or gynecandrous. The strong statistical support for this group in both parsimony and Bayesian analyses (Fig. 1, 2) and the short branch lengths within the clade suggest that the species are closely related and possibly recently evolved. Easily observed characters such as pubescence, which also varies within species in this group (Waterway 1996, 2002), and sheath length, which varies within populations and sometimes even among culms within individuals (MJW pers. obs.) should not be used to arbitrarily divide this natural group into several sections. Our analyses support the inclusion of at least species from sects. *Hallerianae, Hirtifoliae,* and *Longicaules* into an expanded sect. *Porocystis* along with some, but not all, of the species that Kükenthal included in his broadly circumscribed sect. *Hymenochlaenae.* In the present analyses, this strongly supported clade includes only North American species. Whether *C. pallescens,* a widespread Eurasian species that is also apparently native to North America, is part of this group is not clear. In the parsimony tree (Fig. 1), it is placed as a sister group to the ''expanded *Porocystis*'' described above, but in the Bayesian tree (Fig. 2), it is strongly supported as sister to *C. acutiformis* (sect. *Paludosae*), another widespread species with Eurasian and African distribution. Further investigation of this larger group is required before formal changes in sectional circumscription can be made.

The two remaining species sampled from sect. *Hymenochlaenae* are part of two different lineages: *C. prasina* is sister to the small clade formed by two species of sect. *Limosae,* but without statistical support, while *C. sylvatica,* a common species of European forests, is strongly supported as sister to *C. pendula* (sect. *Rhynchocystis*), another common European species. The relationships of these species will be discussed below in the context of their sister groups.

Polyphyly of the ''bladder sedges.''—Species with relatively large, inflated perigynia that are usually long beaked with bidentate apices and contain nutlets with persistent styles, have previously been classified together (Drejer 1844; Bailey 1886; Kükenthal 1909) as sect. *Physocarpae* Drejer. These ''bladder sedges'' are commonly considered to be a natural group (Bailey 1886; Kukkonen 1963), although they are now usually treated as several related sections: *Carex, Lupulinae, Paludosae, Pseudocypereae* (Tuck.) H. Christ, *Rostrales, Squarrosae,* and *Vesicariae.* Distribution of species among sects. *Carex, Paludosae, Pseudocypereae,* and *Vesiciariae* has varied among authors, with Mackenzie (1931–35) being the most divergent. He placed his sect. *Hirtae* Tuck. ex Kük. (= sect. *Carex* p. p.) close to sect. *Virescentes* (= *Porocystis*) rather than with the other ''bladder sedges,'' presumably due to the pubescent perigynia shared by the two groups. Reznicek and Catling (2002) circumscribed sect. *Carex* to include only a small group of species with true vegetative culms and long-beaked perigynia, and noted that even with the removal of these species, sect. *Paludosae* is ''broadly construed'' and needs worldwide revision (Reznicek and Catling 2002). The monotypic sect. *Collinsiae* with multispicate inflorescences and spreading subulate perigynia enclosing nutlets with persistent styles has also frequently been allied with sect. *Lupulinae* or *Rostrales* (Mackenzie 1931–35; Standley 2002). *Carex pauciflora* (sect. *Leucoglochin*), with unispicate inflorescences and treated as part of subgen. *Primocarex* by Kükenthal, has also been allied with the ''bladder sedges'' by some authors (e.g., Bailey 1886; Mackenzie 1931–35; Savile and Calder 1953; Koyama 1962). Savile and Calder (1953), in a phylogenetic scheme that was based in part on relationships with parasitic fungi, went so far as to combine all of the groups mentioned above into the new subgen. *Kuekenthalia* Savile & Calder. Their proposal did not receive much support from other caricologists, but the notion that these species are closely related has persisted to the present, with the relevant sections generally treated near one another in floras that are loosely arranged in a putative phylogenetic sequence (e.g., Chater 1980; Jermy et al. 1982; Gleason and Cronquist 1991; Egorova 1999; Ball and Reznicek 2002).

The analyses presented here clearly demonstrate that this group is polyphyletic, with species represented in six different lineages on the trees resulting from both analyses (Fig. 1, 2). The largest core group (clade D4d; Fig. 1, 2) including members of sects. *Vesicariae, Lupulinae, Paludosae* (sensu Reznicek and Catling 2002 [but not as to type]), and *Carex* is strongly supported as monophyletic in all analyses. These sections consistently have large inflated perigynia, sturdy and usually persistent geniculate styles, and robust growth form. Most, but not all, grow in paludal habitats and many have diploid chromosome numbers higher than 60. *Carex acutiformis,* the type species of sect. *Paludosae,* and *C. vestita,* also usually classified in sect. *Paludosae,* have smaller, thinner perigynia, and less rigid styles, and are not included in this core clade. Instead they show greater sequence similarity to *C. scabrata* and *C. amplifolia* from sect. *Anomalae,* although this relationship is not strongly supported. The placement of *C. acutiformis* in a different clade from *C. riparia* is in accord with the viewpoint of Egorova (1999) who transferred all Eurasian species of sect. *Paludosae,* including *C. riparia,* to sect. *Tumidae* Kük. except for *C. acutiformis.* Sections *Vesicariae* and *Lupulinae,* represented by six and three species, respectively, are paraphyletic within this larger group in both analyses. A close relationship between *C. comosa* and *C. hystericina,* more often segregated from sect. *Vesicariae* as part of sect. *Pseudocypereae* (Mackenzie 1931–35; Fernald 1950; Gleason and Cronquist 1991; Egorova 1999), is strongly supported, but this species pair is nested within a clade that also includes species from sects. *Vesicariae* and *Lupulinae.* Two groups have long been recognized within the small North American sect. *Lupulinae* (Reznicek and Ball 1974), with *C. intumescens* and *C. grayi* differing from the other four species in many characters, including growth form, shape and texture of perigynia and achenes, number and position of spikelets, length of lowest bract sheath, chromosome numbers, and nature of the silica bodies in the epidermal cells of the nutlets (Menapace et al. 1986). Similarly, *C. retrorsa* has been considered ''anomalous'' within sect. *Vesicariae* and shares many features with the *C. lupulina* group as well as hybridizing with both *C. lupulina* and *C. lupuliformis* Sartw. ex Dewey (Reznicek and Ball 1974). Our analyses are consistent with the structural comparisons in both cases: *C. intumescens* and *C. grayi* form the sister clade to the entire core group, rather than grouping with *C. lupulina,* and *C. retrorsa* is well supported as sister to *C. lupulina* rather than to any of the other five species of

Vesicariae. It is clear that at least the currently recognized sects. *Vesicariae, Lupulinae,* and *Paludosae* will need realignment or merger if monophyly is to be the criterion for sectional circumscription. Much broader sampling across sects. *Carex, Paludosae,* and *Vesicariae,* currently in progress by the first author, is required before these decisions can be made.

Two small sections of ''bladder sedges'' are each strongly supported within different lineages from the core assemblage and from each other. Section *Squarrosae,* represented by *C. squarrosa* and *C. typhina,* forms a well-supported clade with *C. shortiana,* a similar species that often hybridizes with at least one of the *Squarrosae* species (Cayouette and Catling 1992; Cochrane 2002). This group is weakly supported in the parsimony analysis and strongly supported in the Bayesian analysis as part of a clade that includes species from sects. *Phacocystis* and *Racemosae,* a relationship that has not been suggested previously. Similarly, the strongly supported clade formed by the two species representing sect. *Rostrales* is well supported (parsimony analysis) or strongly supported (Bayesian analysis) as sister to an assemblage of species with which it has not previously been allied, including representatives of sects. *Ceratocystis, Indicae, Rhynchocystis,* and *Spirostachyae.* Many of these groups are widespread with representatives in Europe, Asia, Africa, and Australia. Broader geographic sampling is needed to test the monophyly of this assemblage and determine relationships within it.

The association of multispicate *C. collinsii* and unispicate *C. pauciflora* (sect. *Leucoglochin*) with the ''bladder sedges,'' as indicated by Mackenzie (1931–35) in his sectional sequence and as advocated by Savile and Calder (1953) in their circumscription of the new subgen. *Kuekenthalia* is not supported by our analyses. *Carex pauciflora* has no apparent relation to the ''bladder sedges,'' instead it groups with other unispicate species in the Core Unispicate clade (clade A, Fig. 1, 2). The placement of the monotypic sect. *Collinsiae* as sister to the *Griseae/Careyanae* clade has not been predicted and is difficult to justify based on morphology.

Relationships among sections Ceratocystis, Spirostachyae, Rhynchocystis, *and* Rostrales.—The relationship between sects. *Ceratocystis* and *Spirostachyae* has also been controversial (Crins and Ball 1988) with some authors uniting the two as sect. *Extensae* Fries (Mackenzie 1931–35) or under the sect. *Spirostachyae* (Kükenthal 1909), while others maintain them as distinct sections (Chater 1980; Jermy et al. 1982; Egorova 1999). Both sections have widespread distribution across three or more continents, although the only species of *Spirostachyae* in North America are recent introductions (Crins 2002). The two sections can be distinguished by the shape and structure of the pistillate spikelets, shape of the nutlets, the presence or absence of reddish-brown crystalline inclusions in the epidermal cells of the perigynia, and the flavonoid composition (Crins and Ball 1988). In both parsimony and Bayesian analyses, the two species of *Ceratocystis* are strongly supported as a subclade distinct from the subclade that includes *C. punctata* (sect. *Spirostachyae*), but all three species are very well supported (94% BS) as a larger clade that also includes the European species, *C. sylvatica* (sect. *Hymenochlaenae* sensu Kükenthal 1909; sect. *Sylvaticae* sensu Jermy et al. 1982) and *C. pendula* (sect. *Rhynchocystis*) as well as the African species, *C. echinochloe* (subgen. *Vigneastra*), and the two species of sect. *Rostrales,* as noted above. Our analyses are thus in agreement with Chater (1980) and Crins and Ball (1988) who recognized the affinity of these two groups while maintaining them as distinct sections. Support for the monophyly of *C. sylvatica* with *C. pendula* is also strong in both analyses. This supports Chater's (1980) placement of both species in sect. *Rhynchocystis,* rather than their classification into different sections as suggested by Kükenthal (1909), Jermy et al. (1982), and Egorova (1999), who specifically said that the two were ''not related phylogenetically'' (Egorova 1999). Despite the very good support for the clade including all eight species and the strong support for each species pair, relationships among the species pairs have only weak support in the combined analysis and weak or no support in the individual analyses, so we can draw no further conclusions about this lineage.

Relationships among sections Laxiflorae, Paniceae, Griseae*, and* Careyanae.—Five species included in the present study belong to sections that are exclusively or predominantly distributed in eastern North America, usually in deciduous forest habitats. *Carex albursina* and *C. blanda* belong to sect. *Laxiflorae,* a group that was formerly circumscribed (Kükenthal 1909; Mackenzie 1931–35; Fernald 1950; Bryson 1980; Manhart 1986; Gleason and Cronquist 1991) to include species now segregated as sect. *Careyanae,* represented here by *C. plantaginea.* Naczi advocated recognition of the two groups (Naczi 1992; Naczi et al. 2002) identified by Bryson (1980), based on morphology, and Manhart (1986), based on secondary chemistry, as two distinct sects. *Laxiflorae* s.s. and *Careyanae.* Phylogenetic analysis using morphological data (Naczi 1992) indicated that sect. *Careyanae,* so defined, did not form a monophyletic group with sect. *Laxiflorae* but was more closely related to species in sects. *Griseae* and *Oligocarpae* (Heuff.) Mack., which together formed a clade. These ideas are fully supported by the present analysis, although sampling from each group was very limited.

The two sampled species of sect. *Laxiflorae* are strongly supported as monophyletic, as is their close relationship to species of sect. *Paniceae.* Affinities between sect. *Laxiflorae* and sect. *Paniceae* have been previously implied or specified (Mackenzie 1931–35; Koyama 1962), and species originally described as part of sect. *Paniceae* (e.g., *C. chapmanii* Steud.) are now classified in sect. *Laxiflorae* (Bryson and Naczi 2002). This relationship between sects. *Laxiflorae* and *Paniceae* was also strongly supported in the analyses of Roalson et al. (2001), where the sections were represented by two different species. Their analyses, as well as those of Waterway et al. (1997) and Heindrichs et al. (2004*a*), also support the inclusion of sect. *Bicolores* (Tuck. ex L. H. Bailey) Rouy in this monophyletic group, as earlier suggested by Bailey (1886) and implied by the sectional sequence of Mackenzie (1931–35), Fernald (1950), and Ball and Reznicek (2002).

The three species representing sects. *Careyanae* (*C. plantaginea*) and *Griseae* (*C. hitchcockiana* and *C. oligocarpa*) are also strongly supported as monophyletic in the combined

analyses and in two of the three individual gene analyses (ETS-1f and *trn*L–*trn*F). There is no support for a sister-group relationship between this clade and the *Laxiflorae/Paniceae* clade. Instead, both the ETS analysis and the combined analysis strongly support a clade formed by the *Careyanae/Griseae* group with the monotypic sect. *Collinsiae* and two western North American endemics from sect. *Aulocystis.* Previous suggestions regarding the relationships of *C. collinsii* have placed it near sect. *Rostrales* based on narrow, elongated, somewhat inflated perigynia, while affinities with sect. *Hymenochlaenae* have been suggested for sect. *Aulocystis.* The novel arrangement shown in our combined analysis may represent a distinct evolutionary lineage from which we have sampled only a small fraction of the species and, as a result, have difficulty recognizing the basis for the relationship. However, since support for this larger clade is only significant in the combined analysis and in the analysis using only ETS-1f, which dominates the combined analyses, we should be cautious in interpreting this set of relationships until more species within the group are sampled and support from other gene regions has been demonstrated.

Other sectional relationships.—Several authors have implicitly (Mackenzie 1931–35; Jermy et al. 1982; Ball and Reznicek 2002) or explicitly (Bailey 1886; Kükenthal 1909) suggested a close relationship among some or all of sects. *Racemosae* (= *Atratae* (Heuff.) H. Christ in older works), *Scitae* Kük., *Limosae,* and *Phacocystis* (= *Acutae* (J. Carey) H. Christ in older works). Egorova (1999) considered species in these sections (except *Phacocystis,* which she placed in a new subgen. *Kreczetoviczia* Egorova) to be related and among the most advanced in subgen. *Carex.* These groups are poorly represented in our analyses, but they do show substantial sequence similarity and are frequently grouped together (but without statistical support) in the clade that includes clade D4c, the *Shortianae/Squarrosae* clade discussed above. The two species of sect. *Limosae* form a well-supported clade in both analyses, but the two species of sect. *Phacocystis* are only supported as monophyletic in the Bayesian analysis and in the parsimony analysis using *trn*L data alone. Section *Racemosae* is polyphyletic with *C. stylosa* forming an unsupported clade with sect. *Limosae* and *C. prasina* and *C. mertensii* supported as sister to *C. aquatilis* (sect. *Phacocystis*) in the combined parsimony analysis and sister to the *C. aquatilis/C. crinita* clade in the Bayesian tree. The polyphyly of sect. *Racemosae* is not surprising given Nannfeldt's (1977) description of this section as ''notoriously unnatural.'' Our analyses provide no support for Egorova's subgen. *Kreczetoviczia,* nor do they support combining sects. *Limosae* and *Paniceae,* as has been suggested by Koyama (1962). However, much more extensive sampling of these large groups is needed to gain an understanding of their evolutionary relationships.

Species from sects. *Clandestinae* (= *Digitatae* (Fries) H. Christ), *Acrocystis, Pictae* Kük., and *Lamprochlaenae* have also been explicitly (Koyama 1962; Savile and Calder 1953) or implicitly (Mackenzie 1931–35; Ball and Reznicek 2002) grouped together. Savile and Calder (1953) noted the similarity of smuts attacking species from these sections while Egorova (1999) considered species in these groups to be among the most advanced in subgen. *Carex,* in part due to their tolerance for dry habitats. The presence of species from all of these groups in clade D3 suggests affinity, but this clade is well supported only in the Bayesian analysis and also includes species from sects. *Hymenochlaenae* s.s., *Abditispicae,* and subgen. *Vigneastra.* The position of *C. depauperata* in this clade is curious, since it has much larger, distinctive perigynia, and the section in which it is placed (*Depauperatae* or *Rhomboidales* Kük.) is often aligned with sect. *Paniceae* (Kükenthal 1909; Jermy et al. 1982) rather than with any of the groups in clade D3. Furthermore, sect. *Clandestinae* appears to be polyphyletic, with *C. pedunculata* nested within clade D4 and *C. digitata* in clade D3. This is in accordance with the findings of Roalson et al. (2001), where the five sampled species of this group belonged to four different lineages, and of Heindrichs et al. (2004*a*) with seven sampled species in three different lineages. Many species in these groups are widely distributed in temperate regions, and a more global approach to sampling will be required to clarify their relationships.

Conclusions and Recommendations for Future Studies

In conclusion, it is worth noting that none of the previous hypotheses regarding the evolution of tribe Cariceae receive significant support from our analyses. Neither the various theories suggesting that different unispicate groups gave rise to different genera or different groups within *Carex* (or vice versa) (Kreczetovicz 1936; Nelmes 1952; Smith and Faulkner 1976), nor the idea that subgen. *Vigneastra* is primitive within the tribe (Nelmes 1951), nor the more recent theory of Reznicek (1990) that species with compound inflorescences similar to those found in some members of subgen. *Vignea* may be the progenitors of *Carex,* are even weakly supported by these or previous molecular analyses. Molecular analyses also fail to support Egorova's (1999) ideas about trends from primitive wetland to more advanced dry land species within subgen. *Carex,* nor the segregation of subgen. *Kreczetoviczia* from subgen. *Carex.* The two largest lineages appear to have diverged early in the evolution of the tribe with the *Vignea* clade and Core *Carex* clade evolving independently from each other and probably from the Core Unispicate and *Schoenoxiphium* clades for a very long time, although the existence and position of a Caricoid clade remains equivocal, particularly the relationship of *Schoenoxiphium* to the other groups. Given the strong monophyly of the tribe, it is likely to have had a single origin, but this group has either not yet been included in molecular studies, or radiation was rapid from a common ancestor that is now extinct (cf. Egorova [1999] who argues for parallel development of the different groups of *Carex* from an extinct ''ProtoCarex'').

On the other hand, our analyses support the monophyly of core groups within some previously recognized sections and they are consistent with several earlier ideas about relationships within subgen. *Vignea* and *Carex.* Although there is no support for species of subgen. *Vigneastra* as primitive within the tribe, there is support for the basal position of at least some species of this group within the Core *Carex* clade. As in the previous analysis of Starr et al. (2004), there is also support for a monophyletic group of unispicate *Kobresia* species and for differential placement of androgynous vs.

dioecious unispicate *Carex* species. Our analyses have also suggested some new groupings within the Core *Carex* and *Vignea* clades, most quite plausible based on morphology, but some novel assemblages require serious investigation.

As we have noted throughout the text, more sampling is needed before we can fully understand relationships among Cariceae. In particular, *Kobresia* and *Schoenoxiphium* need detailed work, and the remaining unispicate carices should be added to the growing list of sequenced species. The species of subgen. *Vigneastra* should also be a very high priority. Within subgen. *Carex,* potentially primitive groups with androgynous lateral spikes (e.g., *Decorae* (Kük.) Ohwi), those with paniculiform inflorescences (e.g., *Fecundae* Kük.), and those with very low chromosome numbers (e.g., *Siderostictae* Franch. ex Ohwi) should be sampled. Within subgen. *Vignea,* more species with three stigmas (e.g., *Macrocephalae, Gibbae* Kük.) and additional species with compound inflorescences should be added to molecular phylogenetic analyses.

While adding these groups should go a long way toward understanding evolutionary relationships and trends in character evolution, more intensive sampling of all groups is needed to achieve the goal of a stable and robust phylogeny that can be used in studies of adaptive radiation, niche conservatism, community assembly, biogeography, and the evolution of diversity. The tribe Cariceae, with its broad geographic distribution, wide range of habitats, and mix of species-rich and species-poor lineages has the potential to become a model system for such studies. To make this possible, we need to broaden the geographic sampling to include species from eastern Asia, subtropical Southeast Asia, Africa, New Zealand, South America, and add more species from Eurasia as well as doing intensive sampling within many of the larger clades.

With more than 2100 species to be sampled, the DNA regions to be sequenced must be chosen to give maximum information with minimal investment in time and expense. All three DNA regions, used independently for the 99-taxon data matrix, were able to recover the two largest major clades with significant statistical support in our analyses, but only the combined data allowed resolution of many other major groups. Our analyses suggest that combined data from the *trn*L–*trn*F region and ETS-1f were most useful at resolving and providing support for interior branches within the tree, while ETS-1f and ITS data were most variable among closely related species. The new cpDNA spacer region used in the *Vignea* analysis was not particularly variable in this group, but substitution rates in all DNA regions were lower in subgen. *Vignea,* suggesting that exploration of variability in the *trn*E–*trn*D region in other parts of the tree would be warranted, especially since homoplasy was extremely low for characters from this gene region.

As more species are included in the analyses, more parsimony-informative characters will be needed. A nested strategy, using different but overlapping sets of DNA regions, will help to resolve the ''backbone'' tree, as well as the resolution within individual clades. The clades that we already know are well supported (e.g., the ''expanded *Porocystis*'' clade, the *Laxiflorae/Paniceae* clade, the *Careyanae/Griseae* clade, or various sections within *Vignea*) and can be the subject of intensive studies (sampling many more species) using the more variable DNA regions (ETS-1f, ITS, *trn*L–*trn*F or other variable nuclear or chloroplast introns to be developed). The ''backbone'' tree can be further developed with somewhat more conservative DNA regions as species from as yet unstudied groups are added to exemplars from each major clade. More conservative regions could include *trn*L–*trn*F, *trn*E–*trn*D, or other variable chloroplast introns and spacer regions, or even rapidly evolving coding genes. A more thorough search for variable DNA regions at both ends of the spectrum and a coordinated sequencing strategy involving researchers from different geographic regions is needed before a fully resolved phylogenetic hypothesis for Cariceae can become a reality.

ACKNOWLEDGMENTS

The analyses presented here could not have been conducted without the competent and cheerful laboratory and field assistance of Tara McKay, Sofia Fuga, Philippe Roof, Yanik Bérubé, Greg Taylor, Nadia Surdek, Marie-Eve Rheault, Charles Latrémouille, Nadia Cavallin, Caroline Gallant, Anna Dabros, and Tracy Eades. We also thank Martin Lechowicz, Tony Reznicek, Phillip Hyatt, Nigel Blackstock, Paul Ashton, Leslie Gold, and Eric Roalson for help in finding *Carex* species to sample or providing samples for DNA sequencing. We are grateful to Alan Yen for sharing the primers he developed to amplify the *trn*E–*trn*D region. Alan Yen, Richard Olmstead, Sean Graham, Bruce Ford, and Tony Reznicek provided advice, assistance, and helpful discussions at various stages of the project. We also acknowledge with thanks financial support from a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (NSERC), team grants from the Fonds Québécois de la Recherche sur la Nature and les Technologies (FQRNT), and the Canadian Foundation for Innovation to MJW, and an NSERC postdoctoral fellowship to JRS.

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