

Phylogenetic Relationships in Cariceae (Cyperaceae) Based on ITS (nrDNA) and *trnT-L-F* (cpDNA) Region Sequences: Assessment of Subgeneric and Sectional Relationships in *Carex* with Emphasis on Section *Acrocystis*

ERIC H. ROALSON^{1,2}, J. TRAVIS COLUMBUS, and ELIZABETH A. FRIAR

Rancho Santa Ana Botanic Garden, 1500 North College Avenue, Claremont, California 91711-3157

¹Present address: LMS, MSC, MRC534, Smithsonian Institution, 4210 Silver Hill Rd., Suitland, Maryland 20746

²Author of correspondence

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ABSTRACT. With approximately 2,000 species, *Carex* is the largest genus in the Cyperaceae and is one of the most widespread genera in the world. Relationships within *Carex* and among the genera of the Cariceae (*Carex*, *Cymophyllus*, *Kobresia*, *Schoenoxiphium*, and *Uncinia*) are unclear. For this reason, a molecular phylogenetic study employing nrDNA ITS and cpDNA *trnT-L-F* spacer sequences was undertaken. In addition to creating hypotheses of relationship for the Cariceae and testing classifications of this tribe, a primary goal of this study was to assess relationships within *Carex* section *Acrocystis* and identify a monophyletic group for more detailed study. These analyses suggest that *Cymophyllus*, *Kobresia*, *Schoenoxiphium*, and *Uncinia* are nested within *Carex*. Three primary clades are suggested: a *Carex* subgenus *Vignea* clade, a clade including *Carex* subgenus *Primocarex* (for the most part) and the other genera of Cariceae, and a clade predominately comprised of *Carex* subgenera *Carex* and *Indocarex*. A large part of *Carex* section *Acrocystis* forms a monophyletic group but several Eurasian species are more closely related to other groups rather than to this core clade. Assessment of chromosome number variation across the Cariceae clade suggests that the ancestor of the Cariceae had a moderate to high chromosome number. In addition, these analyses suggest the sister group of the Cariceae is a clade including *Scirpus* sensu stricto, *Amphiscirpus*, and *Dulichium*.

With approximately 2,000 species, *Carex* is the largest genus in the Cyperaceae and is one of the most widespread genera in the world (Reznicek 1990). More than 600 species occur in North America alone (Hermann 1970). *Carex* is a member of tribe Cariceae Kunth ex Dumort. along with four other genera: *Cymophyllus*, *Kobresia*, *Schoenoxiphium*, and *Uncinia* (Kükenthal 1909; Reznicek 1990). While *Carex* is cosmopolitan in distribution, *Cymophyllus* (1 sp.) is restricted to southeastern North America; *Kobresia* (40 spp.) is best developed in the Himalayas with a few boreal species; *Schoenoxiphium* (17 spp.) is restricted to southern and eastern Africa and Madagascar; and *Uncinia* (50 spp.) is distributed primarily in the southern hemisphere (Reznicek 1990).

Recent molecular phylogenetic work has implied that *Cymophyllus*, *Kobresia*, and *Uncinia* are nested within *Carex* (Waterway et al. 1997; Yen and Olmstead, in press). Unfortunately, the intratribal relationships are still uncertain due to sparse sampling of the 70 or so recognized sections of *Carex* and generally poor cladistic resolution (i.e., low bootstrap support and unresolved polytomies) in these studies.

The Cariceae are well defined morphologically by unisexual flowers, with female flowers subtended by a bract (perigynium, a modified prophyll) the margins of which are partially or fully fused (Reznicek 1990). *Carex*, *Cymophyllus*, and *Uncinia* are characterized by fully fused perigynia, while *Kobresia* and *Schoenoxiphium* have partially to completely fused perigynia. Another character used to circumscribe genera is the morphology of the rachilla (a continuation of the axis that bears the female flower). In *Uncinia* the rachilla is topped by a hooked scale, while in *Kobresia* and *Schoenoxiphium* the rachilla often bears additional flowers. In *Carex* and *Cymophyllus* it is rarely exerted from the perigynium and never bears flowers or a hooked scale.

Carex has been usually split into four subgenera (*Carex*, *Indocarex*, *Primocarex*, and *Vignea*) and 70 or more sections, following the world-wide monograph of the Cariceae by Kükenthal (1909). For detailed historical reviews of supraspecific classification in *Carex* see Reznicek (1990) and Yen and Olmstead (in press). Comparing classifications of *Carex* is problematic because most are geographically restricted (Europe, Chater 1980; North America, Mac-

kenzie 1935; U.S.S.R., Kreczetowicz 1935, Egorova 1999).

The subgenera of *Carex* are distinguished primarily by inflorescence branching patterns and distribution of the unisexual flowers. *Primocarex* is characterized by a terminal spike inflorescence, while the inflorescence in the other subgenera is branched. *Vignea* has short bisexual spikes (following the terminology of Reznicek (1990)) and lacks a cladoprophyll (the prophyll of lateral branches). *Vignea* is also characterized by a false suture on the abaxial side of the perigynium, a feature not present in any of the other subgenera of *Carex* or other genera of the Cariceae. *Indocarex* always has bisexual spikes and perigynium-like cladoprophylls on secondary and tertiary branches, whereas subgenus *Carex* usually has single-sex spikes and ocreaform cladoprophylls (Reznicek 1990).

Homology of reproductive structures used in circumscribing members of Cariceae is uncertain (Smith and Faulkner 1976; Reznicek 1990). Assessing homology of morphological characters is beyond the scope of this paper, and will be dealt with elsewhere.

Although the phylogenetic position of section *Acrocystis* (= *Montanae*) within *Carex* is not well understood, it has played a central role in some early phylogenetic schemes. Heilborn (1924) suggested that *Acrocystis* is primitive within *Carex* owing to its low chromosome numbers. Savile and Calder (1953) postulated a basal position within subgenus *Carex* (as *Eucares*) along with section *Obtusatae* (Tuck.) Mack. They proposed that these sections were derived from members of *Primocarex*. Kukkonen (1963) generally agreed with Savile and Calder, but suggested that there are two lineages in *Carex*, each independently derived from *Kobresia*, one leading from *Kobresia* through *Carex* subgenus *Primocarex* to subgenus *Vignea* and the other from *Kobresia* directly to *Acrocystis* in subgenus *Carex*. Alternatively, Reznicek (1990) postulated that "it now seems clear that the tiny plants in section *Acrocystis*, with xeromorphic adaptations and reduced inflorescences, are highly specialized."

Acrocystis is distributed across Eurasia and North America, with one species in the Andes Mountains of Peru, Bolivia, and Argentina. Members of the section tend to inhabit the dry understory of coniferous and deciduous woodlands and often occur on calcareous or acidic soils (Mackenzie 1935). Circumscription of *Acrocystis* has varied especially in regards to the inclusion or exclusion of several Eurasian taxa. Kükenthal (1909) included *Carex tomen-*

tosa, *C. globularis*, and *C. grioletii* in his section *Pachystylae* rather than in *Acrocystis*, where they are currently treated (e.g., Chater 1980). Ohwi (1936) included nine species from Japan, Korea, and Taiwan; two of these were placed in other sections by Kükenthal, *C. mira* in *Frigidae* and *C. mandshurica* in *Pachystylae*. Koyama (1962) considered *Acrocystis* (as *Montanae*) as part of a more broadly circumscribed section *Digitatae*. Prior to this these groups had been separated by the sheathing base of the inflorescence bracts of *Digitatae* versus the non-sheathing bracts of *Acrocystis*. Koyama (1962) saw this character in conflict with "other characters seemed to be important." He did recognize a split in *Digitatae* s.l. along cytological lines. One group has fewer, larger chromosomes ($2n < 20$), while the other has more numerous, smaller chromosomes ($2n > 38$). These cytological groups do not correspond to the traditionally delimited *Acrocystis* and *Digitatae*. Koyama noted that the data of Savile and Calder (1953) support this circumscription as the smut fungi that infect *Digitatae* and related groups are indistinguishable from those infecting *Acrocystis*.

Some 49 taxa are recognized as belonging to *Acrocystis*, although the section has not been revised since Kükenthal's treatment (1909). Twenty-seven taxa are recognized in North American *Acrocystis*. Mackenzie revised this group (1913a, 1913b; 1935), describing eight new taxa. Since then, several revisions of species groups in the section have been produced. These studies have employed multivariate numerical analysis of morphological characters (Crins and Ball 1983), foliar flavonoid chemistry (Rettig and Giannasi 1990), and achene micromorphology (Rettig 1990). These studies did not include any Eurasian or South American species, nor was the relationship to other North American or Eurasian species postulated.

The goals of this study were five-fold: (1) to assess the relationships among the genera of the Cariceae and subgenera and sections of *Carex*, in particular those in subgenus *Carex*; (2) to assess the relationship of Eurasian *Acrocystis* to North American *Acrocystis* and test for monophyly; (3) to assess what sections or species may be sister to North American members of *Acrocystis*; (4) to assess previous classifications of the Cariceae; and (5) to address chromosome evolution in the Cariceae tribe.

MATERIALS AND METHODS

Sampling. Samples were selected from live plants grown at Rancho Santa Ana Botanic Garden

(RSA), live plants provided by Barb Wilson (OSC) and Takuji Hoshino (OKAY), dried material provided by Alan Yen (including the sample from NU), and herbarium specimens at NMCR, OSC, POM, RSA, TAES, and UCR. Outgroup sampling included live and herbarium material of genera in the Cyperaceae and Juncaceae (17 spp.; Table 1). The ingroup comprised 100 species (one species represented by two varieties) representing all five genera of the Cariceae and all four subgenera of *Carex* (Table 2). Representative species were chosen from as many of the genera, subgenera, and sections as were available from the Cariceae. Sampling favored members of *Carex* subgenus *Carex*. Voucher information is listed in Tables 1 and 2. Given the diversity of the Cariceae (> 2,000 species), 100 species represents only 5% of the total diversity of the tribe.

DNA Sequencing. DNA was isolated using a modified 2X CTAB buffer method (Doyle and Doyle 1987; Porter 1997). Templates of the nrDNA internal transcribed spacer region (ITS) were prepared using a 1:1 ratio of primers ITS5i (5'-AGG TGA CCT GCG GAA GGA TCA TT-3') and ITS4i (5'-GGT AGT CCC GCC TGA CCT GG-3'). The chloroplast spacer regions were amplified using a 1:1 ratio of the primers trnTf (5'-CAT TAC AAA TGC GAT GCT CT-3') and trnLr (5'-TCT ACC GAT TTC GCC ATA TC-3') for the *trnT-trnL* intergenic spacer (igs), trnLc (5'-CGA AAT CCG TAG ACG CTA CG-3') and trnLd (5'-GGG GAT AGA GGG ACT TGA AC-3') for the *trnL* intron (Taberlet et al. 1991), and trnLe (5'-GGT TCA AGT CCC TCT ATC CC-3') and trnLf (5'-ATT TGA ACT GGT GAC ACG AG-3') for the *trnL-trnF* igs (Taberlet et al. 1991). Polymerase chain-reaction (PCR) amplifications followed the procedures described by Baldwin (1992), Baldwin et al. (1995), and Roalson and Friar (2000).

The PCR products were electrophoresed using a 0.8% agarose gel in a 0.5X TBE (pH 8.3) buffer, stained with ethidium bromide to confirm a single product, and purified using the PEG precipitation procedure (Johnson and Soltis 1995).

Sequencing was performed using an Applied Biosystems Model 373A Automated DNA Sequencing System. Direct cycle-sequencing of purified template DNAs followed the manufacturer's specifications, using the PRISM[®] DyeDeoxy[™] Terminator Kit (PerkinElmer, Inc.).

Sequencing of the ITS region utilized the primers ITS5i, ITS4i, ITS2 (5'-GCT GCG TTC TTC ATC GAT GC-3'), and ITS3 (5'-GCA TCG ATG AAG AAC GCA GC-3'). Sequencing of the chloroplast spacers

made use of trnTf, trnLr, "trnLci" (5'-TCG GTA GAC GCT ACG GAC TT-3'), trnLd, trnLe, and trnLf primers.

Automated DNA sequencing chromatograms were proofed, edited, and contigs were assembled using Sequencher 3.0 (Gene Codes Corporation, Inc.). The sequences were truncated to include only ITS1, 5.8S, ITS2, the *trnT-trnL* igs, the *trnL* intron, and the *trnL-trnF* igs. Identification of the ends of ITS1 and ITS2 were based on comparisons with sequences from other species of Cyperaceae (Roalson and Friar 2000) and Genbank sequences, and the ends of the chloroplast spacers were determined by comparisons with other chloroplast spacer sequences (Taberlet et al. 1991; Columbus et al. 2000). The nrDNA sequences were aligned using Clustal W v. 1.4 (Thompson et al. 1994) at a gap cost:gap extension cost ratio of 10:5, followed by manual editing (Baum et al. 1994). The cpDNA sequences were manually aligned.

All sequences were deposited in GenBank (accessions AF284859 to AF285077).

Phylogenetic Analyses—Outgroup. In order to address tree rooting in the Cariceae, a preliminary analysis of genera from the Cyperaceae and Juncaceae was conducted using only the chloroplast *trnL* intron and *trnL-F* igs. Seventeen species were included, six of which are members of the Cariceae. These sequences were aligned with Clustal W v. 1.4 (Thompson et al. 1994) at a gap cost:gap extension cost ratio of 5:1. Analyses employing other gap cost:gap extension cost ratios did not significantly change the topologies, particularly of strongly supported clades. Gaps were not coded in this analysis and all characters received equal weight. *Luzula* was used as the outgroup taxon for this analysis based on the findings of Muasya et al. (1998). Phylogenetic analysis was as described below.

Phylogenetic Analyses—Ingroup. Maximum parsimony (MP) analysis was performed using PAUP* 4.0b2 (Swofford 1999). The analysis used heuristic searches (acctrans; 10 random addition cycles; TBR branch swapping; steepest descent) due to the size of the data set. Clade robustness was estimated using the 10,000 "fast addition" heuristic bootstrap replicates (Felstenstein 1985; Hillis and Bull 1993; Mort et al. 2000; as implemented by PAUP*4.0b2), and parsimony jackknife analysis (10,000 "fast addition" heuristic replicates; Farris et al. 1996; Mort et al. 2000). Three ingroup data sets were analyzed and compared: ITS, *trnL-L-F*, and a combination of these. All ingroup analyses utilized only one outgroup representative based on the out-

TABLE 1. Genera and species included in the outgroup analysis. Specimens deposited in RSA unless otherwise noted. All taxa are in the Cyperaceae except *Luzula* (Juncaceae).

Genus	Specific epithet	Locality, voucher, and GenBank accession number
<i>Amphiscirpus</i> Oteng-ÿéb.	<i>nevadensis</i> (S.Watson) Oteng-ÿéb.	U.S.A., California; <i>Helmkamp</i> s.n.; AF285066
<i>Bulbostylis</i> Kunth	<i>juncoides</i> (Vahl) Kük. ex Osten	Mexico, Sonora; <i>Roalson</i> 1350; AF285063
<i>Carex</i> L.	<i>alma</i> L.H.Bailey	U.S.A., California; <i>Ross</i> 7838; AF284923
	<i>capitata</i> L.	U.S.A., California; <i>DeDecker</i> 4899; AF284942
<i>Cladium</i> P.Browne	<i>communis</i> L.H.Bailey	Canada, Quebec; <i>Roalson</i> 1329; AF284874
<i>Cymophyllus</i> Mack.	<i>californicum</i> (S. Watson) O'Neill	U.S.A., Nevada; <i>Szeeringen</i> 1596; AF285072
<i>Cyperus</i> L.	<i>fraseri</i> Mack.	U.S.A., North Carolina; <i>Thorne</i> 19393; AF284955
<i>Dulichium</i> Rich. ex Pers.	<i>squarrosus</i> L.	Mexico, Sonora; <i>Roalson</i> 1351; AF285071
<i>Gahnia</i> J.R. & G.Forster	<i>arundinaceum</i> (L.) Britton	Canada, Nova Scotia; <i>Williams</i> 1441; AF285067
<i>Isolepis</i> R.Br.	<i>aspera</i> (R.Br.) Spreng. subsp. <i>globosa</i> (mann) Kern	U.S.A., Hawaii; <i>Flynn</i> 2781; AF285073
<i>Kobresia</i> Willd.	<i>cernua</i> (Vahl) Roem. & Schult.	Australia, New South Wales; <i>Roalson</i> s.n.; AF285070
<i>Leptosperma</i> Labill.	<i>sibirica</i> (Turcz. ex Ledeb.) Boeck.	U.S.S.R., Siberia; <i>Elias et al.</i> 7339; AF284884
<i>Luzula</i> DC.	aff. <i>filiforme</i> Labill.	Australia, Tasmania; <i>Morris</i> 8009; AF285074
<i>Rhynchospora</i> Vahl	<i>comosa</i> E.Mey.	U.S.A., California; <i>Roalson</i> 1394; AF285069
<i>Scirpus</i> L.	<i>corniculata</i> (Lam.) A. Gray var. <i>interior</i> Fern.	U.S.A., Texas; <i>Roalson</i> 1276; AF285068
<i>Schoenoplectus</i> Palla	<i>microcarpus</i> C.Presl	U.S.A., California; <i>Roalson</i> 1215; AF284859
	<i>acturus</i> (Muhl. ex Bigelow) Á.Löve & D.Löve var. <i>occidentalis</i> (S.Watson) S.G.Smith	U.S.A., California; <i>Thorne</i> 54937; AF285064
<i>Schoenoxiphium</i> Nees	<i>americanus</i> (Pers.) Volkart ex Schinz & R.Keller	U.S.A., California; <i>Annable</i> 289; AF285065
<i>Schoenus</i> L.	<i>dissachanthus</i> (S.T. Blake) J.Raynal	Australia; <i>Smyth</i> 175; AF285077
<i>Scleria</i> Bergius	<i>burkei</i> C.B.Clarke	South Africa, Lesotho; <i>Browning</i> 689 (NU); AF284992
<i>Uncinia</i> Pers.	<i>nigricans</i> L.	U.S.A., Nevada; <i>DeDecker</i> 4616; AF285075
	<i>reticularis</i> Michx.	Mexico, Sonora; <i>Roalson</i> 1358; AF285076
	<i>uncinata</i> (L.f.) Kük.	U.S.A., Hawaii; <i>Gustafson</i> 2390; AF284886

TABLE 2. Genera, subgenera, sections, and species of Cariceae and the outgroup included in the Cariceae analyses. The classification mostly follows Kükenthal (1909) in content and order. *Cymophyllus fraseri* was treated by Kükenthal (1909) as *Carex* subgenus *Primocarex* section *Leucocephalae* Holm, but is listed here at the generic rank. Species marked with an asterisk (*) are currently placed in section *Acrocystis* by most authors (see text for details). Specimens deposited in RSA unless otherwise noted. Specimens without collection number (noted in "s.n.") are followed by the herbarium and accession number.

Genus	Subgenus	Section-Subsection	Specific epithet	Locality, Voucher, and GenBank accession numbers
<i>Schoenoxiphium</i> Nees			<i>burkei</i> C.B.Clarke	South Africa, Lesotho; <i>Browning</i> 689 (NU); AF285024; AF284922
<i>Kobresia</i> Willd.		<i>Elyma</i> (Schrad.) C.B.Clarke	<i>myosuroides</i> (Vill.) Fiori & Paol. <i>sibirica</i> (Turcz. ex Ledeb.) Boeck. <i>capillifolia</i> (Decne.) C.B.Clarke	U.S.S.R., Siberia; <i>Elias et al.</i> 7337; AF284985; AF284883 U.S.S.R., Siberia; <i>Elias et al.</i> 7339; AF284986; AF284884 China, Xinjiang Uyгур Zizhiq; <i>Morefield</i> 5071; AF284984; AF284882
<i>Uncinia</i> Pers.	<i>Eti-Uncinia</i> Kük.	<i>Platyandrae</i> C.B.Clarke	<i>brevicaulis</i> Thou.	Chile, Juan Fernandez Island; <i>Solbrig</i> 3647; AF284987; AF284885
<i>Carex</i> L.	<i>Primocarex</i> Kük.	<i>Stenandrae</i> C.B.Clarke <i>Microcephalae</i> Holm <i>Scirpiniae</i> Tuck. <i>Pictae</i> Kük. <i>Petranae</i> O.Lang <i>Psilocarpae</i> Kük.– <i>Polytrichoidae</i> Tuck. <i>Psilocarpae–Firmiculmes</i> Kük. <i>Inflatae</i> Kük. <i>Circinatae</i> Meish.	<i>uncinata</i> (L.f.) Kük. <i>capitata</i> L. <i>gigas</i> (Holm) Mack. <i>scirpoidea</i> Michx. subsp. <i>scirpoidea</i> <i>picta</i> Steud. <i>obtusata</i> Lij. <i>leptalea</i> Wahlenb. <i>multicaulis</i> L.H.Bailey <i>brevveri</i> Boott <i>circinata</i> C.A.Mey.	U.S.A., Hawaii; <i>Gustafson</i> 2390; AF284988; AF284886 U.S.A., California; <i>DeDecker</i> 4899; AF285044; AF284942 U.S.A., California; <i>Wilson</i> 8252 (OSC); AF285027; AF284925 U.S.A., Utah; <i>Goodrich</i> 24125; AF285050; AF284948 U.S.A., Alabama; <i>Churchill</i> 73-4170; AF285020; AF284918 U.S.A., New Mexico; <i>Raulson</i> 852 (NMCR); AF284967; AF284865 U.S.A., Michigan; <i>Raulson</i> 1343; AF285060; AF284958 U.S.A., California; <i>Raulson</i> 1402; AF285028; AF284926 U.S.A., Utah; <i>Huber & Goodrich</i> 2265; AF285012; AF284910 U.S.A., Alaska; <i>Talbot & Talbot</i> 1327; AF285013; AF284911 Austria; <i>Polatschek s.n.</i> (RSA 336002); AF284983; AF284881
		<i>Unciniaeformes</i> Kük.– <i>Pauciflorae</i> Tuck.	<i>pauciflora</i> Lightf.	

TABLE 2. Continued.

Genus	Subgenus	Section-Subsection	Specific epithet	Locality, Voucher, and GenBank accession numbers
<i>Vigna</i> (P. Beauv. ex T.Lestib.) Kük	<i>Foetidae</i> Tuck.	<i>Divisae</i> H.Christ. ex Kük.	<i>vernacula</i> L.H.Bailey	U.S.A., Oregon; Mason 9103 (POM); AF285022; AF284920
			<i>divisa</i> Huds.	Greece, Island of Lesbos; Strid 26023; AF284991; AF284889
			<i>macrorrhiza</i> Boeck.	Argentina, Santa Cruz; Roivainen 2630; AF285018; AF284916
	<i>Multiflorae</i> Kunth	<i>Muehlenbergianae</i> Tuck.	<i>vulpinoides</i> Michx.	U.S.A., Texas; Roalson 1294; AF284968; AF284866
			<i>alma</i> L.H.Bailey	U.S.A., California; Ross 7838; AF285025; AF284923
	<i>Stenorrhynchae</i> Holm	<i>otrubae</i> Podp.	<i>leavenworthii</i> Dewey	U.S.A., Texas; Roalson 1299; AF285033; AF284931
			<i>muricata</i> L.	U.S.S.R., Estonia, Muhu Island; Skortsov et al. s.n. (RSA 545711); AF285036; AF284934
			<i>jonesii</i> L.H.Bailey	U.S.A., California; Brunner 4054; AF285038; AF284936
	<i>Paniculatae</i> Kunth	<i>Macrocephalae</i> Kük.	<i>otrubae</i> Podp.	U.S.S.R., Crimea; Korzhenevsky s.n. (RSA 555824); AF284996; AF284894
			<i>diantra</i> Schrank	U.S.A., California; Brunner 3753; AF285026; AF284924
	<i>Scheilhammeria</i> (Moench) Kunth <i>Oxales</i> Kunth	<i>Canescentes</i> Fr.	<i>macrocephala</i> Willd.	U.S.A., Oregon; Halse 1543; AF285017; AF284915
			<i>bohemica</i> Schreb.	Finland; Veltainen 8650; AF284989; AF284887
			<i>ovatis</i> Good	Greece; Strid 24872; AF28502; AF284900
<i>fracta</i> Mack.			U.S.A., California; Roalson 1214; AF285030; AF284928	
<i>Indocarex</i> Baill.	<i>Indicae</i> Tuck.— <i>Turgidulae</i> Kük.	<i>bicknellii</i> Britton	U.S.A., Michigan; Fritsch 1137; AF285039; AF284937	
		<i>canescens</i> L.	U.S.S.R., Siberia; Murray et al. 256; FA284990; AF284888	
		<i>polystachya</i> Wahlenb.	Brazil, Federal District; Irwin et al. 26503; AF285014; AF284912	
<i>Carex</i> L.	<i>Acutae</i> Fr.— <i>Bicolores</i> Tuck.	<i>filicina</i> Nees	China, Sichuan; Boufford & Bartholomew 24364; AF284981; AF284879	
		<i>eleusinioides</i> Turcz. ex Kunth	U.S.S.R., Buryatskaya A.S.S.R.; Elias 12025; AF285006; AF284904	

TABLE 2. Continued.

Genus	Subgenus	Section-Subsection	Specific epithet	Locality, Voucher, and Genbank accession numbers
		<i>Acutiae-Rigidae</i> Fr.	<i>scopulorum</i> Holm var. <i>scopulorum</i>	U.S.A., Nevada; <i>Holmgren & Reveal</i> 1601; AF285056; AF284954
			<i>scopulorum</i> Holm var. <i>bracteosa</i> (L.H.Bailey) F.J.Herm.	U.S.A., Oregon; <i>Mason</i> 9000 (POM); AF285059; AF284957
		<i>Acutiae-Vulgares</i> Asch.	<i>acuta</i> L.	U.S.S.R., Siberia; <i>Elias et al.</i> 8254; AF284992; AF284890
		<i>Acutiae-Caespitosae</i> Fr.	<i>aquatilis</i> Wahlenb.	Finland; <i>Kukonen</i> 12828; AF284994; AF284892
			<i>utiliica</i> Meinsh.	U.S.S.R., Siberia; <i>McNeil</i> 854; AF285010; AF284908
		<i>Acutiae-Forsiculatae</i> Fransh.	<i>angustata</i> Boott	U.S.A., Idaho; <i>Dawidson</i> 6187; AF285015; AF284913
		<i>Acutiae-Praelongae</i> Kük.	<i>torta</i> Boott	U.S.A., Vermont; <i>Boufford & Conant</i> 22950; AF285021; AF284919
		<i>Acutiae-Cryptocarpae</i> Tuck.	<i>schottii</i> Dewey	U.S.A., California; <i>Brunner</i> 3970; AF285037; AF284935
		<i>Atratae</i> Kunth	<i>bella</i> L.H.Bailey	U.S.A., New Mexico; <i>Raulson</i> 823 (NMCR); AF284966; AF284864
			<i>anganae</i> Steud.	U.S.S.R., Magadan Oblast; <i>Elias</i> 11269; AF284980; AF284878
		<i>Fecundae</i> Kük.	<i>donnell-smithii</i> L.H.Bailey	Mexico, Chiapas; <i>Shilom Ton</i> 8199; AF285005; AF284903
		<i>Sciatae</i> Kük.	<i>podocarpa</i> R.Br.	U.S.S.R., Magadan Oblast; <i>Elias</i> 11380; AF284998; AF284896
		<i>Trachyclaenae</i> Drejer	<i>flacca</i> Schreb. subsp. <i>serrulata</i> (Biv.) Greuter	Greece; <i>Hartwig & Branzén</i> 8709; AF284982; AF284880
			<i>spissa</i> L.H.Bailey	U.S.A., California; <i>Tilforth & Wisura</i> 2140; AF285040; AF284938
		<i>Pachystylae</i> Kük.	<i>pallescens</i> L.	Finland; <i>Himmeri</i> s.n. (RSA) 302781; AF284997; AF284895
			<i>torreyi</i> Tuck.	U.S.A., Wyoming; <i>Porter & Porter</i> 7810; AF285051; AF284949
			* <i>globularis</i> L.	U.S.S.R., Magadan Oblast; <i>Elias</i> 11312; AF285049; AF284947
			* <i>grioteitii</i> Roem.	U.S.S.R., S.F.S.R., Sochi; <i>Vásak</i> s.n. (RSA 541299); F285048; AF284946

TABLE 2. Continued.

Genus	Subgenus	Section-Subsection	Specific epithet	Locality, Voucher, and GenBank accession numbers
			<i>*lomentosa</i> L.	U.S.S.R., Kazakhstan; Skornitsin s.n. (RSA 545712); AF285047; AF284945
			<i>*mandshurica</i> Meish.	Korea, Kangwon Province; Tyson 5044 (POM); AF285045; AF284943
	Montanae (Fr.) H. Christ (<i>Acrocystis</i> Dumort.)		<i>pilulifera</i> L.	Sweden, Uppland; Alm 805; AF284975; AF284873
			<i>oxyandra</i> Kudo	Japan, Ikeda 15896 (OKAY); AF285061; AF284959
			<i>ericetorum</i> Pollich	Sweden, Småland; Snogerup & Snogerup 3214; AF284974; AF284872
			<i>montana</i> L.	France; Rosteter 13887 (TAES); AF284979; AF284877
			<i>pensylvanica</i> Lam.	U.S.A., Michigan; Roalson 1341; AF284977; AF284875
			<i>rossii</i> Boott	U.S.A., Oregon; Wilson 6864 (OSC); AF284972; AF284870
			<i>leucodonta</i> Holm	U.S.A., Arizona; Roalson 1224; AF284973; AF284871
			<i>rugosperma</i> Mack.	U.S.A., Pennsylvania; Roalson 1323; AF284978; AF284876
			<i>communis</i> L.H.Bailey	Canada, Quebec; Roalson 1329; AF284976; AF284874
			<i>supina</i> Willd.	U.S.A., Alaska; Marvitt 1623; AF284999; AF284897
	<i>Lamprochlaenae</i> Drejer		<i>umbrosa</i> Host subsp. <i>subymensis</i>	U.S.S.R., Siberia; Murray et al. 344; AF285042; AF284940
			Less. ex Kunth	
			<i>concinnooides</i> Mach.	U.S.A., Oregon; Wilson 7637 (OSC); AF284965; AF284863
			<i>pedunculata</i> Muhl. ex Willd.	U.S.A., Michigan; Roalson 1346; AF284969; AF284867
			<i>digitata</i> L.	Sweden, Gotland; Snogerup & Snogerup 4181; AF285004; AF284902
			<i>humilis</i> Leyss.	Greece; Hartwig & Franzén 8844; AF285008; AF284906
			<i>lanccolata</i> Boott	Japan, Kanagawa Pref.; Amano s.n. (RSA 328480); AF2850098; AF284907

TABLE 2. Continued.

Genus	Subgenus	Section-Subsection	Specific epithet	Locality, Voucher, and Genbank accession numbers
	(assumed)		<i>planostachyus</i> Kunze	U.S.A., Texas; Wilson 7592 (OSC); AF285034; AF284932
	<i>Albae</i> Asch. & Graebn.		<i>eburnea</i> Boott	Canada, British Columbia; Calder & Kukkonen 27538; AF285000; AF284898
	<i>Limosae</i> Tuck.		<i>limosa</i> L.	U.S.A., Alaska; Miramin 1707; AF285001; AF284899
	<i>Panicaceae</i> Tuck.		<i>paupercula</i> Michx. subsp. <i>irrigua</i> (Wahlenb.) Á.L.öve & D.Löve	Finland; <i>Alto</i> s.n. (RSA 276147); AF285019; AF284917
			<i>falcata</i> Turcz.	
			<i>aurea</i> Nutt.	U.S.S.R., Magadan Oblast; <i>Dokuchaeva</i> s.n. (RSA 533281); AF285016; AF284914
	<i>Griseae</i> L.H.Bailey		<i>microdonta</i> Torr. & Hook.	U.S.A., California; DeDecker 5605; AF285062; AF284960
	<i>Careyanae</i> Tuck.		<i>laxiflora</i> Lam.	U.S.A., Texas; Ertter 4744; AF285052; AF284950
			<i>digitalis</i> Willd.	U.S.A., Texas; Roalson 1291; AF284964; AF284862
	<i>Frigidae</i> Fr.– <i>Mucronatae</i> Nyman		<i>*mira</i> Kük.	U.S.A., North Carolina; Roalson 1322; AF285035; AF284933
	<i>Frigidae</i> – <i>Ferruginae</i> Tuck.		<i>firma</i> Host	Korea, Kangwon Province; Tjyson 4983 (POM); AF285046; AF284944
	<i>Hymenochlaenae</i> Drejer– <i>Pubescentes</i> Kük.		<i>whitneyi</i> Olney	France; Charpin s.n. (RSA 244689); AF284995; AF284893
	<i>Hymenochlaenae</i> – <i>Cracillinae</i> Carey		<i>gracillina</i> Schwein.	U.S.A., Nevada; Tiehm 12035; AF285053; AF284951
			<i>prasina</i> Wehlenb.	U.S.A., Vermont; Boufford & Conant 22925; AF285054; AF284952
	<i>Hymenochlaenae</i> – <i>Debiles</i> Carey		<i>debilis</i> Michx.	U.S.A., North Carolina; Radford 43094; AF285043; AF284941
	<i>Hymenochlaenae</i> – <i>Graciles</i> Tuck.		<i>brunnea</i> Thunb.	U.S.A., Texas; Roalson 1285; AF285029; AF284927
	<i>Hymenochlaenae</i> – <i>Longirostres</i> Kük.		<i>castanea</i> Wahlenb.	China, Guizhou; Sino-American Guizhou Botanical Expedition 1574; AF285003; AF284901
				U.S.A., Vermont; Boufford & Conant 22965; AF285058; AF284956

TABLE 2. Continued.

Genus	Subgenus	Section-Subsection	Specific epithet	Locality, Voucher, and GenBank accession numbers
		<i>Rhomboidales</i> Kük.	<i>brevicollis</i> DC.	U.S.S.R., Moldavian S.S.R.; Elias et al. 6241; AF285011; AF284909
			<i>waltuensis</i> C.A.Mey. subsp. <i>robusta</i> (Fr. & Sav.) T.Koyama	Japan, Shizuoka Pref.; <i>Amatio</i> s.n. (RSA 328501); AF285023; AF284921
		<i>Phyllostachyae</i> Tuck.	<i>basiantha</i> Steud.	U.S.A., Texas; Roalson 1283; AF284970; AF284868
		<i>Spirostachyae</i> Dreijer	<i>lemmonii</i> W.Boott	U.S.A., California; Sanders 15031 (UCR); AF284971; AF284869
			<i>flata</i> L.	U.S.S.R., Russia; Skovtsov s.n. (RSA 545715); AF285007; AF284905
		<i>Pseudo-cyperae</i> Tuck.	<i>antoniensis</i> A.Chev.	Cape Verde Islands, Santo Antao; Kilian & Leyens 2988 (Berling-Dahlem Bot. Gard.); AF285041; AF284939
		<i>Physocarpae</i> Drejer-Lupulinae Tuck.	<i>lupulina</i> Muhl. ex Willd.	U.S.A., Texas; Roalson 1300; AF284963; AF284861
		<i>Physocarpae-Tentaculatae</i> Tuck.	<i>lurida</i> Wahlenb.	U.S.A., North Carolina; Roalson 1315; AF284962; AF284860
			<i>typhina</i> Michx.	U.S.A., Texas; Roalson 1298; AF285032; AF284930
		<i>Physocarpae-Vesicariae</i> Tuck.	<i>exsiccata</i> L.H.Bailey	Canada, British Columbia; Calder & Taylor 36732; AF285055; AF284953
		<i>Paludosae</i> Fr.	<i>acutiformis</i> Ehrh.	U.S.S.R., Kazakh S.S.R.; Elias 9527; AF284993; AF284891
		<i>Hirtae</i> Tuck.	<i>lanuginosa</i> Michx.	U.S.A., California; Roalson 1216; AF285031; AF284929
<i>Cymophyllus</i> Mack.			<i>fraseri</i> Mack.	U.S.A., North Carolina; Thorne 19393; AF285057; AF284955
<i>Scirpus</i> L.			<i>microcarpus</i> C.Presl	U.S.A., California; Roalson 1215; AF284961; AF284859

TABLE 3. DNA sequence statistics for outgroup *trnL-F* sequences and Cariceae ITS and *trnT-L-F* sequences. Abbreviations are as follows: bp, base pairs; TI, transitions; and TV, transversions.

	Outgroup <i>trnL-F</i>	Cariceae ITS	Cariceae <i>trnT-L-F</i>
Length range (bp)	580–1,071	585–663	647–1,121
Aligned length (bp)	1,401	691	2,050
GC content average (%)	26	62	24
Sequence divergence (%)	2–33	0–16	0–8
Number of indels	113	26	99
Number of variable sites	699	298	640
Number of potentially informative sites	381	211	295
Number of constant sites	702	393	1,410
Number of autapomorphic sites	318	87	345
TI/TV bias	1:0.89	1:2.34	1:0.76

group analysis (*Scirpus microcarpus*). All outgroup samples were not included in ingroup analyses due to a combination of difficulties in alignment given the degree of divergence and computational limitations due to data set size.

Maximum likelihood estimates of transition/transversion (TI/TV) biases were measured for the nrDNA and cpDNA data sets individually as implemented in PAUP*4.0b2 utilizing the general time-reversible model. These estimates were used to weight the MP analyses and the weighted analyses were compared to analyses of unweighted data sets. This weighting scheme was implemented to more closely model the MP analyses to the data set.

Homogeneity of the ITS and *trnT-L-F* data sets was assessed using the partition homogeneity test (Farris et al. 1995) as implemented in PAUP*4.0b2. Thirty-thousand replicate data partitions were run employing the TI/TV weighting schemes (heuristic search; simple addition; no branch swapping), excluding constant characters. This test measures character congruence by comparing tree length differences among trees derived from resampled data partitions of the combined data sets and trees derived from the defined data partition.

Some gaps (indels) in the data matrices were mapped onto trees from the combined data analy-

sis to show additional support for clades. Autapomorphic gaps, complex gaps associated with single base repeat regions, and homoplastic gaps were not mapped.

RESULTS

Outgroup Data Set. The aligned outgroup *trnL-F* data set was 1,401 base pairs (bp) long with 699 variable sites, of which 381 were potentially parsimony-informative. The length of the unaligned *trnL-F* region varied from 580 to 1,071 bp (Table 3). Alignment resulted in 113 gap regions, ranging in size from 1 to 67 bp.

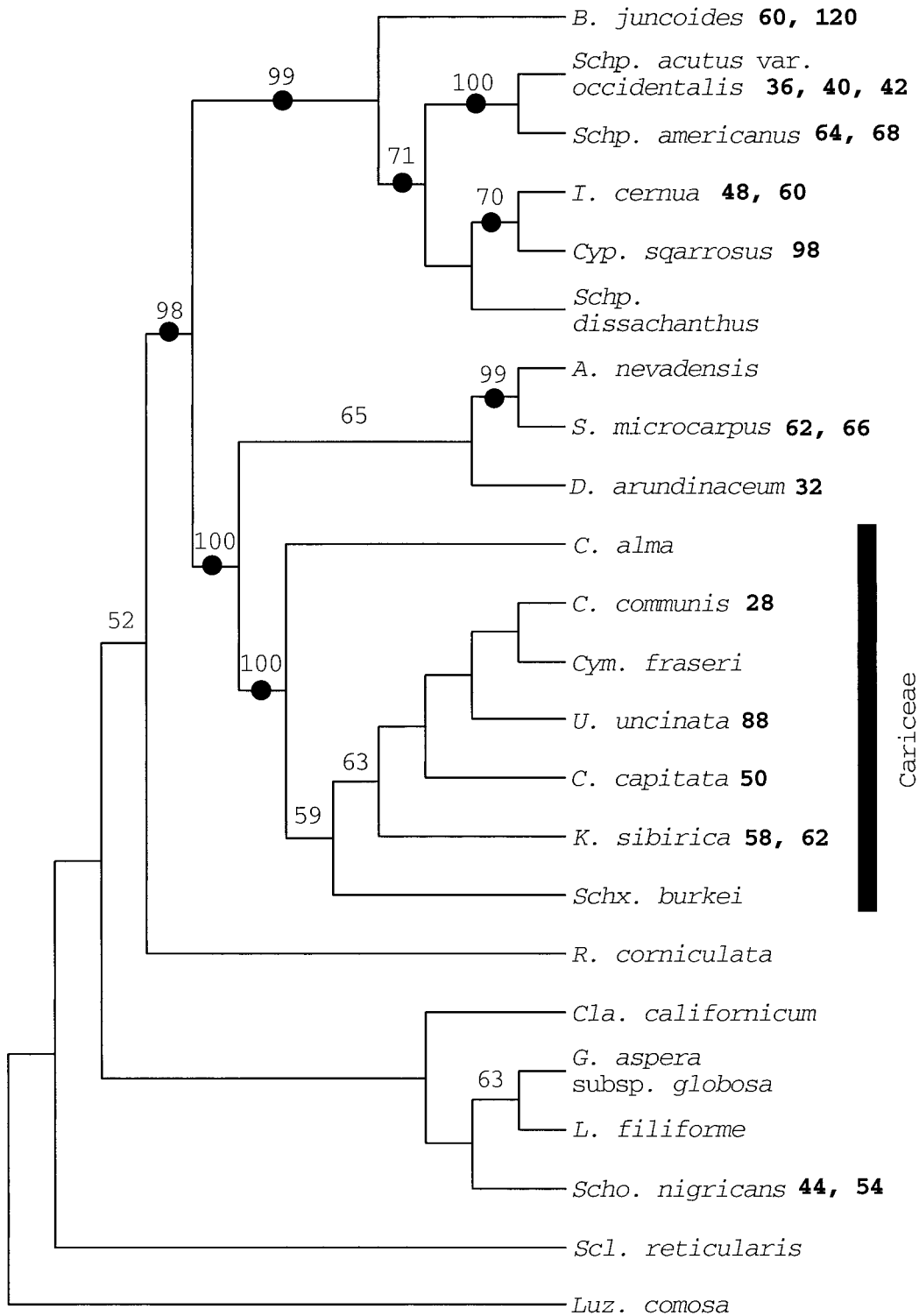
Four most-parsimonious trees (length = 1,476 steps, CI = 0.70, RI = 0.68, RC = 0.48) were found. The strict consensus of these trees is presented in Fig. 1.

Base Change Biases. Transition/transversion biases were calculated to be 2.34 for the ITS data set and 0.76 for the *trnT-L-F* data set (Table 3).

ITS Data Set. The four ITS sequencing primers produced overlapping fragments that collectively covered the entire spacer and 5.8S rDNA regions along both strands. The aligned ITS data matrix was 691 bp long with 298 variable sites, of which 211 were potentially parsimony-informative. The length of the unaligned sequences varied from 585

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FIG. 1. Analysis of outgroups to the Cariceae using the cpDNA *trnL-F* spacer regions. Strict (MP) consensus tree of 4 most parsimonious trees of 1,476 steps (CI=0.703; RI=0.680; RC=0.478). Numbers above branches are bootstrap percentages. Bullets on branches refer to nodes supported by jackknife analysis with at least 67%. Reported chromosome number (2n) of each species (when known) is to the right of the species name. Generic names are abbreviated as follows: B. = *Bulbostylis*, C. = *Carex*, Cla. = *Cladium*, Cym. = *Cymophyllus*, Cyp. = *Cyperus*, D. = *Dulichium*, G. = *Gahnia*, I. = *Isolepis*, K. = *Kobresia*, L. = *Lepidosperma*, Luz. = *Luzula*, R. = *Rhynchospora*, S. = *Scirpus*, Scho. = *Schoenus*, Schp. = *Schoenoplectus*, Schx. = *Schoenoxiphium*, Scl. = *Scleria*, and U. = *Uncinia*.



to 663 bp (Table 3). There were 26 gaps ranging from 1 to 27 bp in length.

Maximum parsimony analysis of the ITS Cariceae data set resulted in 20,300 most-parsimonious trees (length = 1,368 steps, CI = 0.34, RI = 0.63, RC = 0.21). The search was terminated prematurely due to insufficient computer memory. Figure 2 is the strict consensus of these trees.

trnT-L-F Data Set. The chloroplast primers produced overlapping fragments that covered the majority of the intron and igs, with only a small portion of the 5' end of the *trnL* intron and 3' end of the *trnT-L* igs missing. Some sequences were lacking portions of the 3' end of the *trnL* intron and 5' end of the *trnL-F* igs due to difficulty in sequencing this region. The aligned *trnT-L-F* data set was 2,050 bp long with 640 variable sites, of which 295 were potentially parsimony-informative. The length of the unaligned data set varied from 647 to 1,121 bp (Table 3). There were 99 gaps ranging from 1 to 206 bp in length.

Maximum parsimony analysis of the *trnT-L-F* Cariceae data resulted in 15,200 most-parsimonious trees (length = 1,196 steps, CI = 0.66, RI = 0.77, RC = 0.51). The search was terminated prematurely due to insufficient computer memory. The strict consensus of these trees is presented in Fig. 3.

Partition Homogeneity Test. The partition homogeneity test found a significant difference between the ITS/*trnT-L-F* partition and random partitioning ($P = 0.009392$).

Combined Analysis. The aligned combined ITS and *trnT-L-F* data set was 2,741 bp long with 938 variable sites, of which 506 were potentially parsimony-informative.

Maximum parsimony analysis of the combined ITS and *trnT-L-F* Cariceae data sets employing the TI/TV bias matrix resulted in 168 most-parsimonious trees (length = 2,991.44 steps, CI = 0.45, RI = 0.60, RC = 0.27). The differences between the weighted and unweighted analyses were minimal, with no conflicting well-supported nodes. The strict consensus of these trees is presented in Fig. 4.

Comparison of ITS, trnT-L-F, and Combined Analyses. The ITS strict consensus tree has several strongly supported clades of closely related species, but except for the *Carex* subgenus *Vignea* clade (bs 73%, jk \geq 67%), internal nodes lack bootstrap/jackknife support (Fig. 2). The chloroplast strict consensus tree has similarly poor statistical support, although it also provides strong support for a *Carex* subgenera *Carex* and *Indocarex* clade (bs 87%, jk \geq 67%) as well as the *Vignea* clade (bs 100%,

jk \geq 67%; Fig. 3). The primary difference between the results of these two analyses is that the *trnT-L-F* analysis places the genera *Cymophyllus*, *Kobresia*, and *Uncinia*, as well as most of *Carex* subgenus *Primocarex*, in a clade sister to the subgenera *Carex* and *Indocarex* clade, while the ITS analysis places these taxa as a grade leading to the *Carex/Indocarex* clade (Figs. 2, 3). Individual data set analyses under different weighting schemes (results not shown) did not significantly alter the topology of the resultant trees, but did resolve some polytomies present in the unweighted analyses. Statistical support for clades in the unweighted analysis did not change significantly with weighting.

There is yet no consensus on when data sets should be combined (for a review, see de Queiroz et al. 1995). While the partition homogeneity test provides a test of congruence among data sets, it is not clear how the test is affected by differences in gene history versus homoplasy (Miller et al. 1999). Additionally, many authors consider simultaneous analysis of all data to be the most effective way to study evolutionary descent (Thornton and DeSalle 2000, and references therein). In this study, the incongruences between the data sets do not involve well-supported branches, and, therefore, the data sets were combined to assess the resolving power of all of the data in a single analysis.

The structure of the combined analysis strict consensus tree (Fig. 4) is most similar to the *trnT-L-F* strict consensus tree in terms of internal branch structure, but strongly supported clades in both the ITS and *trnT-L-F* analyses are also strongly supported in the combined analysis. In addition, while the ITS strict consensus produced 20 branches with moderate to high statistical support (jk \geq 67%), and the *trnT-L-F* analysis resulted in 23 (12 the same as in ITS), 33 resulted from the combined analysis. Furthermore, many nodes with low bootstrap/jackknife support in the separate analyses had greater support when the data were combined (Figs. 2–4). Hence, we consider the combined strict consensus tree to be a better estimate of relationships for the Cariceae than either the ITS or *trnT-L-F* phylogenies.

Gaps, while not coded in the analyses, did provide some additional support for some clades (Fig. 5). However, most gaps were homoplastic or autapomorphic.

DISCUSSION

Analysis of Outgroups to the Cariceae. Generic relationships in the Cyperaceae have long been un-

certain despite two major suprageneric revisions in the last 15 years (Goetghebeur 1985; Bruhl 1995). In these and previous treatments, the Cariceae have been placed in the same subfamily as *Scleria* and relatives (e.g., Bruhl 1995), or in a subfamily of its own (e.g., Goetghebeur 1985). In a recent molecular phylogenetic study of the Cyperaceae utilizing chloroplast *rbcl* sequences (Muasya et al. 1998), *Carex* grouped with members of *Eriophorum* and *Scirpus*. Unfortunately, this relationship was poorly supported (bs 57%). Although no definite sister group of the Cariceae was identified, a distant relationship between *Scleria* and *Carex* was evident. Recent phylogenetic studies in the Cariceae based on cpDNA coding and non-coding regions (Yen and Olmstead, in press) have employed members of *Dulichium*, *Eriophorum*, and *Scirpus* as outgroups, although none of these genera was favored as the Cariceae sister group. For these reasons additional analyses of outgroup relationships were included here.

Again, in the present study, no one particular genus was found to be strongly supported as the sister of the Cariceae. However, a clade including *Scirpus microcarpus*, *Amphiscirpus nevadensis*, and *Dulichium arundinaceum* forms a sister clade to the Cariceae lineage (Fig. 1), a relationship strongly supported by bootstrap (100%) and jackknife (100%) statistics. *Eriophorum* was not included in this analysis. There is no support for a close relationship between *Scleria* and *Carex*.

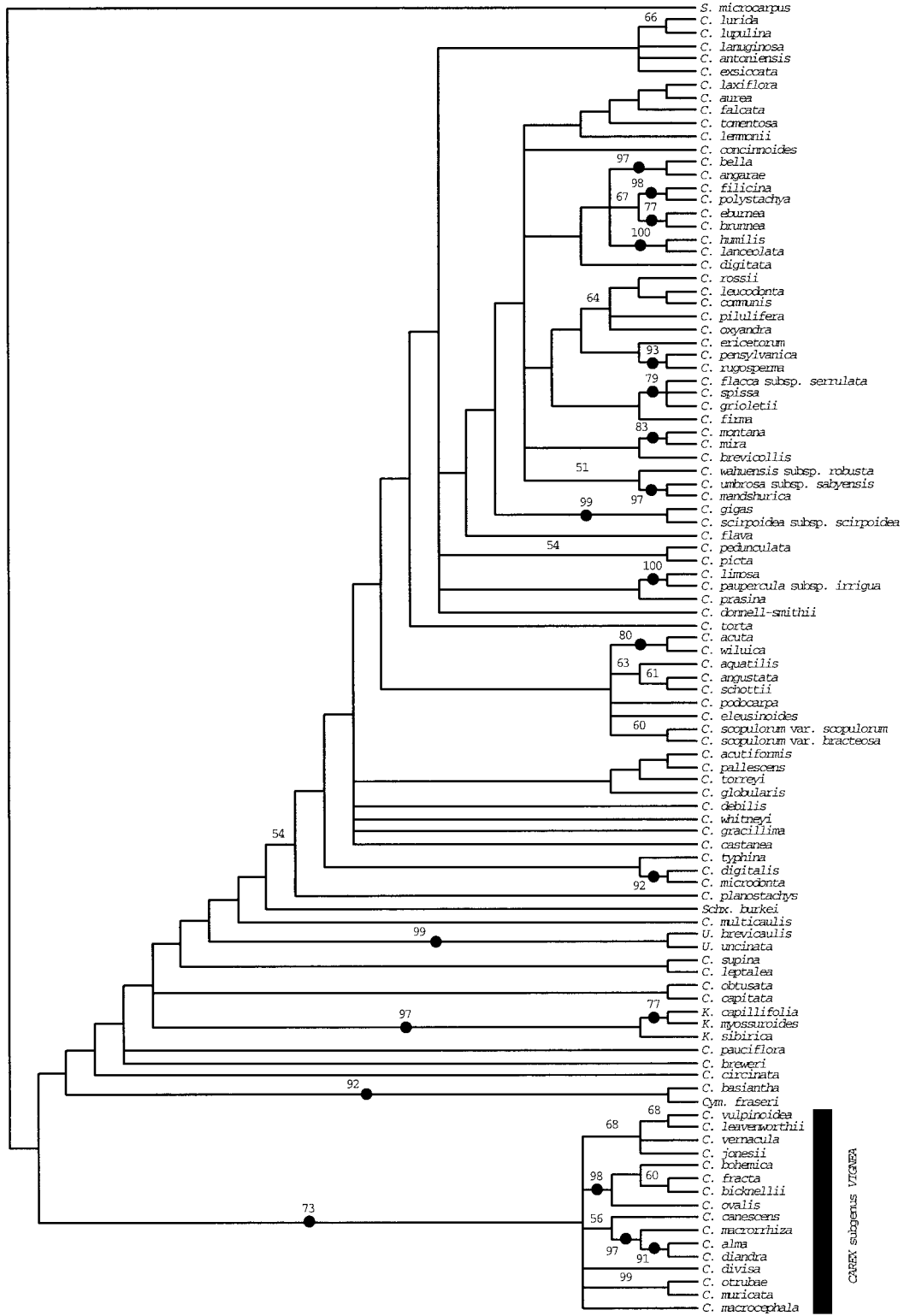
Generic Relationships in the Cariceae. The only world-wide classification of the Cariceae is that of Kükenthal (1909; overlaid on the phylogeny in Fig. 4). For this reason, it is the principal classification tested here, although more recent subgeneric and sectional circumscriptions are also addressed. The results presented here show that the genera *Cymophyllus*, *Kobresia*, *Schoenoxiphium*, and *Uncinia* are nested within *Carex*. These four genera form a clade with most of the members of *Carex* subgenus *Primocarex* (Fig. 4, clade B; further reference to clades A, B, and C all regard Fig. 4), although this clade is not strongly supported (bs < 50%). *Schoenoxiphium* appears to be sister to the other members of this clade, with each of the other genera allied with different members of subgenus *Primocarex* (or odd members of subgenus *Carex*). *Cymophyllus* is paired with *Carex basiantha*, commonly considered a member of subgenus *Carex*; *Kobresia* is grouped with *C. pauciflora*; and *Uncinia* is placed with *C. multicaulis*. None of the relationships among these genera and members of *Carex* are supported by bootstrap/jack-

knife statistics except the pairing of *Cymophyllus* with *Carex basiantha* (bs 70%, jk \geq 67%). However, *Kobresia* and *Uncinia* are strongly supported as monophyletic (*Kobresia*—bs 100%, jk \geq 67%, one indel; *Uncinia*—bs 100%, jk \geq 67%). *Kobresia* is made up of two groups of species. One group is circumboreal in distribution and includes the species sampled here. The other group is largely endemic to the Himalayan Mountains region. Because the second group was not sampled here, monophyly of the genus has not been demonstrated. In other studies (Yen and Olmstead, in press) that sampled both groups, though, *Kobresia* species from both groups fall within a clade of *Cymophyllus*, *Kobresia*, *Uncinia*, and *Carex* subgenus *Primocarex*, but do not form a monophyletic group. In addition, sampling within subgenus *Primocarex* was minimal, therefore, relationships among these genera and species of *Carex* should be viewed with caution.

Some authors have suggested that *Kobresia* and *Schoenoxiphium* are congeneric (Kern 1958; Koyama 1961). While these genera are members of the same clade in the analyses presented here, they are not sister taxa. This is congruent with the findings of Yen and Olmstead (in press). The shared character of open perigynia, which has been used as evidence of close relationship, is likely plesiomorphic or homoplastic and not direct evidence of close relationship.

Infrageneric Classification in Carex. The three major clades in the ITS/*trnT-L-F* strict consensus tree correlate roughly with the subgenera of Kükenthal (1909). Clade A is composed of members of subgenus *Vignea*, which is strongly supported (bs 100%, jk \geq 67%), and is placed sister to the rest of the tribe (Fig. 4). Of the sections of *Vignea* with multiple samples (*Divisae*, *Ovales*, *Muehlenbergianae*, and *Multiflorae*), only *Ovales* is possibly monophyletic. *Carex bohemica*, placed by Kükenthal (1909) in section *Schellhammeria*, forms an unresolved polytomy with members of *Ovales* (Fig. 4). Current classification (Reznicek 1993) includes *C. bohemica* in *Ovales*, which is congruent with these analyses. Only half of the sections recognized by Kükenthal (1909) as belonging to *Vignea* were included in this analysis. Therefore, more detailed studies of *Vignea* are required to address phylogenetic relationships within the subgenus. Strong evidence is presented here, though, that *Vignea* is a monophyletic lineage and this corroborates previous hypotheses regarding the subgenus (Reznicek 1990).

Clade B is composed of subgenus *Primocarex*, two members of subgenus *Carex*, and the other genera



of the Cariceae. Discussion surrounding genera other than *Carex* is addressed above. Nine of 14 sections of *Primocarex* recognized by Kükenthal (1909) were sampled. With one exception, only one member of each section of *Primocarex* was sampled. One member each of two subsections (*C. leptalea* of *Polytrichoideae* and *C. multicaulis* of *Firmiculmes*) of section *Psilocarpae* were sampled. In the ITS/*trnT-L-F* phylogeny this section is not monophyletic. This supports Mackenzie (1935), who did not infer the two species to be closely related. He suggested that *C. leptalea* is related to section *Phyllostachyae* in subgenus *Carex*. While *C. leptalea* and *Phyllostachyae* are both in clade B, neither are associated with the rest of subgenus *Carex* (clade C). *Carex multicaulis* was suggested by Mackenzie (1935) to be allied with *Petraeae* (as *Rupestres* Tuck.) and *Albae*, with affinities to subgenus *Carex*. Both *C. multicaulis* and *Petraeae* are in clade B, though not sister taxa, while *Albae* is associated with the rest of subgenus *Carex* in clade C.

Two species in clade B are commonly treated as belonging to subgenus *Carex*: *C. basiantha* (section *Phyllostachyae*) and *C. supina* (section *Lamprochlaenae*). The association of *Phyllostachyae* with a clade comprised of *Primocarex* and other genera of the Cariceae is novel, but the association of this section with some members of *Primocarex* is not (Starr et al. 1999). The placement of *C. supina* with members of *Primocarex* is not unprecedented—Mackenzie (1935) placed *C. supina* in section *Petraeae* (as section *Obtusatae*) instead of *Lamprochlaenae*.

Clade C is composed of the subgenera *Carex* and *Indocarex*, as well as three species of subgenus *Primocarex*, and is well supported (bs 97%; jk \geq 67%, one indel). Only two species (*C. filicina* and *C. polystachya*) from one section (*Indicae*) of subgenus *Indocarex* are included in this study. Although these species form a well-supported monophyletic group (bs 100%, jk \geq 67%), Yen and Olmstead (in press) found, based on analysis of chloroplast sequences and better sampling of the subgenus, that *Indocarex* is polyphyletic with respect to subgenus *Carex*.

Kükenthal (1909) recognized 29 sections in subgenus *Carex*, and many subsections; 24 of these sec-

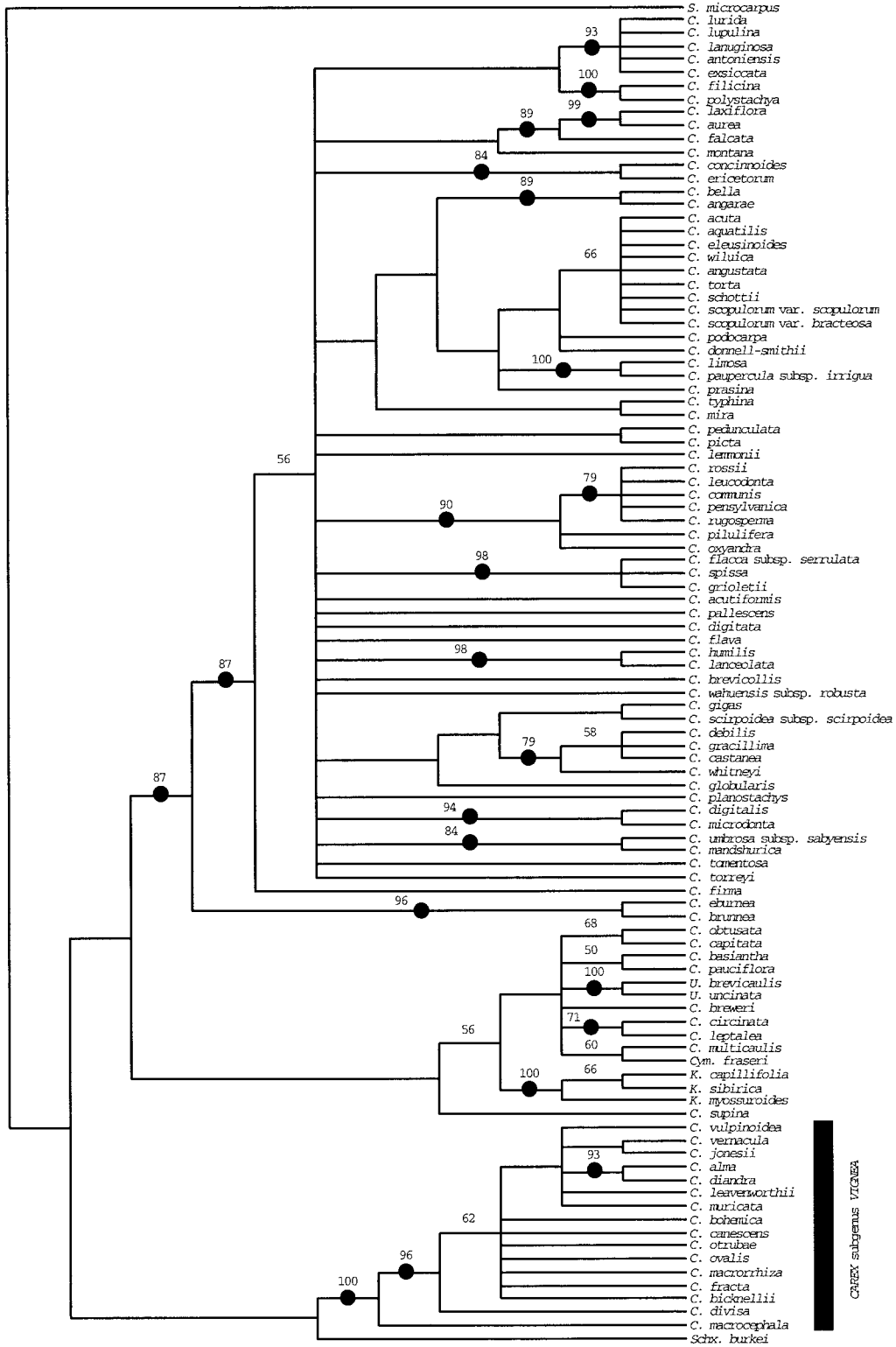
tions were sampled here. As sampling within most sections is poor, the question of monophyly of each section as a whole cannot be addressed in this paper. Instead, the monophyly or non-monophyly of sections as suggested by the data presented here is addressed with the caution that hypotheses of monophyly may change with additional data. Only two of the sections sampled here (*Limosae* and *Atratae*; two samples each) are monophyletic (Fig. 4). In more detailed analyses of *Limosae* with more taxa sampled using ITS and chloroplast spacer sequences (Waterway et al. 1997), the section is not monophyletic. The two representatives of *Atratae* (*C. angarae* and *C. bella*) are well-supported (bs 100%, jk \geq 67%, one indel) as forming a clade, and are positioned in a tritomy with a portion of *Digitatae* and a clade including most of subgenus *Carex*. Section *Trachychlaenae* is potentially monophyletic, forming an unresolved polytomy with *C. grioletii* (section *Pachystylae*; Fig. 4).

Section *Acutae* is represented by nine taxa (*C. acuta*, *C. angustata*, *C. aquatalis*, *C. elusinoides*, *C. schottii*, *C. scopulorum* var. *scopulorum*, *C. scopulorum* var. *bracteosa*, *C. torta*, and *C. wiluica*) representing all seven of the subsections recognized by Kükenthal (*Bicolores*, *Caespitosae*, *Cryptocarpae*, *Forsiculatae*, *Praelongae*, *Rigidae*, and *Vulgares*). This section is paraphyletic, with a species of section *Scitae* (*C. podocarpa*) nested within. Otherwise, this group forms a well-resolved clade that is sister to a clade composed of several other sections. Subsection *Rigidae* is represented by two taxa and is monophyletic, but subsection *Vulgares*, also represented by two taxa, is polyphyletic. *Acutae* is currently treated as *Phacocystis* Dumort. (e.g., Standley 1989; Luceño and Aedo 1994) with no subsectional division. *Carex aurea* (in Kükenthal's *Paniceae*) is currently treated in section *Bicolores* Tuck. (Mackenzie 1935; Hermann 1970) but does not group with the *Acutae* in this analysis (treated by Kükenthal (1909) as a subsection of *Acutae*), instead forming a well-supported clade (bs 91%, jk \geq 67%) with *C. falcata* (*Paniceae*) and *C. laxiflora* (*Careyanae*; Fig. 4).

Of the remaining sections sampled, nine of these

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FIG. 2. Analysis of relationships within Cariceae using the nrDNA ITS data set. Strict (MP) consensus tree of 20,300 most parsimonious trees of 1,368 steps (CI=0.336; RI=0.634; RC=0.213). Numbers above branches are bootstrap percentages. Bullets on branches refer to nodes supported by jackknife analysis with at least 67%. Generic names are abbreviated as follows: C. = *Carex*, Cym. = *Cymophyllus*, K. = *Kobresia*, S. = *Scirpus*, Schx. = *Schoenoxiphium*, and U. = *Uncinia*.



appear to be polyphyletic. One, *Acrocystis* (Kükenthal's *Montanae*), is addressed in more detail below.

Section *Physocarpae* is represented by four species (*C. exsuccata*, *C. lupulina*, *C. lurida*, and *C. typhina*). Kükenthal separated this section into three subsections—*Lupulinae*, *Tentaculatae*, and *Vesicariae*. Three of the four species fall within a well-supported clade (bs 91%, jk \geq 67%) that also includes species from sections *Hirtae* (*C. lanuginosa*) and *Pseudo-cyperae* (*C. antoniensis*). Both of these other sections are placed close to the *Physocarpae* in Kükenthal's treatment, as they share septate-nodulose leaves. Another section with septate-nodulose leaves, *Paludosae*, does not appear to be closely related to this clade. Subsection *Tentaculatae* (represented by *C. lurida* and *C. typhina*) is not monophyletic, with *C. typhina* forming a clade with members of sections *Careyanae* and *Griseae*. However, Mackenzie (1935) treated *C. typhina* in section *Squarrosae* Carey, separated from the rest of the *Tentaculatae*.

Section *Hymenochlaenae* is represented by six species from five of six subsections recognized by Kükenthal (1909) (*Debiles*, *Graciles*, *Gracillimae*, *Longirostres*, and *Pubescentes*; *Capillares* not sampled). Four species form a well-supported clade (bs 84%, jk \geq 67%; *C. castanea*, *C. debilis*, *C. gracillima*, and *C. whitneyi*) while the other two (*C. brunnea* and *C. prasina*) appear distantly related from this clade and each other. *Carex brunnea* forms a well-supported clade (bs 99%, jk \geq 67%, 2 indels) with *C. eburnea* of section *Albae*, while *C. prasina* forms a clade with section *Limosae*. More detailed study of the *Hymenochlaenae* is being carried out by others (M. Waterway, pers. comm.).

Section *Digitatae* is represented by six species, all from subsection *Eu-Digitatae* (subsection *Radicales* not sampled). This group is scattered across clade C (Fig. 4). Only two species group together, *C. humilis* and *C. lanceolata* and are well-supported (bs 100%, jk \geq 67%, one indel), and this clade forms a polytomy with other lineages towards the base of clade C. Of the other taxa, *C. pedunculata* forms a well-supported clade (bs 80%, jk \geq 67%) with *C. picta* (*Pictae*); *C. concinoides* is placed alone in a large polytomy; *C. digitata* is placed alone in the

grade of subgenus *Carex* species; and *C. planostachys* is sister to a clade composed of members of sections *Physocarpae*, *Careyanae*, and *Griseae* (Fig. 4).

Section *Pachystylae* is represented by six species (*C. globularis*, *C. grioletii*, *C. mandshurica*, *C. pallescens*, *C. tomentosa*, and *C. torreyi*). This group is also scattered across clade C. No monophyletic groups are apparent. *Carex globularis*, *C. pallescens*, and *C. torreyi* form a grade leading to *C. acutiformis* (section *Paludosae*). This clade of four species is supported by an indel. *Carex tomentosa* and *C. mandshurica* form a clade with *C. umbrosa* subsp. *sabyensis* (section *Mitratae*) and *C. wahuensis* subsp. *robusta* (section *Rhomboidales*). *Carex mandshurica* and *C. umbrosa* subsp. *sabyensis* are further supported by an indel. *Carex grioletii* forms a well-supported tritomy (bs 99%, jk \geq 67%, one indel) with *C. flacca* subsp. *serrulata* and *C. spissa* of section *Trachychlaenae*. Several of these taxa have been placed in *Acrocystis* by other authors and will be addressed in more detail below.

Section *Careyanae*, represented by two taxa, *C. digitalis* and *C. laxiflora*, is polyphyletic. *Carex digitalis* is paired with *C. microdonta* of section *Griseae* and *C. laxiflora* is grouped with *C. aurea* and *C. falcata* of section *Paniceae*.

Section *Spirostachyae*, represented by two species (*C. flava* and *C. lemmonii*), does not form a monophyletic group. *Carex flava* is placed as a single branch in the grade of subgenus *Carex* sections, and *C. lemmonii* is sister to a clade composed of *Careyanae* and *Paniceae*.

Section *Frigidae* is represented by two species (*C. mira* and *C. firma*) that also do not form a clade. *Carex mira* is well-supported (bs 86%, jk \geq 67%) as sister to *C. montana* of *Acrocystis* and *C. firma* is sister to a clade composed of *C. laxiflora* (*Careyanae*), *C. aurea* (*Paniceae*) and *C. falcata* (*Paniceae*). *Carex mira* is considered by some to belong to *Acrocystis* and this will be discussed below.

Section *Rhomboidales* is represented by two taxa (*C. brevicollis* and *C. wahuensis* subsp. *robusta*). These species do not form a clade. *Carex brevicollis* is part of a tritomy, and *C. wahuensis* subsp. *robusta* forms a clade with members of *Mitratae* and *Pachystylae*.

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FIG. 3. Analysis of relationships within Cariceae using the cpDNA *trnT-L-F* data set. Strict (MP) consensus tree of 15,200 most parsimonious trees of 1,196 steps (CI=0.659; RI=0.770; RC=0.507). Numbers above branches are bootstrap percentages. Bullets on branches refer to nodes supported by jackknife analysis with at least 67%. Generic names are abbreviated as follows: C. = *Carex*, Cym. = *Cymophyllus*, K. = *Kobresia*, S. = *Scirpus*, Schx. = *Schoenoxiphium*, and U. = *Uncinia*.

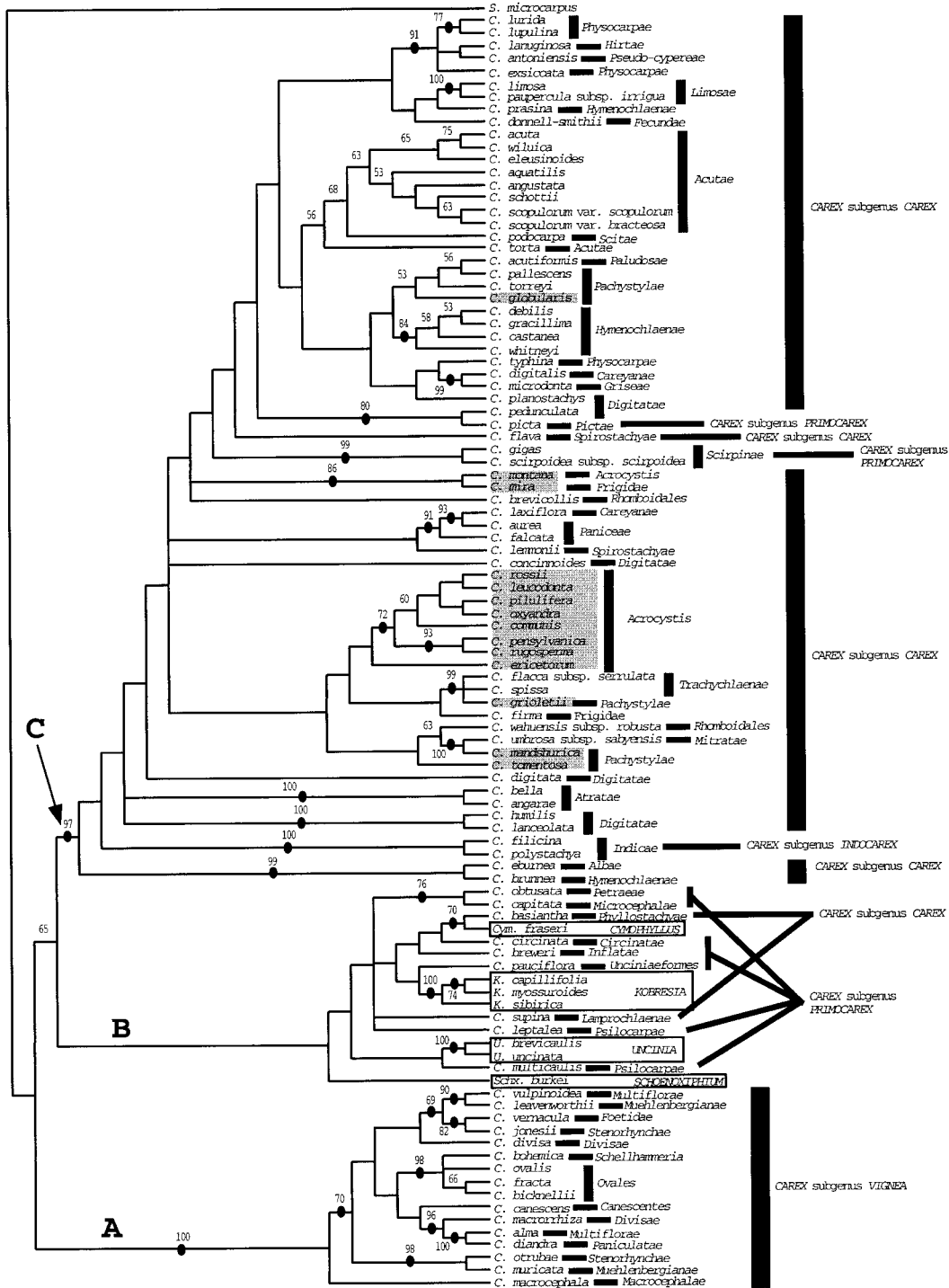


FIG. 4. Analysis of relationships within Cariceae using combined ITS and *trnT-L-F* data sets. Strict (MP) consensus tree of 168 most parsimonious trees of 2,991.44 steps (CI=0.454; RI=0.600; RC=0.272). Numbers above branches are bootstrap percentages. Filled circles on branches refer to nodes supported by jackknife analysis with at least 67%. The classification of Kükenthal (1909) is overlaid on the phylogeny. *Cymophyllus fraseri* was treated by Kükenthal as *Carex*

Three taxa considered to belong to subgenus *Primocarex* are members of clade C. These are *C. picta* (section *Pictae*) and *C. gigas* and *C. scirpoidea* subsp. *scirpoidea* (section *Scirpinae*). *Carex picta* is placed sister to *C. pedunculata* (section *Digitatae*). The taxa of *Scirpinae* comprise a clade sister to a large clade containing several sections of subgenus *Carex*. Recently, Dunlop and Crow (1999) revised *Scirpinae* and excluded *C. gigas* and *C. scabriuscula* Mack. from the section on the basis of morphological, anatomical, ecological, and chromosome number data. While the circumscription of *Scirpinae* cannot be addressed here, it seems clear that at least *C. gigas* is closely related to *C. scirpoidea*, given the strong statistical support for their pairing (bs 99%, jk \geq 67%). Mackenzie (1935) and others have considered sections *Pictae* and *Scirpinae* to be more closely related to various subgenus *Carex* sections than to other *Primocarex* groups, which is supported by our study.

The implication of our findings is that morphological characters used to circumscribe sections in *Carex* are likely homoplastic or plesiomorphic. Detailed reassessment of morphological character states and the search for physical characters other than macromorphology (e.g., micromorphology, Menapace and Wujek 1985; leaf transverse anatomy, Shepherd 1977; etc.) may help to address these incongruities. Non-macromorphological characters, such as micromorphology and leaf transverse anatomy, have proven to be helpful in resolving incongruities between macromorphology and molecular phylogenetics in other graminoid monocots (e.g., Columbus 1999).

Relationships in Section *Acrocystis*. *Acrocystis*, as it is currently treated, appears to be polyphyletic. A majority of the species of this section forms a core *Acrocystis* clade, but six species are not in this clade (Fig. 4). Of the species included in *Acrocystis* by Kükenthal (1909), only *C. montana* is not in this core clade. It is possible, given the low statistical support for branches separating the core *Acrocystis* clade from other members of *Acrocystis*, that with additional data *Acrocystis* might prove to be monophyletic, but these analyses suggest otherwise. The species not associated with the core clade are all of Eurasian origin, while the core clade includes the

North American species (*C. communis*, *C. leucodonta*, *C. pensylvanica*, *C. rossii*, and *C. rugosperma*), two Eurasian species (*C. ericetorum* and *C. pilulifera*), and a species restricted to far eastern Asia (*C. oxyandra*). Only five of the 27 currently recognized taxa of North American *Acrocystis* were included in this analysis. More detailed sampling (Roalson, unpubl. data) places all of the North American taxa in the core *Acrocystis* clade.

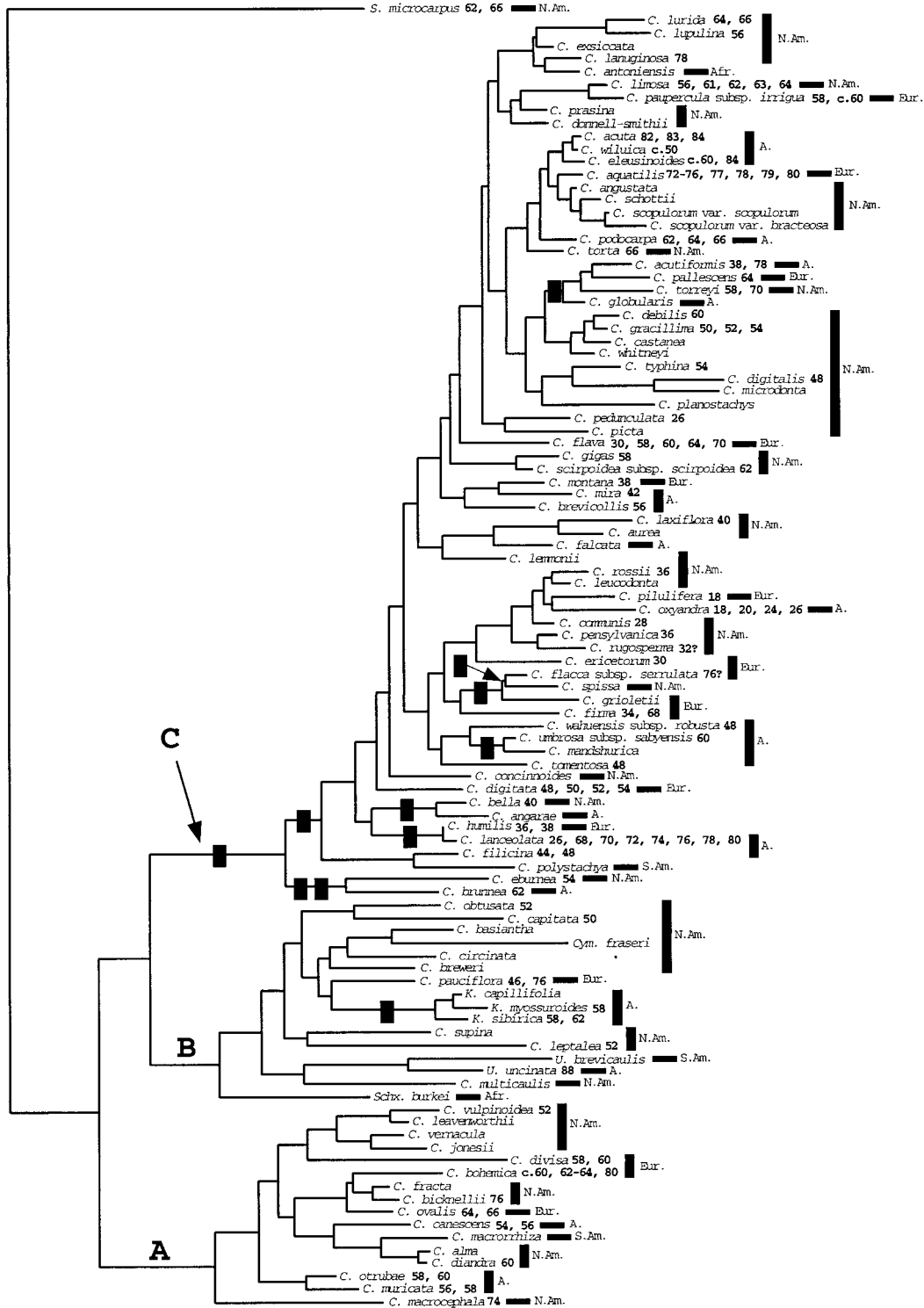
Kükenthal's exclusion of *C. globularis*, *C. grioletii*, *C. mandshurica*, and *C. tomentosa* (all section *Pachystylae*), and *C. mira* (section *Frigidae*) from *Acrocystis* appears justified based on the ITS/*trnT-L-F* phylogeny. *Carex montana*, though, has always been placed in *Acrocystis*, and its absence from the core clade is surprising. Instead, it is sister to *C. mira*, an Asian member of section *Frigidae*/*Acrocystis*. *Carex ericetorum*, *C. pilulifera*, and *C. oxyandra* form a group with the North American *Acrocystis* members—*C. ericetorum* as sister to the rest of the core *Acrocystis* clade, and *C. pilulifera* and *C. oxyandra* nested within the North American members. The placement of *C. pilulifera* with North American species is not novel as Fernald (1902) commented on the similarity of *C. pilulifera* to the North American *C. communis*. The placement of *C. ericetorum*, *C. pilulifera*, and *C. oxyandra* in this clade is consistent, regardless of how many of the North American *Acrocystis* species are included (Roalson, unpubl. data).

While *Acrocystis* is not monophyletic in the ITS/*trnT-L-F* phylogeny, relationships to other sections of *Carex* and genera of Cariceae as proposed by previous authors can be addressed. Heilborn's (1924) suggestion that *Acrocystis* is primitive in *Carex* owing to its low chromosome number is unlikely. Given the position of the taxa (particularly the core clade), this group does not appear to hold a "primitive" (i.e., basal clade) location in the genus. Chromosome evolution in *Carex* will be discussed below. Savile and Calder (1953) suggested *Acrocystis* is primitive within subgenus *Carex* along with *Obtusatae*, but most of the species of *Acrocystis* are not near the base of the subgenus *Carex* clade, nor are any allied with *Obtusatae*.

Kukkonen (1963) suggested that *Acrocystis* was derived from a portion of *Kobresia* leading to sub-

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subgenus *Primocarex* section *Leucocephalae*, but is treated here at the generic rank. Taxa in shaded boxes are species currently placed in *Acrocystis*. Generic names are abbreviated as follows: C. = *Carex*, Cym. = *Cymophyllus*, K. = *Kobresia*, S. = *Scirpus*, Schx. = *Schoenoxiphium*, and U. = *Uncinia*.



- 10 changes

genus *Carex*, but *Acrocystis* does not appear to be closely related to any members of *Kobresia*. Reznicek (1990) inferred that *Acrocystis* held a "derived" position in subgenus *Carex*, which appears accurate, at least for most of the species.

Koyama (1962) considered *Acrocystis* to be part of a more broadly defined *Digitatae*. In light of the combined ITS/*trnT-L-F* phylogeny, this grouping is artificial, with both *Acrocystis* and *Digitatae* being polyphyletic whether considered together or apart.

The relationship among *Acrocystis* and other sections is difficult to address, particularly given the absence of statistical support for the relationship of the core *Acrocystis* clade and neighboring clades. The two other clades that appear closest to the core *Acrocystis* clade comprise a diversity of *Carex* species. The sister clade includes *C. firma* (*Frigidae*), *C. flacca* subsp. *serrulata* (*Trachychlaenae*), *C. spissa* (*Trachychlaenae*), and *C. grioletii* (*Pachystylae/Acrocystis*). The next clade includes *C. tomentosa* (*Pachystylae/Acrocystis*), *C. wahuensis* subsp. *robusta* (*Rhomboidales*), *C. umbrosa* subsp. *sabyensis* (*Mitratae*), and *C. mandshurica* (*Pachystylae/Acrocystis*). Given the lack of statistical support for sister-group relationships, resolving this issue will depend on expanded sampling and additional data.

Chromosome Evolution and Biogeography.

Chromosome number variation has played a central role in hypotheses of diversification of *Carex*. *Carex*, as in many if not all genera of the Cyperaceae and Juncaceae, has diffuse centromeres or polycentric chromosomes (Greilhuber 1995). This condition, in association with extreme levels of aneuploid chromosome number change ($n = 6-68$ with nearly every aneuploid number in-between present; Nishikawa et al. 1984), has been suggested as the driving mechanism of speciation in *Carex* and has been described as agmatoploidy, or qualitative aneuploid change where the change is mediated by chromosome fission and fusion (Davies 1956; Grant 1971; Nishikawa et al. 1984; Whitkus 1987). This hypothesis has yet to be explicitly tested. Given this ram-

phant chromosome number variation, clear indications of how chromosome number change has taken place is not readily apparent. Given the phylogenetic hypothesis at hand, though, some idea of chromosome evolution can be inferred.

Chromosome number reports have been overlaid on one of the combined analysis trees (Fig. 5). Unfortunately, chromosome numbers have not been reported for many species. Chromosome numbers of outgroups are given next to species names in the outgroup analysis (Fig. 1).

Genera surrounding the Cariceae in the outgroup analysis generally have high chromosome counts ($2n = 62, 66$), although *Dulichium arundinaceum* in the sister clade to the Cariceae has a fairly low chromosome number ($2n = 32$). In addition, species of Cariceae in clades A and B generally have moderate to high chromosome numbers ($2n = 46-88$), and the basal branches of clade C also have moderate to high chromosome numbers ($2n = 44-62$). These data suggest that the ancestor of Cariceae had a moderate to high chromosome number and that the low chromosome numbers of some species of *Carex* are a derived condition. This is contrary to the hypotheses of Heilborn (1924) and others. Given the hypothesis that agmatoploid chromosome number change is the driving force of chromosome variation in *Carex*, if agmatoploid chromosome number change is occurring, chromosome fusion might be as common or more so than fission.

Traditional revisionary studies in *Carex* have focused on species groups over a subcontinental range. The phylogenetic hypothesis of relationships provided here calls into question the ability of geographically restricted revisionary studies to resolve relationships among closely related species. The continent of origin of specimens included in this study has been mapped onto one of the combined analysis trees in Fig. 5, although some species occur on more than one continent. Nearly all clades include individuals from multiple continents. This suggests that for revisionary studies to be effective

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FIG. 5. Analysis of relationships within Cariceae using combined ITS and *trnT-L-F* data sets with chromosome variation and biogeography overlaid on the phylogeny. One of 168 most parsimonious trees displayed as a phylogram to indicate branch lengths. Bars on branches refer to nodes supported by a gap (indel). Reported chromosome number ($2n$) of species (when known) is to the right of taxon names. Species that have variation in chromosome number are noted with the appropriate range of numbers. Continent of origin of the specimen is to the right of taxon names and chromosome numbers. Continents are abbreviated as follows: A. = Asia, Afr. = Africa, Eur. = Europe, N.Am. = North America, and S.Am. = South America. Generic names are abbreviated as follows: C. = *Carex*, Cym. = *Cymophyllus*, K. = *Kobresia*, S. = *Scirpus*, Schx. = *Schoenoxiphium*, and U. = *Ucinia*.

at elucidating relationships in species groups, a world-wide view must be taken.

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