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THE PHYLOGENETIC POSITION OF *CAREX* SECTION *PHYLLOSTACHYS* AND ITS IMPLICATIONS FOR PHYLOGENY AND SUBGENERIC CIRCUMSCRIPTION IN *CAREX* (CYPERACEAE)[1]

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Wide speculation surrounds the origin and phylogenetic relationships of the most highly reduced sections in the genus *Carex.* In order to gain a better understanding of phylogeny in *Carex,* the relationship of the reduced sect. *Phyllostachys* to 12 putatively related sections, representing all four subgenera (*Primocarex, Indocarex, Carex, Vignea*), was inferred from sequences of the ITS (internal transcribed spacer) region of nrDNA. Phylogenetic reconstructions identified two main clades: (1) a ''compound'' clade composed of sections from subg. *Indocarex, Primocarex,* and a portion of subg. *Carex,* and (2) a ''reduced'' clade consisting of sections from subg. *Carex* (*Phyllostachys*) and *Primocarex* (*Filifoliae* and *Firmiculmes*). Subgenus *Indocarex* was paraphyletic within the ''compound'' clade supporting classifications that have merged it within a wider subg. *Indocarex*/*Carex*/*Primocarex* line. Subgenus *Primocarex* was polyphyletic. This result was consistent with theories that extreme reduction has occurred along several different evolutionary lines in *Carex.* Phylogenetic theories inferred from the presence or abnormal growth of the rachilla were not supported by tree topologies. Difficult sectional circumscriptions, such as the separation of sections *Laxiflorae* and *Careyanae,* were strongly upheld by sequence data. The ITS region is an effective tool for defining sectional limits and for estimating relationships among sections in *Carex,* but does not provide enough phylogenetic information to fully resolve relationships below the sectional level.

Key words: *Carex*; Cyperaceae; ITS phylogeny; sectional delimitation; subgeneric circumscription.

The genus *Carex* L. is one of the largest (~2000 spp.) and most widespread of all flowering plant genera (Reznicek, 1990). Although cosmopolitan, most of its species are north temperate in distribution with centers of diversity in North America and eastern Asia (Ball, 1990; Reznicek, 1990; Naczi, 1992). The habitats of *Carex* species are as diverse as the genus and range from dry open savannas and rain forests, to wet meadows, deciduous and coniferous forests, and Arctic tundra. The genus and its tribe *Cariceae* Kunth ex Dumort. are clearly distinguished within the Cyperaceae Juss. by the possession of consistently unisexual flowers and a perigynium, which is a sac-like structure of prophyllar origin that surrounds the naked gynoecium (Blaser, 1944). The perigynium provides the primary source of taxonomic characters in *Carex,* and it is largely through its subtle differences in shape, size, texture, and nervation that the enormous number of species in *Carex* are distinguished (Nelmes, 1951).

Despite the global distribution and ecological importance of this genus, evolutionary relationships within *Carex* are poorly understood (Reznicek, 1990). This lack of understanding can be attributed to the nature of morphological and anatomical characters in *Carex.* Floral reduction (Smith and Faulkner, 1976), uniform vegetative morphology and anatomy (Metcalfe, 1971), and repeated events of parallelism and reversal (Reznicek, 1990; Naczi, 1992; Starr, 1997) have obscured phylogenetic trends and have led to the recognition of many artificial taxa at the sectional and subgeneric level. The extreme reduction in the genus and the almost complete lack of knowledge of its ontogeny (Alexeev, 1988), have further raised homology problems that complicate phylogenetic reconstruction at all levels.

DNA characters offer an obvious source of variation whose use is just beginning to be explored in *Carex.* The advantages afforded by these characters in terms of character number, homology assessment, reduced homoplasy, and ease of scoring character states suggest that DNA characters could make a substantial contribution to the systematics of this genus.

In this study we investigate the usefulness of DNA characters in the systematics of *Carex* by using sequences from the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (nrDNA). These loci have become the sequences of choice for addressing phylogenetic questions within genera because they are easily aligned and amplified, they have a high mutation rate, and they are relatively small (for a review see Baldwin et al., 1995).

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Because of the size of the genus, we chose to focus our study on *Carex* section *Phyllostachys* (J. Carey) L.H. Bailey. Several arguments favor this approach. First, the small size of the section (eight species) allows for both interspecific and intraspecific variation to be assessed. Second, its clear delimitation from other sections and the recent resolution of a number of its taxonomic problems (Catling, Reznicek, and Crins, 1993; Naczi, Reznicek, and Ford, 1998; Ford et al., 1998) suggest that the interpretation of results should be not confounded by a poorly resolved taxonomy. Third, the evolutionarily reduced and unconventional inflorescence morphology of the *Phyllostachys* has led many authors to consider the section as pivotal to our understanding of subgeneric limits and evolution in the genus. Thus a clarification of the phylogenetic position of this section will allow us to address these wider systematic issues in *Carex*.

The specific objectives of this study were therefore to: (1) assess the utility of the ITS region for resolving both phylogenetic and taxonomic questions in *Carex*; (2) determine whether *Carex* section *Phyllostachys* is monophyletic; and (3) determine the systematic position of this section within the genus *Carex* by comparing sequence data of section *Phyllostachys* with species representative of putatively related sections.

SYSTEMATIC BACKGROUND

Subgeneric classification—In the only worldwide monograph of the genus, Kükenthal (1909) divided *Carex* into four subgenera: (1) *Primocarex* Kük., characterized by solitary terminal spikes; (2) *Vignea* (P. Beauv. ex Lestib. f.) Peterm., distinguished by sessile, bisexual inflorescence units, the lack of a cladoprophyll (a tubular structure subtending an inflorescence), and distigmatic flowers; (3) *Indocarex* Baillon, a mainly tropical group with paniculate inflorescences, bisexual inflorescence units, cladoprophylls, inflorescence prophylls (perigynium-like organs subtending lateral inflorescence units), and tristigmatic flowers; and (4) *Carex*, characterized by mostly peduncled, unisexual inflorescence units, cladoprophylls, and usually tristigmatic flowers.

Kükenthal (1909) considered subg. *Primocarex* primitive within *Carex* because its spikelets typically possessed a conspicuous secondary axis (rachilla): a trait common to the other genera in the *Cariceae*, but normally lacking in subg. *Indocarex*, *Carex*, and *Vignea*. Most subsequent authors (Reznicek, 1990, and papers cited therein) have criticized this arrangement, believing that the species in subg. *Primocarex* had evolved independently from multispicate species in the other subgenera or from closely related genera in the *Cariceae*. Based on these conclusions, most recent authors recognize three subgenera (*Carex*, *Indocarex*, and *Vignea*) with members of *Primocarex* divided between subg. *Vignea* or *Carex* or placed in closely related genera (Reznicek, 1990). Contrasting opinions, however, have been expressed by researchers such as Ohwi (1936) and Koyama (1962) who recognized only two subgenera, *Carex* and *Vignea*, based on the observation that some species in sections *Hymenochlaenae* (Drejer) L.H. Bailey and *Frigidae* Christ subsect. *Decorae* Kük. are transitional in their morphology between subg. *Carex* and *Indocarex*.

Carex sect. Phyllostachys and its position within the genus Carex—*Carex* section *Phyllostachys* is a clearly defined group of eight species (*C. backii* Boott; *C. juniperorum* Catling, Reznicek, & Crins; *C. latebracteata* Waterfall; *C. jamesii* Schwein., *C. saximontana* Mack.; *C. willdenowii* Willd.; *C. basiantha* Steud.; and *C. superata* Naczi, Reznicek, & B. A. Ford) restricted to North America north of Mexico. The section is easily distinguished by features such as large foliaceous pistillate scales and culms that are dilated at the apex (Crins, 1990; Catling, Reznicek, and Crins, 1993).

The strongly marked nature of the *Phyllostachys* and the lack of an obvious outgroup have led to wide speculation regarding its position within the genus. Bailey (1885, 1886) postulated that *Phyllostachys* was composed of two subsections: (1) *Bractoideae* L.H. Bailey, which included three of the species (*C. backii*, *C. jamesii*, and *C. willdenowii*) presently recognized in section *Phyllostachys*; and (2) *Phyllostachyae* L.H. Bailey, composed of species currently placed in section *Firmiculmes* Kük. (*C. multicaulis* L.H. Bailey, *C. geyeri* Boott). Bailey postulated that *Phyllostachys* was an offshoot of sect. *Acrocystis* Dumort. and that the section was connected to sect. *Laxiflorae* Kunth via *C. multicaulis*. Features such as dilated culm apices in many species of the *Laxiflorae* and its nearest allies (Mackenzie, 1935) lend some credibility to Bailey's hypothesis.

Holm (1900, 1903) argued that sect. *Phyllostachys* was not closely related to sections *Acrocystis*, *Laxiflorae*, or *Firmiculmes*, but was instead an extreme form of sect. *Hymenochlaenae* that demonstrated transitional characters between subg. *Carex* and *Indocarex*. His hypothesis was partly based on the fact that sect. *Phyllostachys* emulates the indocaricoid condition when the abnormal growth of the rachilla within basal perigynia leads to the production of lateral androgynous inflorescence units.

Kreczetovicz (1936) expressed views similar to Holm's but believed that sect. *Phyllostachys* was a highly derived group that had evolved only recently (Pliocene or Pleistocene) from subg. *Indocarex* via transmutive reduction. He defined transmutive reduction as reduction where fusion and/or a transformation of parts (e.g., of a bract into a scale) obscured a recent origin for species from subg. *Indocarex*. He cited the leafy pistillate scales and dilated culms of the *Phyllostachys* as clear evidence that such a reduction had taken place.

Based on smut host-parasite studies, Savile and Calder (1953) proposed that section *Phyllostachys* was a highly derived section within a lineage that included the multispicate section *Limosae* (Tuckerm.) Christ and the unispicate section *Scirpinae* Tuckerman. Although they conceded that subgenus *Primocarex* sensu Kükenthal was artificial, their studies of smut infections suggested that nearly half its species comprised a natural group. Other reduced taxa, like sections *Phyllostachys* and *Scirpinae*, were considered highly evolved because their smuts appeared to be related to smuts that infected multispicate carices typical of advanced subgenera. In addition, species in these sections seemed to lack a rachilla, a feature present in ''true'' primocarices.

Although authors rarely discuss the intricacies of their evolutionary system, the arrangement of taxa within their monographs presents an implicit phylogenetic hypothesis.

TABLE 1. Collection data for populations of species sampled from *Carex* section *Phyllostachys*. Vouchers are deposited at WIN unless otherwise noted. Individuals sampled from the same population are numbered (1) and (2). GenBank accession numbers are given in succession for ITS 1 sequences and for combined sequences consisting of ITS 2 and 17 bp at the 3′ end of the 5.8S gene.[a] Sequences for the entire ITS region (ITS 1, 5.8S, ITS 2) were determined in those individuals with a single GenBank accession number.

Subgenus	Species studied	Voucher	GenBank accession numbers
1. *Carex*	*C. backii* Boott	CANADA. Manitoba: Treesbank, *Ford* 9502 & *Starr*.	GBANAF027409, GBANAF027451
		Ontario. Simcoe Co., *Reznicek* 6364 & *Reznicek* MICH.	GBANAF027410, GBANAF027452
		Ontario. Niagara R.M., North Grimsby Twp., *Ball* s.n.	GBANAF027411, GBANAF027453
	C. saximontana Mackenzie	CANADA. Saskatchewan. Cypress Hills, *Ford* 9526 & *Starr*.	GBANAF027412, GBANAF027454
		(1) Manitoba. Treesbank, *Ford* 9501 & *Starr*.	GBANAF027413, GBANAF027455
		(2) *Ford* 9501 & *Starr*.	GBANAF027414, GBANAF027456
	C. latebracteata Waterfall	U.S.A. Arkansas. Polk Co., *Naczi* 3948 & *Ford*.	GBANAF027415, GBANAF027457
		Oklahoma. McCurtain Co., *Naczi* 3953 & *Ford*.	GBANAF027416, GBANAF027458
	C. juniperorum Catling, Reznicek, & Crins	CANADA. Ontario. Hastings Co., *Oldham* s.n. et al.	GBANAF027417, GBANAF027459
		U.S.A. Ohio. Adams Co., *Naczi* 3878.	GBANAF027418, GBANAF027460
		Kentucky. Bath Co., *Naczi* 3890.	GBANAF027419, GBANAF027461
	C. jamesii Schweinitz	CANADA. Ontario. Essex Co., *Oldham* 16117.	GBANAF027420, GBANAF027462
		U.S.A. Virginia. Bath Co., *Naczi* 4482 & *Thieret*.	GBANAF027421, GBANAF027463
		Arkansas. Polk Co., *Naczi* 3949 and *Ford*.	GBANAF027422, GBANAF027464
		Kentucky. Boone Co., *Naczi* 4096.	GBANAF027423, GBANAF027465
	C. willdenowii Willdenow	U.S.A. Ohio. Pike Co., *Naczi* 3887.	GBANAF027424, GBANAF027466
		Pennsylvania. Bradford Co., *Naczi* 4287 & *Thieret*.	GBANAF027425
		Kentucky. Franklin Co., *Naczi* 3835 & *Borne*.	GBANAF027426, GBANAF027467
	C. superata Naczi, Reznicek & B.A. Ford	(1) U.S.A. Mississippi. Tishomingo Co., *Naczi* 4013 et al.	GBANAF027427, GBANAF027468
		(2) *Naczi* 4013 et al.	GBANAF027428
		Alabama. Butler Co., *Naczi* 3990 & *Ford*.	GBANAF027429, GBANAF027469
	C. basiantha Steudel	U.S.A. Alabama. Butler Co., *Naczi* 3991 & *Ford*.	GBANAF027430, GBANAF027470
		Mississippi. Itawamba Co., *Naczi* 4005 et al.	GBANAF027431, GBANAF027471
		Louisiana. West Feliciana Parish, *Naczi* 3987 & *Ford*.	GBANAF027432, GBANAF027472

[a] The prefix GBAN has been added for linking the on-line version of *American Journal of Botany* to GenBank and is not part of the actual GenBank accession number.

The position of sect. *Phyllostachys* in the monographs of Mackenzie (1935) and Kükenthal (1909) is worthy of mention owing to the great influence of these works on the present systematics of the genus. Mackenzie (1935) did not divide his 71 North American sections into subgenera but arranged them in a system where sectional affinities were vaguely expressed through a conspectus. Although the first five sections, referable to subg. *Primocarex*, were thought to be ‘‘primitive,’’ other solitary-spiked species were regarded as ‘‘closely related to more developed sections’’ and aligned with representatives of the other subgenera. Section *Phyllostachys* was positioned between the unispicate sections *Polytrichoideae* (Tuckerm.) Mack. and *Filifoliae* (Tuckerm.) Mack. at the beginning of a large group of sections variously placed in subg. *Indocarex, Carex,* and *Primocarex.* Mackenzie (1935) did not directly align the *Phyllostachys* or *Polytrichoideae* with any other section, but grouped the *Filifoliae* with sections *Obtusatae* (Tuckerm.) Mack., *Praecoces* Christ, and *Acrocystis.* Kükenthal (1909) placed sect. *Phyllostachys* between two Old World sections, the *Rhomboidales* Kük. and the *Elatae* Kükenthal. Species in these sections are generally characterized by many-flowered, multispicate inflorescences, sheathing inflorescence bracts, and many-nerved perigynia. These characters are either absent or weakly developed in sect. *Phyllostachys.*

MATERIALS AND METHODS

Choice of taxa—Specimen citations for all individuals sequenced in this study and the nomenclature of the taxa employed are given in Tables 1 and 2. Taxa were chosen as representatives of specific groups

TABLE 2. Taxonomy and collections of the taxa used in this study. Vouchers are deposited at WIN unless otherwise noted. GenBank accession numbers are given in succession for ITS 1 sequences and for combined sequences consisting of ITS 2 and 17 bp at the 3′ end of the 5.8S gene. Sequences for the entire ITS region (ITS 1, 5.8S, ITS 2) were determined in those species with a single GenBank accession number.

Subgenus	Section	Species studied and voucher	GenBank accession numbers
1. *Carex*	*Acrocystis* Dumort.	*C. albicans* Willd. U.S.A. Arkansas, Scott Co., *Ford* 9440 & *Naczi*.	GBANAF027439, GBANAF027478
		C. peckii Howe CANADA. Alberta, Edmonton, *Starr* 96010.	GBANAF027441, GBANAF027480
		C. pensylvanica Lam. Manitoba, Portage la Prairie, *Ford* 9604 et al.	GBANAF027440, GBANAF027479
		C. tonsa (Fernald) E.P. Bicknell var. *rugosperma* (Mack.) Crins. Manitoba, Whiteshell Provincial Park., *Ford* 94111.	GBANAF027438, GBANAF027477
	Careyanae Tuckerm. ex Kük.	*C. careyana* Torr. CANADA. Ontario, Halton Co., *Ball* s.n.	GBANAF027443, GBANAF027482
		C. plantaginea Lam. Ontario, Peterborough Co., *Bakowsky* #96–174.	GBANAF027442, GBANAF027481
	Hymenochlaenae (Drejer) L.H. Bailey	*C. arctata* Boott CANADA. Manitoba, Whiteshell Provincial Park, *Ford* 9624 et al.	GBANAF027446, GBANAF027485
	Laxiflorae Kunth	*C. laxiflora* Lam. U.S.A. Arkansas, Polk Co., *Ford* 9443 & *Naczi*.	GBANAF027444, GBANAF027483
		C. blanda Dewey CANADA. Ontario, Peterborough Co., *Bakowsky* #96–176.	GBANAF027445, GBANAF027484
2. *Indocarex* Baill.	*Cruciatae* (C.B. Clark) Nelmes	*C. cruciata* Wahlenb. MALAYSIA. Mulu National Park, Sarawak, *Yen* 075 WTU.	GBANAF027450, GBANAF027489
	Indicae Tuckerm.	*C. polystachya* Sw. BELIZE. Cayo district, *Jones* 11275 & *Wipff* MICH.	GBANAF027448, GBANAF027487
	Polystachyae Tuckerm.	*C. baccans* Nees TAIWAN. Wu Lai, Taipei, *Yen* 078 WTU.	GBANAF027449, GBANAF027488
3. *Primocarex* Kük.	*Filifoliae* (Tuckerm.) Mack.	*C. filifolia* Nutt. CANADA. Manitoba, Lauder Sand Hills, *Punter* & *Punter* s.n.	GBANAF027433, GBANAF027473
	Firmiculmes Kük.	*C. multicaulis* L. H. Bailey U.S.A. California, San Diego Co., *Ford* 9567 & *Starr*.	GBANAF027435, GBANAF027475
		C. geyeri Boott Montana, Cascade Co., *Starr* MT96039 et al.	GBANAF027434, GBANAF027474
	Scirpinae Tuckerm.	*C. scirpoidea* Michx. CANADA. Alberta, Jasper National Park, *Bayer* AB-96010 et al.	GBANAF027447, GBANAF027486
4. *Vignea* (P. Beauv. ex Lestib. f.) Peterm.	*Deweyanae* Tuckerm.	*C. deweyana* Schw. CANADA. Alberta, Edmonton, *Starr* 96007.	GBANAF027437, GBANAF027476
	Divisae Christ	*C. eleocharis* L. H. Bailey Alberta, Jasper National Park, *Bayer* AB-96004 et al.	GBANAF027436

or putative lineages to which *Carex* section *Phyllostachys* has previously been associated (see Systematic Background section). Some of the taxa placed close to sect. *Phyllostachys* by Mackenzie (1935) and Kükenthal (1909) could not be included in this study due to the unavailability of material or to problems encountered in amplification and sequencing.

Choice of outgroup—Efforts to obtain complete sequences for the ITS 1 and ITS 2 regions of a member of the closely related genus *Kobresia* Wahlenb. were unsuccessful. As a result, the two species sequenced from subgenus *Vignea* were chosen as the outgroup. This decision was based on the general agreement that this subgenus is natural (e.g., Nelmes, 1951; Koyama, 1962; Reznicek, 1990; etc.) and not closely related to sect. *Phyllostachys.* Unrooted searches supported the assumption of monophyly for the ingroup, i.e., a single branch partitioned the ingroup from the outgroup (Swofford et al., 1996).

DNA isolation and PCR amplification—Total genomic DNA was extracted from live or dried leaf tissue of a single individual (Table 1) following a modified CTAB method (Doyle and Doyle, 1987) with 1.0% beta-mercaptoethanol in the extraction buffer. To assess the amount of infraspecific variation that might be expected for the ITS region in *Carex,* two to four individuals from across the range of each of the eight species in section *Phyllostachys* were sequenced. In two cases, individuals from single populations (*C. saximontana, Ford* 9501 & *Starr*; *C. superata, Naczi* 4013 et al.; Table 1) were sequenced to determine whether any variation might exist at the population level.

PCR amplification and sequencing of ITS—Double-stranded DNA for the complete ITS region (3′ 18S to 5′ 26S fragment) was PCR (polymerase chain reaction) amplified from total genomic DNA using the forward primer ITS-L (Hsiao et al., 1994), and the reverse primer ITS-4 (White et al., 1990). As in grasses (Hsiao et al., 1994), ITS-L provided better amplification than the forward primer ITS-5 (White et al., 1990). Each 100-μL reaction mixture contained 10 μL of 10X *Taq* reaction buffer, 6 μL of 25 mmol/L magnesium chloride solution, 65–70 μL of de-ionized water, 5 μL of each of the primers in 5-pmol concentrations, 2 μL of a 10 mmol/L dNTP solution in equimolar ratio, 1μL of *Taq* DNA polymerase (1 unit), and 10–50 ng of template DNA. Double-stranded PCR products were produced on a GeneE® thermal cycler (Techne Inc., Princeton, New Jersey) via 30 cycles of DNA denaturation at 95°C for 1 min, primer annealing at 48–55°C for 1 min, and DNA strand extension by *Taq* DNA polymerase at 72°C for 2 min. The PCR was terminated at the end of 30 cycles by a final extension at 72°C for 7 min. The double-stranded DNAs were then purified by differential filtration using ULTRAFREE®-MC filter units (30000 NMWL, Millipore Corporation, Bedford, Massachusetts) and diluted with de-ionized water based on the strength of the amplification.

Cycle sequencing of the purified amplification product was performed using the dideoxy chain termination method (Sanger, Nicklen, and Coulson, 1977) employed by the *fmol*®*1 Sequencing System (Promega Corporation, Madison, Wisconsin). All sequencing primers were 5′ end-labeled in a preliminary reaction involving T4 polynucleotide kinase and [γ-^{32}P]-dATP (Amersham Life Sciences Inc., Cleveland, Ohio). Termination products for both ITS 1 and ITS 2 were produced from the double-stranded template DNA with the terminal primers ITS-L and ITS-4, and the internal primers ITS-2 and ITS-3 of White et al. (1990). Fragments were separated electrophoretically on 0.4 mm, denaturing, 6% polyacrylamide gels (8mol/L urea) at 2300 v/60 W. Gels were fixed in 10% acetic acid for 20 min, washed in distilled water, and patted dry with paper towels to remove excess fluid. The gels were then placed in an oven at 65°C for 3 h to dry, and exposed to Kodak Biomax-100 or Bio-Max MR film for 24–48 h depending on the activity of the gel.

Sequence analysis—The boundaries of the coding (18S, 5.8S, and 26S rDNA) and spacer regions were determined by comparison to the published sequences for rice (Takaiwa, Oono, and Sugiura, 1985). Complete sequences for the entire ITS region (ITS 1, 5.8S gene, and ITS 2) were not obtained for all taxa, therefore, only ITS 1, ITS 2, and a small, 17-bp portion at the 3′ end of the 5.8S gene that included two point mutations, were included in the analysis. DNA sequences were aligned initially using CLUSTAL V (Higgins, Bleasby, and Fuchs, 1992), then adjusted manually to minimize gap number using SeqApp version 1.8a (Gilbert, 1992). Regions of ambiguous alignment were excluded from all distance calculations and phylogenetic analyses in order to reduce systematic error (Swofford et al., 1996). Absolute pairwise distances between sequences were determined in PAUP 3.1.1. (Swofford, 1993) using the DISTANCE MATRIX option. Gaps were coded as missing for all phylogenetic analyses as recommended by Wojciechowski et al. (1993). Primary sequence lengths and GC contents were determined in Amplify 1.2 (Engels, 1993). These values were manually recalculated for those sequences with ambiguous nucleotide characters (e.g., N, Y, R), which are unacceptable to the program.

Phylogenetic analysis—All phylogenetic reconstructions were performed using the computer program PAUP version 3.1.1. (Swofford, 1993) run on a Power Macintosh. Heuristic searches were performed on equally weighted characters using Fitch parsimony (1971) and a SIMPLE stepwise addition of taxa. The ‘‘save all minimal trees’’ (MULPARS), ‘‘tree-bisection-reconnection’’ (TBR), and ‘‘collapse all zero length branches (COLLAPSE)’’ commands were used in searches for optimal trees. Five hundred replicates employing a RANDOM addition sequence were also used in heuristic searches for islands of equally most parsimonious trees (Maddison, 1991). In order to assess the confidence that could be placed in the monophyly of clades, bootstrap (Felsenstein, 1985) and decay analyses (Bremer, 1988; Donoghue et al., 1992) were performed. Decay indices (DI) were estimated using the ‘‘converse constraint’’ method of Baum, Sytsma, and Hoch (1994). Bootstrap (BS) values were determined from 500 replicates using heuristic searches and a SIMPLE stepwise addition of taxa. The amount of phylogenetic information in the parsimony analysis was assessed by use of the consistency index (CI; Kluge and Farris, 1969), and the retention index (RI; Farris, 1989). The number of unambiguous character state changes along branches was determined in MacClade 3.0 (Maddison and Maddison, 1992). A preliminary analysis was conducted to assess the effect of intraspecific variation in *Carex* section *Phyllostachys* on the topology of trees. The strict consensus trees of heuristic searches involving all 42 sequences were compared to those trees produced from searches using only the first, second, or third individuals (and combinations thereof) of each of the species sampled more than once (data not shown). No topological differences were observed in these analyses; therefore, only the first individual sequenced from each species (26 sequences in total; see Fig. 1) was used in subsequent analyses to reduce computational time.

The phylogenetic utility of insertions/deletions (indels) was also explored by incorporating indels into the data set as binary characters (bases present = 1/absent = 0). A small portion (17 bp) of the 3′ end of the 5.8S gene that contained the only variability between species in this coding sequence was also included.

To evaluate the contribution of each of the spacers to the analysis and to determine their level of congruence, ITS 1 and ITS 2 sequences were analyzed separately. Heuristic tree searches only employed a SIMPLE addition sequence owing to the length of search times.

RESULTS

Sequence analysis—Aligned sequences for ITS 1, ITS 2, and 17 bp at the 3′ end of the 5.8S gene of the 26 individuals used in the final analysis are presented in Fig. 1. The sequence for the complete ITS region, including both spacers and the entire 5.8S gene of *Carex superata,* is given in Fig. 2. Summary statistics for all 42 individuals sequenced in this study are given in Table 3.

Spacer lengths for all carices examined ranged from 217 bp (*C. peckii* and *C. baccans*) to 223 bp (*C. tonsa* var. *rugosperma*) for ITS 1, and from 211 bp (*C. filifolia*) to 231 bp (*C. pensylvanica* and *C. peckii*) for ITS 2. On average, ITS 2 was 3 bp longer than ITS 1. GC contents ranged from 56.4 to 69.5% (mean = 63.4%) for ITS 1 and from 58.8 to 72.7% (mean = 67.8%) for ITS 2. The 5.8S subunit in carices, as determined from three complete sequences (*C. superata, Naczi* 4013 et al., *C. willdenowii, Naczi* 4287 & *Thieret,* and *C. eleocharis, Bayer* AB-96004 et al.; Tables 1, 2), is 166 bp in length. Align-

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                                 10         20         30         40         50         60         70         80         90         100
                                 .          .          .          .          .          .          .          .          .          .
backii (MB)             TCGTTGCCTT TCAAAAAACA CGACCGTTGC ACACGTGACA GAATGCTGCC GGGGTGGTGC TTGCTGCCTC CCCCGGCCAC A-CCGGCCTC TTCCTTCTCG
saximontana (SASK)      TCGTTGCCTT TCAAAAAACA CGACCGTTGC ACACGTGACA GAATGCTGCC GGGGTGGTGC TTGCTGCCTC CCCCGGCCAC A-CCGGCCTC TTCCTTCTCG
latebracteata (ARK)     TCGTTGCCTC TAAAAAAACA CGACCGTTGC ACATGTGACA GAATGCTGCC GGGGAGGTTC TTGCTGCCTC CCCCGGCCAC A-CCGGCCTC TTCCTTCTCG
juniperorum (ONT)       TCGTTGCCTT TCAAAAAACA CGACCGTTGC ACACGTGACA GAATGCTGCC GGGGAGGTGC TTGCTGCCTC CCCCGGCCAC A-CCGGCCTC TTCCTTCTCG
jamesii (ONT)           TCGTTGCCTT TCAAAAAACA CGACCGTTGC ACACGTGACA GAATGCTGCC GGGGAGGTGC TTGCTGCCTC CCCCGGCCAC A-CCGGCCTC TTCCTTCTCG
willdenowii (OH)        TCGTTGCCTT TCAAAAAACA CGACCGTTGC ACACGTGACA GAATGCTGCC GGGGAGGTGC TTGCTGCCTC CCCCGGCCAC A-CCGGCCTC TTCCTTCTCG
superata (MS)           TCGTTGCCTT TCAAAAAACA CGACCGTTGC ACACGTGACA GAATGCTGCC GGGGAGGTGC TTGCTGCCTC CCCCGGCCAC A-CCGGCCTC TTCCTTCTCG
basiantha (AL)          TCGTTGCCTT TCAAAAAACA CGACCGTTGC ACACGTGACA GAATGCTGCC GGGGAGGTGC TTGCTGCCTC CCCCGGCCAC A-CCGGCCTC TTCCTTCTCG
filifolia               TCGCTGCCTT -GAAAAAACA CGACCGTTCC ACACGTGATA GAATGCTACC AAAGAGGTGC TTGCTGCCTC CTT-GGCCCC AGCCGGCCTC TTCCCTCTCG
geyeri                  TCGTTGCCTC TGAAAA--CA CGACCGCTGA ACACGTGACA CAACGCTGCC GGGGAGGTGC TTGCTGCCTC CTC-GGCCCT A-CCGGCCTC TTCCCTCGCG
multicaulis             TCGTTGCCTT TAAAAAA-CA CGACCGTCGA ACACGTGACA GAACGCTGCC GGGGAGGTGC TCGCCACCTC CTC-GGCCCA A-CCGGCCTC TTCCCTCGCG
eleocharis              TCGTTGCCTC TCGAAAAACA CGACCGTTGC ACAAGTGACA GAATGCTGCC GGAGAGGTGC TTGCTGCCTC CTC-GGCCCC A-CCGGCCTC GTCCCTCTAG
deweyana                TCGTTGCCTC TCGAAAAACA CGACCGTTGA ACACGTGACA GAACGCTGCC GGAGAGGCGC TCGCCGCCTC CTC-GGCCCC A-CCGGCCTC GTCCCTCTAG
tonsa                   TCGTTGCCTT TCCAAAAACA CGACCGTCGA ACACGTGACA GAATGCTGCC GCGGAGGCGC CTGCCGCCTC CTC-GGCCCC A-CCGGCCTC CTCCGTCTCG
albicans                TCGTTGCCTT TCCAAAAACA CGACCGTCGA ACACGTGACA GAATGCTGCC GCGGAGGCGC CTGCCGCCTC CTC-GGCCCC A-CCGGCCTC CTCCCTCTCG
pensylvanica            TCGTTGCCTT TCCAAAA-CA CGACCGTCGA ACACGTGACA GAATGCTGCC GCGGAGGCGC YTGCCGCCTC CTC-GGCCCC A-CCGGCCTC ACCCCTCTCG
peckii                  TCGTTGCCTT TCCAAAA-CA CGACCGTTGA ACACGTGACA GAATGCTGCC GCGGAGGTGC YTGCCGCCTC CTC-GGCCCC A-CCGGCCTC ACCCCTCTCG
plantaginea             CCGCCGCCTT TTCGAAAACA CGACCGTCGA ACAAGTGACA GAATGCCGCC GCGGAGGCGC CTGCCGCCTC CCC-GGCCCC A-CCGGCCTC CTCCCTCACG
careyana                CCGTCGCCTT TCCGAAAACA CGACCGTCGA ACACGTGACA GAATGCCGCC GCGGAGGCGC CCGCCGCCTC CCC-GGCCCC A-CCGGCCTC CTCCCTCACG
laxiflora               TCGTTGCCTT TCCAAAAACA CGACCGTCGA ACACGTGACA GAATGCTGCC GCGGAGGCGC TGGCCGCCTC CTC-GGCCCC A-CCGGCCTC CTCCCTCGCG
blanda                  TCGTTGCCTT TCCAAAAACA CGACCGTCGA ACACGTGACA GAATGCTGCC GCGGAGGCGC TGGCCGCCTC CTC-GGCCCC G-CCGGCCTC CTCCCTCGCG
arctata                 TCGTTGCCTT TCCAGAAACA CGACCGTCGA ACACGTGACA GAATGCTGCC GCGGAGGCGC TCGCCGCCTC CTC-GGCCCC G-CCGGCCTC CTCCCTCTCG
scirpoidea              TCGTTGCCTT TACAAAAACA CGACCGTCGA ACACGTGATA GAATGCTGCC GTGGTGGCGC TTGTCGCCTC CTC-GGCCCC A-CCGGCCTC CTCCCTCTCG
polystachya             TCGTTGCCTT TCCCAAAACA CGACCGTCGA ACACGTGATA GAATGCTGCC GTGGTGGCGC TTGCCGCCTC CTC-GGCCCC A-CCGGCCAC CTCCCTCTCG
baccans                 TCGTTGCCTT TCCAAAA-CA CGACCGTCGA ACACGTGACA AAATGCTGCC GCGGGGGTGG TTGCTGCCTC CTC-GGCCCC A-CCGGCCTC AACCCTCTCG
cruciata                TCGTTGCCTT TCCAAAAACA CGACCGTTGA ACACGTGACA GAATGCTGCC GCGGAGGTGC CTGCTGCCTC CCC-GGCCCC A-CCGGCCTC ATCCCTCTCG
                                   ********                                                              1        2

                                 110        120        130        140        150        160        170        180        190        200
                                 .          .          .          .          .          .          .          .          .          .
backii (MB)             CCCTCT-GGG CGCGTTGGTC GCTGGCCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTG AGGCACTGGC GAGCCGCTCA GTGGTTG-CG
saximontana (SASK)      CCCTCT-GGG CGCGTTGGTC GCTGGCCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTG AGGCACTGGC GAGCCGCTCA GTGGTTG-CG
latebracteata (ARK)     CCCTCT-AGG CGTGTTGGTC GTTGGCCGGA ATACGGCGCG GGATGACGCC AAGGAATACG AG-AAAGCTG AGGCACTGGC GAGCCGCTCA GTGGTTG-CG
juniperorum (ONT)       CCCTCT-GGG CGCGTTGGTC GCTGGCCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTG AGGCACTGGC GAGCCGCTCA GCGGTTG-CG
jamesii (ONT)           CCCTCT-GGG CGCGTTGGTC GCTGGCCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTG AGGCACTGGC GAGCCGCTCA GCGGTTG-CG
willdenowii (OH)        CCCTCT-GGG CGCGTTGGTC GCTGGCCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTG AGGCACTGGC GAGCCGCTCA GTGGTTG-CG
superata (MS)           CCCTCT-GGG CGCGTTGGTC GCTGGCCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTG AGGCACTGGC GAGCCGCTCA GTGGTTG-CG
basiantha (AL)          CCCTCT-GGG CGCGTTGGTC GCTGGCCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTG AGGCACTGGC GAGCCGCTCA GTGGTTG-CG
filifolia               CCTTGT-GGG CGCGTTGGTG GCTGACCGAA ATACGACGCG GGATGACACC AAAGAACAAC AT-AAAGATG AGGCATCGGC GAGGCGCTCA ATGGTTG-CG
geyeri                  CCCCTC-GGG CGCGTCGGTC GCTGTCCGGA ATACGGCGCG GGATGACGCC AAGGAACACG GT-AGAGCCG GGGCACCGGC GAGCCGCTCA AGGCTCG-CG
multicaulis             CCCCCA-GGG CGCGTCGGTC GCTGTCCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AT-AAAGCTG AGGCACCGGC GAGCCTCTCA AGGTTTG-CG
eleocharis              CCTTTC-GGG CGAGTTGGAT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG ATAGAAGCTG AGGCACCGGC AGGYCGCTAA AGGGCTGGCG
deweyana                CCCTCC-GGG CGAGTTGGAT GCTGGCCGGA ACACGGCGCG GGATGACGCC AAGGAACACG ATAAAAGCTG AGGCACCGGC CGGCCGCTTA AGGGCCTGCG
tonsa                   CCCTTCGGGG CGCGTCGGTT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG ATAAAAGTCG AGGCACCGGC GAGCCGCTCG AGGGCTC-CG
albicans                CCCTTCGGGG CGCGTCGGTT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG ATAAAAGTCG AGGCACCGGC GAGCCGCTCG AGGGCAC-CG
pensylvanica            CCCTC--GGG CGCGTCGGTT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTG AGGCACCGAC GAGCCGCACA AGGGCTC-CG
peckii                  CCCTC--GGG CGCGTCGGTT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTG AGGCACCGGC GAGCCGCACA AGGGCTC-CG
plantaginea             CCCTGCGGGG C-CGTCGGTT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG GT-AAAGCTG AGGCACCGGC GAGCCGCTCA AGGGCTC-TG
careyana                CCCTGC-GGG CGCGTCGGTT GCTGGTCGGA AAACGGCGCG GGATGACGCC AAGGAACACG GT-AAAGCCG AGGCACCGGC GAGCCGCTCA AGGGCTC-CG
laxiflora               CCCTTCGGGG C-CGTCGGTT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG GTAAGAGCTG AGGCGCCGGC GAGCCGCTCG AGGGTTC-CG
blanda                  CCCTTCGGGG C-CGTCGGTT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG GTAAAAGCTG AGGCGCCGGC GAGCCGCTCG AGGGTTC-CG
arctata                 CCCTTCGGGG CGCGTCGGAT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG GT-AAAGCCG AGGCACTGGC GAGCCGCTCA AGGGCTC-CG
scirpoidea              CCCTTC-GGG CGCGTTGGTT GTTGGTTGGA ATACGGCGCG GGATGACGCC AAGGAACACG AT-AAAGCTA AGGCACCGGG GATCCGCTCA AGGGCTC-YG
polystachya             CCCTCC-GGG CGCGTTGGTT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTA AGGCACCGTC GAGCCGCGAA AGGGCTG-CG
baccans                 CCCTC--GGG CGCGTTGGTT GCTGGTCGGA ATACGGCGCG GGATGACGCC AAGGAACACG AG-AAAGCTG AGGCACCGGC GAGCTGCACA AGGGCTC-TG
cruciata                CCCTCTCGGG CGCGCTGGGT GTTGGCCGGA ATACGGCGCG GGTTGACGCC AAGGAACACG AG-AAAGCTG AGGCACCGGC GAGCCGCACT GGGGCTT-TG
                           ****     3                                                       4                                     5

                                 210        220        230        240        250        260        270        280        290        300
                                 .          .          .          .          .          .          .          .          .          .
backii (MB)             CCGG-TTGCC -AAGGCCAA- TGAAAAAAA- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTC AC----GGCA AA-GATGCGG ACATTGGCCC
saximontana (SASK)      CCGG-TTGCC -AAGGCCAA- TGAAAAAA-- CCTCATGGGC GTTAGAAGCC CATCCACGCT CAGTTGCCTC AC----GGCA AA-GATGCGG ACATTGGCCC
latebracteata (ARK)     CCGG-TTGCC -AAGGCCAA- TGAAAAAAA- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGATTGCCTC AT----GGCA AA-GATGCGG ACATTGGCCC
juniperorum (ONT)       CCGG-TTGCC -AAGGCCAA- TGAAAAAA-- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTC AC----GGCA AA-GATGCGG ACATTGGCCC
jamesii (ONT)           CCGG-TTGCC -AAGGCCAA- TGAAAAAA-- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTC AC----GGCA AA-GATGCGG ACATTGGCCC
willdenowii (OH)        CCGG-TTGCC -AAGGCCAA- TGAAAAAAA- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTC AC----GGCA AA-GATGCGG ACATTGGCCC
superata (MS)           CCGG-TTGCC -GAGGCCAA- TGAAAAAA-- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTC AC----GGCA AA-GATGCGG ACATTGGCCC
basiantha (AL)          CCGG-TTGCC -GAGGCCAA- TGAAAAAA-- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTC AC----GGCA AA-GATGCGG ACATTGGCCC
filifolia               CCGG-TTGCC -GAGGCCAA- TGAAAAAA-- CCTCATGGGG GTTAGAAGCC CATCCACGCT CGGTTGCCTC AT----GGCA AA-GATTCGG ACATTG-CCC
geyeri                  TCGG-TCGCC -GAGGCCAA- TGAAAAACAA CCTCACGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTC AC----GGCA GA-GATGCGG ACATTGGCCC
multicaulis             TCGG-TCGCC -AACGCCAA- TGAAAAAAA- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTC AC----GGCA AA-GATGCGG AAATTGGCCC
eleocharis              TCGGG-TGCC -AAGGCCAA- CGAAAAAA-- CCTCATGGGC GTTAGAAGCC CATTCACGCT CGGTTGCCTC AC----GGCA AA-GATGCGG ACATTGGCCC
deweyana                CCGGG--GCC -GAGGCCAA- CGAAAA---- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA AC----GGCA GA-GATGCGG ACAATGGCCC
tonsa                   TCGG-TTGCC TGAGGCCAAA CAAAAAA--- CCTCACGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA ACTAACGGCA AAAGATGCGG ACATTGGCCC
albicans                TCGG-TTGCC GAAGGCCAA- TAAAAA---- CCTCACGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA ACTAACGGCA AAAGATGCGG ACATTGGCCC
pensylvanica            TCGG-TTGCC -AAGGCCAAA CAAAAAAA-- CCTCACGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA ACTAACGGCA AAAGATGCGG ACATTGGCCC
peckii                  TCGG-TTGCC -AAGGCCAAA CAAAAA---- CCTCACGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA ACTAACGGCA AAAGATGCGG ACATTGGCCC
plantaginea             CCGG-TTGCT -GAGGCCGG- CAAAAAAGA- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA ACTAACGGCA AA-GATGCGG ACATTGGCCC
careyana                CCGG-TTGCC -GAGGCCGG- AAAAAAAA-- CCTCACGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA ACTAACGGCA AA-GATGCGG ACATTGGCCC
laxiflora               TCGG-TTGCC -GATG-CAAA CAAAAA---- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA ACCAACGGCA AA-GATGCGG ACGTTGGCCC
blanda                  TCGG-TTGCC -GATG-CAAA CAAAAA---- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA ACCAACGGCA AA-GATGCGG TCGTTGGCCC
arctata                 TCGG-TTGCC -AAGGCCAA- CAAGAGA--- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA AC----GGCA AA-GATGCGG ACATTGGCCC
scirpoidea              TCGG-TTGCC -AAGGCCAA- CAAAAAA--- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTA ACTAACGGTA AA-GATGCGG ATATTGGCCC
polystachya             TCGGGTTGCC -AAGGCCAA- TAAAAAAA-- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGTTTGCCTC ACTAATGGCA AAAGATGCGG ACATTGGCCC
baccans                 TCGG-TTGCC -AAGGCCAA- CAAAAAA--- CCTCATGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCCA ACTAACGGTA AAAGATGCGG AAATTGGCCC
cruciata                TCGG-TTGCC -AAGGCCGA- TAGAAAA--- CCTCACGGGC GTTAGAAGCC CATCCACGCT CGGTTGCCTG ACTAACGGCA AAAKATGCGG ACATTGGCCC
                            678    9    1   * **********                                                 1        1              1
                                        0                                                                1        2              3
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                                  310        320        330        340        350        360        370        380        390        400
                                   .          .          .          .          .          .          .          .          .          .
backii (MB)               TCCGAACCGC GAGGTGCGGT GGGCCTAAGT GTACGGCCGT CGTATGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCATA
saximontana (SASK)        TCCGAACCGC GAGGTGCGGT GGGCCTAAGT GTACGGCCGT CATATGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCATA
latebracteata (ARK)       TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTACGGCCGT CGTATGTGGC CTGGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCTC GAGCCCCATA
juniperorum (ONT)         TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTACGGCCGT CGTATGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCATA
jamesii (ONT)             TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTACGGCCGT CGTATGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCATA
willdenowii (OH)          TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTACGGCCGT CGTATGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCATA
superata (MS)             TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTACGGCCGT CGTATGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCATA
basiantha (AL)            TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTACGGCCGT CGTATGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCATA
filifolia                 TCCGAGCCGC GAGGTGCGGT GGACCTAAGT GTGCAGTTAT CGTATGTGGC C-AGGAGCGG TGAC------ ----CTGCGC ACGTCACCCC GAGCGCCATA
geyeri                    TCCGAACCGC GAGGTGCGGT GGGCCCAAGT GAACGGCCGC CGTATGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCGCCCC GAGCCCCGTA
multicaulis               TCCGAACCGC GAGGCGCGGT GGGCCCAAGT GTACGGCCGT CGTACGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGTCCCGTA
eleocharis                TCCGAACCTC GAGGTGCGGT GGGCCTAAGT GTGCGGCCGT CGTATGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GCGCCTCGTA
deweyana                  TCCGAACCTT GAGGTGCGGT GGGCCTAAGT GTGCGGCCGT CGTACGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCGTA
tonsa                     TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGTACGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCGTA
albicans                  TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGTACGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCGTA
pensylvanica              TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGTACGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCGTA
peckii                    TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGTACGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCGTA
plantaginea               TCCGAACCGC GAGGCGCGGT GGGCCCAAGT GTGCGGCCGT CGTACGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCGTG
careyana                  TCCGAACCGC GAGGCGCGGT GGGCCCAAGT GTGCGGCCGT CGTACGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCGTG
laxiflora                 TCCGAGCCGC GAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGTGCGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGCCGCCCC GAGCCCCGTA
blanda                    TCCGAGCCGC GAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGTGCGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGCCGCCCC GAGCCCCGTA
arctata                   TCCGAGCCGC GAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGTACGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCTGTA
scirpoidea                TCCGAACCGC GAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGTGCGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCGTA
polystachya               TCCGAACCGT AAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGCATGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCCCGTA
baccans                   TCCGAACCGT GAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGTACGTGGC C-GGGAGCGG CGAGTGGTGG GCTACTGCGC ACGTCACCCC GAGCCTCGTA
cruciata                  TCCGAACCGT AAGGCGCGGT GGGCCTAAGT GTGCGGCCGT CGTACATGGC C-GGGAGCGG CGAGTGGTGG GNTACTGCGC ACGCCACCCC GAGCCCCGTA
                                                                                  1                  1
                                                                                  4                  5

                                  410        420        430        440        450        460        470        480
                                   .          .          .          .          .          .          .          .
backii (MB)               AGGACACAGG GCCTTGTTTG ACCCCCTAAC GAGGAGC--- -ATGCTGTCG CGGCTTGTGC --TGTGCGGC ACCTTCGGAC C [444]
saximontana (SASK)        CCGACACAGG GCCTTGTTTG ACCCCCTAAC GAGGAGC--- -ATGCTGTCA CGGCCTGTGC --TGTGCGGC ACCTTCGGAC C [443]
latebracteata (ARK)       CCGACACAGG GCCTTGTTTG ACCCCTGAAC GAGGAGC--- -ATGCTGTCG CGGCCTGTGC --TGTGCGGC ACCTTCGGAC C [445]
juniperorum (ONT)         CCGACACGGG GCCTTGTTTG ACCCC-TAAC GAGGAGC--- -ATGCTGTCG CGGCCTGTGC --TGTGCGGC ACCTTCGGAC C [442]
jamesii (ONT)             CCGACACGGG GCCTTGTTTG ACCCC-TAAC GAGGAGC--- -ATGCTGTCG CGGCCTGTGC --TGTGCGGC ACCTTCGGAC C [442]
willdenowii (OH)          CCGACACAGG GCCTTGTTTG ACCCC-TAAC GAGGAGC--- -ATGCTGTCG CGGCCTGTGC --TGCGCGGC ACCTTCGGAC C [443]
superata (MS)             CCGACACAGG GCCTTGTTTG ACCCC-TAAC GAGGAGC--- -ATGCTGTCG CGGCCTGTGC --TGTGCGGC ACCTTCGGAC C [443]
basiantha (AL)            CCGACACAGG GCCTTGTTTG ACCCC-TAAC GAGGAGC--- -ATGCTGTCG CGGCCTGTGC --TGTGCGGC ACCTTCGGAC C [443]
filifolia                 TCTACATAGT GCCTTGTTTG ATCCCCTAAC AATGAGC--- -ATGCTGTCG CGGCTTATGC --TGCGCAGC ACCTTCGAAC C [431]
geyeri                    CGAACACAGG GCCTTGTTCG ACCCCCTAAC GAGGAGC--- -ATGCCGTCG CGGCTTGCGC --CGTGCGGC ACCTTCGGAC C [442]
multicaulis               CGCACACAGG GCCTTGTTTG ACCCCCTAAC GAGGAGC--- -ATGCCGCCG CGGCTCGCGC --CGCGCGGC ACCTTCGGAC C [442]
eleocharis                CCGACACAGG GCCTTTTTTG ACCCC-TAAC GAGGAGC--- -TTGCCGTTG CGGCTTG-GC --TGCGCGGC GCCTTCGGAC C [442]
deweyana                  CCGACACAGG GCCTCTTATG ACCCC-TCAC GAGGAGC--- -TTGCCGT-- CGGCTCGCGC --CGCGCGGC GCCTTCGGAC C [438]
tonsa                     ATGACGCAGG GCCTTGTTGG AACCCCTAAC GAGGAGC--- -ATGCTGCCG CGGCTTCGGC --TGCGCGGC GTCTTCGGAC C [450]
albicans                  CCGACGCAGG GCCTTGTTGG AACCCCTAAC GAGGAGC--- -AASCTGCCG CGGCTTCGGC --TGCGCGGC GTCTTCGGAC C [448]
pensylvanica              CCGACGCAGG GCCTTGTTGG AACCCCTAAC GAGGAGCCAA ATCGCTGCCG CGGCTTCGGC --TGCGCGGC GTCTTCGGAC C [450]
peckii                    CCGACGCAGG GCCTTGTTGG AACCCCTAAC GAGGAGCCAA TTCGCTGCCG CGGCTTCGGC --TGCGCGGC GTCTTCGGAC C [448]
plantaginea               CCGATGCAGG GCCTTGTTCG ACCCC-TAAC GAGGAG---- -GCG-CGCCG CGGCTTCTGC CGTGCGTGGC GCCTTCGGAC C [446]
careyana                  CCGACGCAGG GCCCCGTTCG ACCCC-TAAC GAGGAGC--- -GCGGCGCCG CGGCTTCTGC CGCGCGCGGC GCCTTCGGAC C [447]
laxiflora                 GCGACGCAGG GCCTTGTTGG AACCCCTAAC GAGGAGC--- -GCGCCGCCG CGGCTTGTGC --TGCCGTGC GCCTTCGGAC C [445]
blanda                    ACGACGCAGG GCCTTGTTGG AACCCCTAAC GAGGAGC--- -GCGCCGCCG CGGCTTGTGC --TGCCGTGC GCCTTCGGAC C [445]
arctata                   CCGACGCAGG GCCTTGTACG ACCCCCTAAC GAGGAGC--- -ACGTCGCCG CGGCCTGCGC --CGCGCGGC ACCTTCGGAC C [442]
scirpoidea                CCGACGTAGG GCCTTGTACG ACCCCCTAAC GAGGAGC--- -ATGCCGCTG CAGCTTCGGC --CGCACGGC GCCTTCGGAC C [445]
polystachya               CCGACGCAGG GCCTTCTTTG AACCCCTAAC GAGGAGC--- -ACGCCGCTG CGGCTTCCGC --TGCGCGGC GCCTTCGGAC C [448]
baccans                   CCGATGCAGG GCCTTGTTTG ATCCC-TAAC GAGGAGC--- -ATGTCGTTG CGGCTTNTGC --TGCGCGGT GCCTTCGGAC C [443]
cruciata                  CCGATGCAGG GCCTTGTTTG AACCCCTAAC GAGGAGC--- -ACGTCGCTG CGGCTTNTGC --TGCGCGGC GCCTTCGGAC C [447]
                                                 ******          1  1     1    1        2   2
                                                                 6  7     8    9        0   1
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Fig. 1. Continued.

ment of the 5.8S gene with sequences from the Cyperaceae (*Kobresia simpliciuscula* (Wahlb.) Mack., *Keleher* & *Punter* #94/155, WIN; GenBank number GBAN-AF027490), Poaceae (*Oryza sativa*; Takaiwa, Oono, and Sugiura, 1985), and two dicot families (i.e., Fabaceae, *Vicia faba*; Tanaka, Dyer, and Brownlee, 1980; and Cucurbitaceae, *Cucumis sativus*; Torres, Ganal, and Hemleben, 1990) identified a 3-bp insertion (5′>CAT<3′) near the 3′ end that was shared by the genera *Carex* and *Kobresia* (Figs. 2, 3). With the exception of an autapomorphic mutation in *C. filifolia,* the only variation observed between 5.8S sequences in *Carex* was a T/C transition at the third position of this insertion. In order to align sequences, an equal number of unambiguous insertion/deletion (indel) events had to be inferred for each spacer (ITS 1 = 11; ITS 2 = 11). All of the indels in

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←

Fig. 1. The complete sequence for ITS 1 (positions 1–230), ITS 2 (positions 248–481), and 17 bp at the 3′ end of the 5.8S gene (base pairs 231–247), for each of the 26 taxa used in the final analysis. Regions of ambiguous alignment that were not used in phylogenetic analyses are indicated by asterisks below sequences. Insertions/deletions (indels) used in the analysis are indicated by number below sequences. An autapomorphic insertion (position 113) of a single base pair in one of the three individuals of *C. backii* (*Reznicek* 6364 & *Reznicek*; Table 1) sequenced is not shown. The complete length of the combined ITS 1 and ITS 2 spacers (excluding the 17 bp of the 5.8S gene), for each taxon, is given in brackets at the end of sequences.

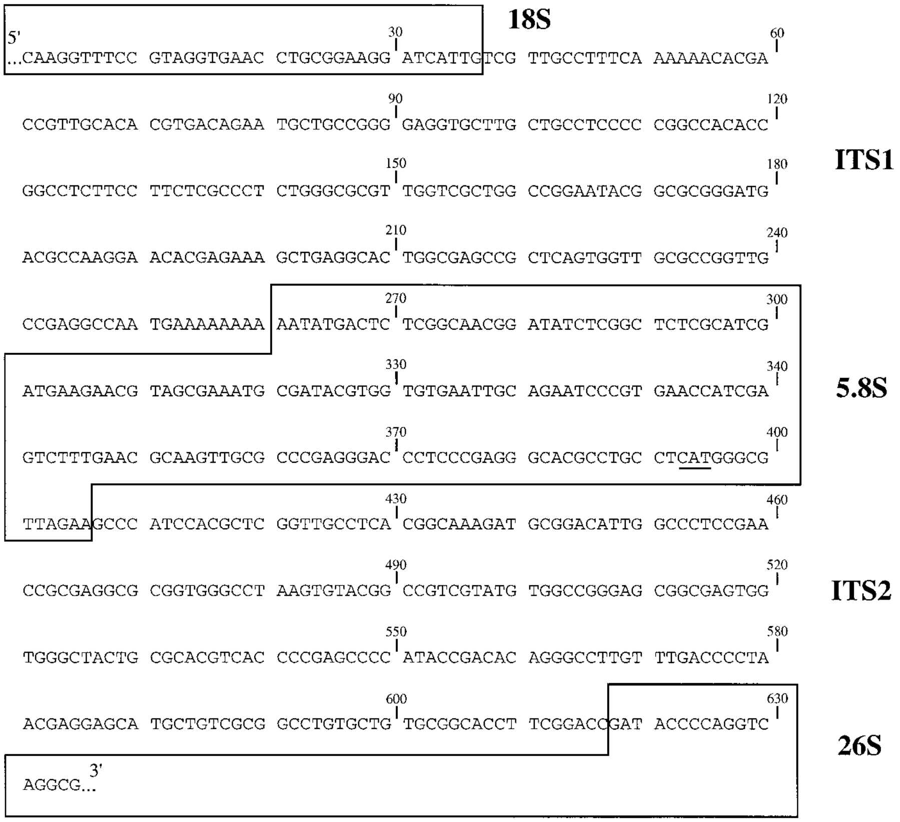

Fig. 2. The complete sequence for the ITS region of *Carex superata,* showing the ITS 1 and ITS 2 spacers, the complete 5.8S gene, and portions of the 18S and 26S genes of nrDNA. Coding sequences are boxed. The position of the 5′>CAT<3′ insertion in the 5.8S gene is indicated by underlining.

ITS 1 were 1 bp in length, whereas five of the 11 indels in ITS 2 were greater than 1 bp, including a unique 10-bp deletion in the ITS 2 spacer of *C. filifolia.* Ten of the 22 indels in the analysis were autapomorphic, while seven of the remaining 12 had a CI = 1.0.

The levels of nucleotide divergence in *Carex* ranged from 0.0 to 20.90% in ITS 1 and from 0.0 to 18.72% in ITS 2 (Table 3). When both spacers were considered, the pairwise sequence divergence between carices ranged from 0.0 to 19.70%. With the exception of the *Firmiculmes* (7.19%), sequence divergence within sections for which more than one species was sequenced was low (0.0–3.83%; Table 4). In contrast, variation among these sections was considerably higher, with the lowest values being found in comparisons between sections *Careyanae* and *Acrocystis* (6.41–7.13%; Table 5), and the highest between sections *Phyllostachys* and *Laxiflorae* (11.08–14.22%; Table 5). In all cases, the highest divergence values were obtained when members of sections in the "reduced" clade were compared to those in the "compound" clade (see below). Within section *Phyllostachys,* sequence divergence between species was low, ranging from complete identity (e.g., *C. basiantha* vs. *C. superata*; but see below) to 3.83%. Infraspecific variation was

TABLE 3. General sequence statistics for ITS 1 and ITS 2 (alone and combined).

	ITS 1	ITS 2	Combined (ITS 1 + ITS 2)
Length range (bp)	217–223	211–231	431–450
Length mean (bp)	220.27	223.50	443.77
Aligned length (bp)	231	234	465
GC content range (%)	56.4–69.5	58.8–72.7	57.5–71.1
GC content mean (%)	63.4	67.8	65.6
Sequence divergence (%)	0.00–20.90	0.00–18.72	0.00–19.70
Number of excluded sites	23 (10.0%)	6 (2.6%)	29 (6.2%)
Number of indels	11	11	22
Number of variable sites	94 (45.2%)	87 (38.2%)	181 (41.5%)
Number of potentially informative sites	57 (27.4%)	48 (21.1%)	105 (24.1%)
Number of constant sites	114 (54.8%)	141 (61.8%)	255 (51.6%)
Number of autapomorphic sites	37 (17.8%)	37 (16.2%)	74 (17.0%)

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3' end of 5.8S rDNA

Oryza sativa      GCCGAGGGCACGCCTGCCT---GGGCGTCACGC

Cucumis sativus   ............T......---..........
                                                    ITS2
Vicia faba        ............T......---...T....AT ───▶

Kobresia          ...................CAT.....T.NAA

Carex backii      ...................CAT.....T.GAA
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Fig. 3. The aligned 3′ end of the 5.8S gene for the Cyperaceae (*Kobresia* and *Carex*; Starr, this study), Poaceae (*Oryza sativa*; Takaiwa, Oono, and Sugiura, 1985), Fabaceae (*Vicia faba*; Tanaka, Dyer, and Brownlee, 1980), and Cucurbitaceae (*Cucumis sativus*; Torres, Ganal, and Hemleben, 1990). Note the 3-bp insertion (5′>CAT<3′) shared by *Carex* and *Kobresia.*

extremely low and never exceeded 1% of the sequences compared (Table 6). The highest divergence between populations within a species was seen in *C. backii* where divergence between the three populations examined ranged from 0.48 to 0.96%. A single point mutation difference was seen in one of the three individuals sampled for both *C. saximontana* and *C. willdenowii.* All other variation within species was due to ambiguous characters that may or may not be true polymorphisms. It should be noted, however, that length variation (± 1 bp) between tandem repeats within individuals (e.g., *C. juniperorum* and *C. superata*), within populations (*C. superata* − 2 bp), and within species (*C. basiantha* and *C. superata*) was commonly seen at the 3′ poly-A tail of ITS 1. Because of the volatility of this region, it was excluded from pairwise divergence calculations and phylogenetic analyses.

Phylogenetic analysis—The alignment of ITS spacer sequences resulted in a matrix of 465 characters of which 23 positions in ITS 1 and six positions in ITS 2 were excluded due to alignment ambiguities (marked by asterisks in Fig. 1). Of the remaining 436 characters, 181 (41.5%) were variable (94 in ITS 1; 87 in ITS 2) and 105 (24.0%) were potentially phylogenetically informative (57 in ITS 1; 48 in ITS 2). Heuristic searches of the reduced 26 taxa data matrix using either a STEPWISE or a RANDOM addition sequence of taxa recovered the same five most parsimonious trees of 341 steps each. All of the most parsimonious trees had a consistency index of 0.66 and a retention index of 0.75.

The single tree of five most parsimonious trees, topologically identical to the 50% majority rule tree, is presented in Fig. 4 along with bootstrap values, decay indices, and unambiguous character changes per branch. The only difference between this tree and the strict consensus of all five trees was seen in the loss of resolution in section *Phyllostachys* (dotted branches in Fig. 4). Two major clades were evident on this tree: (1) a ‘‘reduced’’ clade consisting of sections *Phyllostachys, Filifoliae,* and *Firmiculmes,* and (2) a ‘‘compound’’ clade comprising sections *Careyanae, Laxiflorae, Hymenochlaenae, Acrocystis, Scirpinae, Indicae, Cruciatae,* and *Polystachyae.* Other strongly supported groups in this analysis included the sister group to the ‘‘compound’’ clade composed of two Southeast Asian indocarices, *C. baccans* and *C. cruciata,* and a robust clade (DI = 5; 86% BS) consisting of sections *Phyllostachys* and *Filifoliae.* All sections for which more than one individual was sequenced were monophyletic and strongly supported (DI = 6–12; 89–100% BS), except for section *Acrocystis* (DI = 3; 69% BS). Although the data did not provide firm evidence for the monophyly of section *Acrocystis,* they did support the existence of two strong clades within it; viz. *C. tonsa* var. *rugosperma* and *C. albicans,* and *C. pensylvanica* and *C. peckii.* Most of the internal branches in the ‘‘compound’’ clade were weak, including the *Careyanae*/*Laxiflorae* lineage. Within section *Phyllostachys* three species pairs were recognized: *C. backii* and *C. saximontana, C. juniperorum* and *C. jamesii,* and *C. basiantha* and *C. superata. Carex juniperorum* and *C. jamesii* formed the best supported clade in the section (DI = 2; 86% BS). Little resolution was seen within section *Phyllostachys,* and the separation of the section into ‘‘narrow-’’ and ‘‘wide-scaled’’ clades (sensu Starr and Ford, 1995; Starr, 1997) was poorly supported. *Carex latebracteata,* a member of the wide-scaled clade, was present in a group that included the members of the narrow-scaled clade.

TABLE 4. Sequence divergence within sections for which more than one species was sequenced. *N* denotes the number of individuals sequenced per section.

Section	*N*	Within sections (%)
Phyllostachys	24	0.00–3.83
Careyanae	2	2.86
Laxiflorae	2	0.95
Firmiculmes	2	7.19
Acrocystis	4	0.94–3.56

Reanalyzing the data with the inclusion of insertion/deletions (indels) and the single variable character found at the 3′ end of the 5.8S gene had little effect on parsimony analyses. The five trees obtained from these searches were topologically identical to the five found using point mutations alone. The trees from both of these analyses had the same consistency index (CI = 0.66) and retention index (RI = 0.75) and differed only marginally by certain bootstrap and decay values and by the number of their steps (data not shown).

Heuristic searches of a separate ITS 1 data set produced 79 trees, 180 steps long with a CI = 0.68 and an RI = 0.78. The strict consensus of this analysis was entirely compatible with the combined analysis and differed only in the level of resolution achieved. The strict consensus of the four trees derived from the analysis of ITS 2 sequences (154 steps; CI = 0.67 and RI = 0.74), however, was slightly different from the combined analysis in the placement of *C. polystachya* (subg. *Indocarex*) as sister to the ‘‘compound clade’’ instead of the *C. baccans*/*C. cruciata* clade (subg. *Indocarex*). Nonetheless, the two analyses were complementary, and the combination of data sets resulted in increased resolution and support for clades that were otherwise absent or weak in separate analyses (data not shown).

DISCUSSION

ITS sequence evolution in Carex—The sizes of the ITS region (602–617 bp) and spacers (ITS 1 = 217–223 bp; ITS 2 = 211–231 bp) in *Carex* are similar to those reported for a wide variety of flowering plants (reviewed

TABLE 5. Sequence divergence between sections for which more than one species was sequenced. Percentage divergence is given above the diagonal, and absolute differences are given below the diagonal.

Section	*Phyllostachys*	*Careyanae*	*Laxiflorae*	*Firmiculmes*	*Acrocystis*
Phyllostachys	—	11.11–14.15%	11.08–14.22%	8.15–11.27%	7.43–11.75%
Careyanae	46–59	—	8.15–8.59%	11.03–13.53%	6.41–7.13%
Laxiflorae	46–59	34–36	—	10.60–12.77%	6.67–7.40%
Firmiculmes	34–47	46–56	44–53	—	9.11–12.23%
Acrocystis	31–49	27–30	28–31	38–51	—

in Baldwin et al., 1995). Sequences from the Poaceae showed the greatest similarities to *Carex* in both the size of the ITS region (Poaceae, 585–603 bp; Hsiao et al., 1995a, b) and its spacers (ITS 1, 214–221 bp; ITS 2, 205–221 bp; Hsiao et al., 1995a, b). However, the range of sequence divergence within *Carex* is generally much higher than seen in the Poaceae and is comparable to the levels of variation observed between relatively distant genera in grasses (cf. Hsiao et al., 1995a, b). In general, the range of ITS sequence divergence within *Carex* (0.00–19.70%) was much higher than that observed within other genera (e.g., *Epilobium* 0.0–12.9%; Baum, Sytsma, and Hoch, 1994; and *Antennaria* 14%; Bayer, Soltis, and Soltis, 1996; but cf. *Arceuthobium*; Nickrent, Schuette, and Starr, 1994) and was comparable to the values that are commonly observed between genera within families (e.g., *Maloideae* 2.7–16.1%; Campbell et al., 1995). The reasons for this are unknown, although the age of the genus and/or poor circumscription (see below) may be involved.

All of the highest pairwise sequence divergence values were observed between *C. filifolia* and the other members of the genus sampled. The sequence of this species had a number of features that made it distinct. It had the shortest combined ITS 1 and ITS 2 sequence by 7 bp, it possessed a considerably lower GC content (57.5%) than other carices (62–71.1%), it had a unique 10-bp deletion in ITS 2, and it possessed very long branch lengths on all trees. *Carex filifolia* represents one of many reduced species that have blurred generic limits within the *Cariceae.* Its generally well-developed rachilla prompted Torrey (1836) to treat it as an *Uncinia,* while its unusual inflorescence and vegetative habit suggested to Ivanova (1939) that it was best placed in the genus *Kobresia.* Although this analysis strongly suggests that *C. filifolia* (sect. *Filifoliae*) is sister to sect. *Phyllostachys* (see below), its relationship to the section and to the rest of the genus will not be understood until a larger phylogenetic study of *Carex* and the *Cariceae* has been conducted.

TABLE 6. Sequence divergence within species of *Carex* sect. *Phyllostachys.*

Species	*N*	Location of populations sampled	Sequence variation (%)
C. backii	3	MB(2), ONT	0.48–0.96
C. saximontana	3[a]	MB(2), SASK	0.00–0.48
C. latebracteata	2	ARK, OK	0.00
C. juniperorum	3	ONT, OH, KY	0.00
C. jamesii	4	ONT, VA, ARK, KY	0.00–0.24
C. willdenowii	3	KY, OH, PA	0.00–0.24
C. superata	3[a]	AL, MS(2)	0.00
C. basiantha	3	AL, LA, MS	0.00

[a] Two individuals are from the same population.

The poor support seen in the lower branches of the compound clade is most likely due to poor taxonomic sampling, but it could also be an indication of rapid radiation. Many authors have felt that widespread speciation has taken place as recently as the Pleistocene (Kreczetovicz, 1936; Whitkus, 1981; Kukkonen and Toivonen, 1988; Ball, 1990), and based on phytogeographical evidence, Stebbins (1981) has suggested that most speciation in *Carex* has taken place within the last 15 million years.

The alignment of *Carex* 5.8S sequences with representatives of several families including the Poaceae and Cyperaceae, indicated that a 3-bp insertion had occurred at the 3′ end of this gene (Figs. 2, 3). Interestingly this insertion was shared with the genus *Kobresia,* a member of the *Cariceae,* but not with any other taxon investigated thus far (Starr, unpublished data). To our knowledge this is the largest insertion known to have occurred within the 5.8S gene of flowering plants, and it may prove useful for helping to delimit the family, the *Cariceae,* or a clade within the tribe itself.

Subgeneric circumscriptions in Carex—Two major clades in *Carex* were identified in this study: (1) an evolutionarily ''reduced'' clade consisting of sections that are traditionally placed in either subg. *Carex* or subg. *Primocarex* and (2) a ''compound'' clade comprising sections typically placed in subg. *Carex, Primocarex,* and *Indocarex* (Fig. 4). These two groups suggest that three of the four subgenera recognized by Kükenthal (1909) are artificial.

Subgenus *Carex* with its enormous number of species and high degree of variability in the sexual expression of the spikes is, not surprisingly, polyphyletic in this analysis (Nannfeldt, 1977; Reznicek, 1990). Although the subgenus is unnatural, this is only due to the placement of sect. *Phyllostachys* within the reduced clade. The multispicate ''core'' sections (i.e., sections *Careyanae, Laxiflorae, Hymenochlaenae,* and *Acrocystis*) typical of subgenus *Carex,* with terminally staminate and laterally pistillate inflorescence units, still form a weak, albeit consistent clade in our analysis. This clade, however, forms a part of a wider ''compound'' lineage that includes a paraphyletic subg. *Indocarex* and a section representative of subg. *Primocarex,* a result that is consistent with classifications that have merged subg. *Indocarex, Carex,* and *Primocarex* in part (e.g., Ohwi, 1936; Koyama, 1962). The placement of sections *Cruciatae* and *Polystachyae* (subg. *Indocarex*) as sister to the remainder of the compound clade is furthermore significant because it is com-

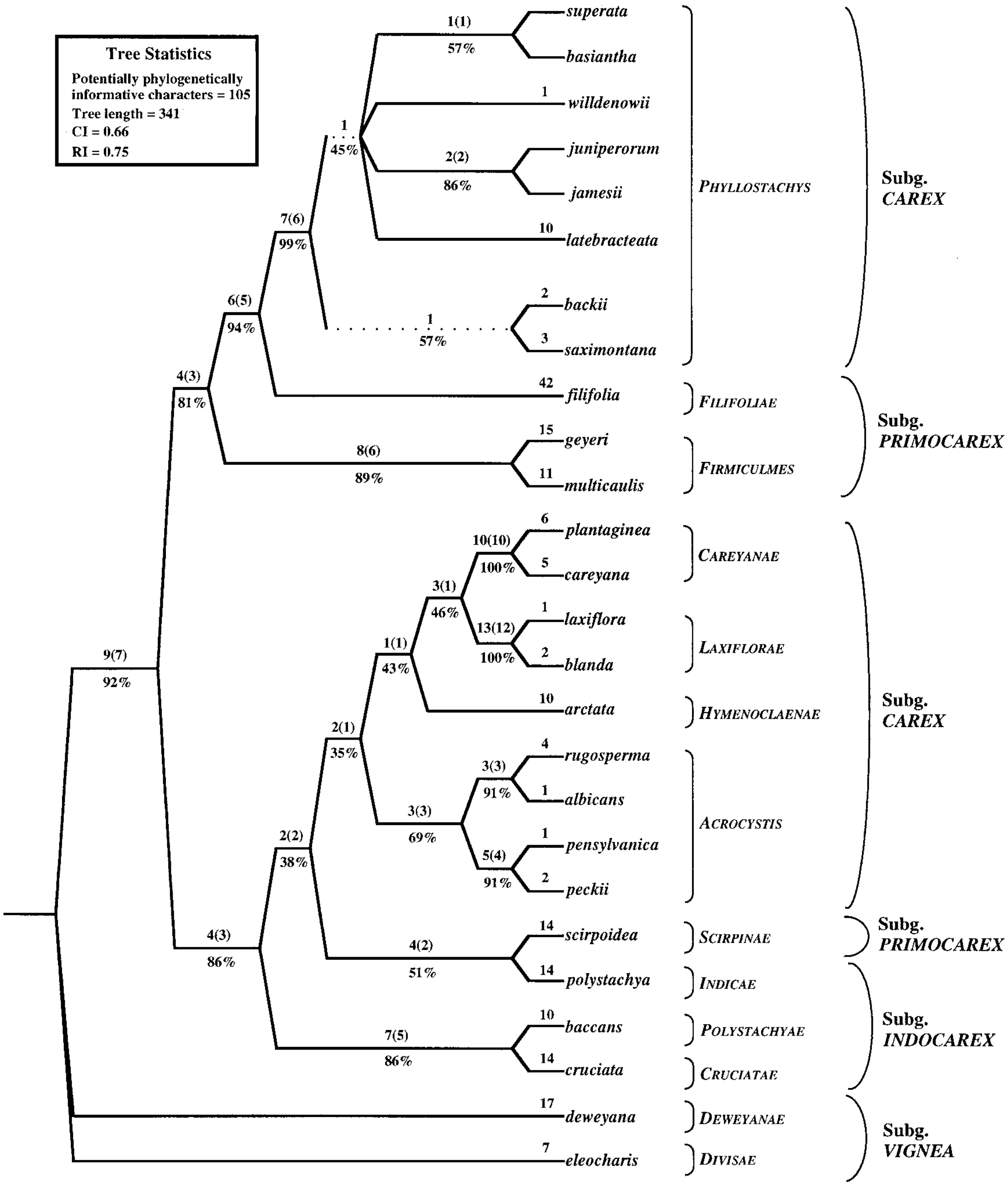

Fig. 4. The single tree of five most parsimonious trees resulting from the phylogenetic analysis of *Carex* section *Phyllostachys* and ten putatively related sections that is topologically identical to the 50% majority rule tree. The members of subgenus *Vignea* were used as an outgroup. The names of the sections and subgenera are given in succession after the brackets to the right of the specific epithets. Branches that collapse in the strict consensus of the five most parsimonious trees are dotted. Unambiguous base pair changes and decay indices (in parentheses) are given above the branch, and bootstrap values are given below the branch. The consistency index (CI), retention index (RI), tree length, and number of potentially phylogenetically informative characters are given in the box at the top left.

patible with many arguments based on phytogeography (Kreczetovicz, 1936; Nelmes, 1951; Ball, 1990) and inflorescence structure (Kreczetovicz, 1936; Koyama, 1962; Smith and Faulkner, 1976), that have suggested subg. *Indocarex* was the possible progenitor of a wider subg. *Indocarex*/*Carex*/*Primocarex* line.

The other major finding of this study is that subg. *Primocarex* appears to be polyphyletic. In all our analyses sect. *Scirpinae* (subg. *Primocarex*) was present in the compound clade and formed a weak group with *C. polystachya*, a North American species in subg. *Indocarex*. The clear separation of section *Scirpinae* from the "reduced" clade is significant in that it confirms the common belief that extreme reduction has occurred along several independent evolutionary lines in *Carex* (Mackenzie, 1935; Kreczetovicz, 1936; Nelmes, 1952; Smith and

Faulkner, 1976). However, a clearer understanding of the relationships of the *Scirpinae* and the reduced clade to the rest of *Carex* and the *Cariceae* await a much wider tribal level study.

Sectional delimitation and taxonomic utility of the ITS region in Carex—One of the main obstacles to a phylogenetic classification of *Carex* at the subgeneric level is the artificial nature of many sections (Crins, 1990; Naczi, 1992). The discovery in this study of low infrasectional and high intersectional ITS sequence divergence suggests, however, that the ITS region is an effective tool for circumscribing sections in *Carex* and determining their relationships. This point is best exemplified by the differences found between species in sections *Laxiflorae* (*C. laxiflora* and *C. blanda*) and *Careyanae* (*C. plantaginea* and *C. careyana*). Most modern authors (e.g., Kükenthal, 1909; Mackenzie, 1935; Fernald, 1950) have not recognized section *Careyanae* as being distinct from the *Laxiflorae.* Recent morphological (Bryson, 1980) and biochemical (Manhart, 1986) treatments of the *Laxiflorae* have likewise continued to recognize only a single section, but do concede that the *Laxiflorae* s.l. are composed of two distinctive groups. Naczi (1992) recognized the *Laxiflorae* and *Careyanae* as distinct sections and demonstrated in a phylogenetic analysis that the *Careyanae* were sister to section *Griseae* and not to the *Laxiflorae* s.s. The present phylogenetic analysis supports the conclusions of Naczi (1992) in that it indicates weak support for a *Careyanae*/*Laxiflorae* clade, but provides strong evidence (DI = 10–15; 100% BS) for the recognition of two distinct sections. Sequence divergence within (0.95%, *Laxiflorae*; 2.86%, *Careyanae*; Table 4) and between (8.15–8.59%; Table 5) sections *Laxiflorae* and *Careyanae* further supports this conclusion.

Despite the low levels of sequence divergence observed within sections, the ITS region can, in some instances, provide conserved characters that are useful for circumscribing critical groups below the sectional level. The clear separation of *C. albicans* from *C. peckii* (sect. *Acrocystis*) provides a good example. Although these two taxa are distinct at the species level, some authors (Gleason, 1952; Gleason and Cronquist, 1963) have treated them as varieties of *C. nigromarginata* Schw. The present analysis, however, places these taxa into two separate and strongly supported clades (DI = 3–4; $>$ 90% BS), and identifies at least 12 mutational differences in their ITS regions. While the above example may seem trivial, it does suggest that DNA sequences could provide important characters to future taxonomic studies in the genus. Section *Acrocystis,* in particular, has numerous unresolved taxonomic problems (e.g., the *C. tonsa* and *C. rossii* Boott complexes) where the ITS region could be an effective aid in resolving the intricate taxonomy of this group.

The phylogenetic position of Carex section Phyllostachys—The results of our study provide little support for the phylogenetic hypotheses proposed by earlier authors (see subsection ‘‘*Carex* sect. Phyllostachys and its position within the genus *Carex*’’ in Systematic Background). Bailey's (1885, 1886) enlarged concept of the *Phyllostachys* that included species currently placed in section *Firmiculmes* (subsection *Phyllostachyae*) is polyphyletic in this analysis. Equally unsupported is Bailey's placement of sect. *Phyllostachys* close to sections *Acrocystis* and *Laxiflorae*; both of these sections are placed in a clade that is separate from the *Phyllostachys.* Holm's (1900) claim that the *Phyllostachys* were not ‘‘sufficiently characteristic’’ to be considered a section is clearly contradicted by the frequent recognition of its singularity in *Carex* (e.g., Bailey, 1886; Mackenzie, 1935; Catling, Reznicek, and Crins, 1993), and by the robust statistical (DI = 6; 99% BS) and character support (seven synapomorphies) substantiating its monophyly in this molecular study. Holm's decision to consider the *Phyllostachys* as primitive within section *Hymenochlaenae* is equally untenable and is not supported.

Our results support decisions to merge subg. *Indocarex* and *Primocarex* in part with subg. *Carex* (Holm, 1900, 1903; Kreczetovicz, 1936; Ohwi, 1936; Koyama, 1962), but they do not support arguments that have used the presence or abnormal growth of the rachilla to justify this merger. Kreczetovicz (1936) considered the rachilla to be a derived character whose aberrant growth, among other characters, pointed to a recent and direct origin for sections *Firmiculmes* and *Phyllostachys* from subg. *Indocarex* via transmutive reduction. Similarly, Holm (1900, 1903) used a teratological specimen of *C. backii* to argue for an expanded subg. *Carex,* noting that sect. *Phyllostachys* was ‘‘in no wise to be distinguished from the *Vigneastra* (= subg. *Indocarex*).’’ The present analysis, however, fails to find evidence to suggest that the presence of rachillae, or their abnormal growth, is any direct indication of phylogenetic relatedness, atavism, or primitiveness. The same type of abnormal growth of the rachilla present in sections *Phyllostachys* and *Firmiculmes* is also prevalent in groups in the compound clade such as sections *Hymenochlaenae* (Koyama, 1962) and *Acrocystis* (Svenson, 1972). In fact, the presence of rachillae and the abnormal growth of rachillae occur sporadically in all four of the subgenera in *Carex* (Snell, 1936; Smith and Faulkner, 1972; Svenson, 1972; Reznicek, 1990), suggesting that these characters do not confer any special insight into the phylogeny of this genus (see Reznicek, 1990).

Equally discordant with our data were Savile and Calder's (1953) phylogenetic hypotheses based on smut parasitism. In their arrangement, sect. *Phyllostachys* is placed in an advanced lineage within subg. *Carex* that includes sect. *Scirpinae,* while sect. *Firmiculmes* is far removed from the *Phyllostachys* in subg. *Carex,* and sect. *Filifoliae* is distantly situated at the base of subg. *Primocarex.* Our ITS data indicate that sections *Phyllostachys* and *Filifoliae* are sister groups, that they are closely related to sect. *Firmiculmes,* and that the *Scirpinae* are at best a distantly related section in a completely separate lineage. Contradictions between smut infection records and other data sets are common (Reznicek, 1990), but they do not mean that smut data are systematically uninformative (e.g., Nannfeldt, 1977; Kukkonen and Timonen, 1979; Savile, 1990). The value of these characters for studying phylogeny in *Carex* is limited, however, by an uncertain knowledge of smut phylogeny (Kukkonen and Timonen, 1979; Reznicek, 1990) and by a poor understanding of whether smut associations and morphology could be linked to other factors such as the ecology or physiology of host species (Hamlin, 1959; Smith and Faulkner, 1976).

Although Kükenthal's (1909) alignment of sect. *Phyllostachys* with sections *Rhomboidales* and *Elatae* cannot be ruled out due to a lack of data, our results would indicate that a close relationship between these three sections is unlikely. The inflorescence morphology of sections *Rhomboidales* and *Elatae* clearly aligns them with the subg. *Carex* group of the compound clade and not with sect. *Phyllostachys* and its allies.

Of all the treatments examined, Mackenzie's (1935) is the most similar to our molecular results by its suggestion of a wider subg. *Indocarex*/*Carex*/*Primocarex* line and by its juxtaposition of sections *Phyllostachys* and *Filifoliae.* Unfortunately Mackenzie did not recognize subgenera and his views on evolutionary relationships in *Carex* are only expressed through a terse and vague conspectus. It is therefore difficult to determine the extent of his disagreement with Kükenthal's (1909) subgeneric circumscriptions or whether the proximity of sections *Phyllostachys* and *Filifoliae* in his system implies a relationship. Our hypothesis that sections *Phyllostachys, Filifoliae,* and *Firmiculmes* might form an evolutionarily reduced clade within the genus that is separate from the members of subg. *Carex* is novel, but the concept of a wider, evolutionarily reduced group in *Carex* is not.

Phylogeny in Carex section Phyllostachys—Although our study helps clarify the phylogenetic position of section *Phyllostachys* within *Carex,* the ITS region was not sufficiently variable to adequately resolve infrasectional relationships. The topology of our tree does show some correlation to a previous analysis based on morphology (Starr and Ford, 1995) in the recognition of three species pairs (i.e., *C. backii*/*C. saximontana*, *C. juniperorum*/*C. jamesii,* and *C. basiantha*/*C. superata*), but it differs by the placement of *Carex latebracteata* in a separate clade from *C. backii* and *C. saximontana.* These results, however, are unreliable since statistical support for the phylogeny is weak and only two clades, the species pairs of *C. juniperorum*/*C. jamesii* and *C. basiantha*/*C. superata,* do not collapse in the strict consensus tree.

The notable differences in anatomy, micromorphology, and macromorphology within section *Phyllostachys* (Catling, Reznicek, and Crins, 1993; Starr, 1997; Naczi, Reznicek, and Ford, 1998) suggested that the rate of evolution in these characters was higher than the mutation rate of ITS. By combining the above characters with ITS sequence data we achieved a much better understanding of infrasectional relationships within the *Phyllostachys.* The results of this study will be presented in a companion paper (Starr, Ford, and Bayer, unpublished data).

CONCLUSIONS

Our study provides evidence to suggest that subgenera *Indocarex, Carex,* and *Primocarex* are artificial as traditionally circumscribed. As many previous authors have believed (e.g., Bailey, 1886; Kreczetovicz, 1936; Ohwi, 1936; Koyama, 1962), subg. *Indocarex, Carex* (in part), and a portion of subg. *Primocarex* form a monophyletic group. Phylogenetic hypotheses and relationships based on the presence or abnormal growth of the rachilla are not supported in this analysis. Extreme reduction appears to have occurred along several different lineages in *Carex.* Our data support the conclusion that an evolutionarily ''reduced'' clade, made up of sections *Phyllostachys, Filifoliae,* and *Firmiculmes,* is separate from a main lineage composed of species in subg. *Carex, Indocarex,* and *Primocarex.* Unfortunately, a clearer understanding of the relationships of the reduced clade to the rest of *Carex* and the *Cariceae* could not be determined due to the small taxonomic sample. Whether this clade is more closely related to elements within *Carex* or outside of the genus as suggested for *Carex filifolia* (e.g., Ivanova, 1939) can only be resolved in a much wider tribal level study.

One of the most enduring problems in the systematics of *Carex* has been the circumscription of sections. Although the ITS region was not variable enough to resolve relationships within sect. *Phyllostachys,* it shows considerable potential for clarifying sectional limits and for hypothesizing relationships between sections in *Carex.* The strong support for the monophyly of the morphologically similar sections *Laxiflorae* and *Careyanae* illustrates the potential of the region for sectional delimitation.

Any study that includes only a small fraction of a group as large as *Carex* must come with a caveat. Many of the relationships that have been enumerated herein can only be expected to change as a wider taxonomic sample is introduced. Phylogenetic relationships within *Carex* will not be completely resolved by 450 bp, and 26 taxa will not be sufficient to unravel subgeneric problems that have existed for over a hundred years. Phylogenetic relationships within *Carex* can only be resolved through a multidisciplinary approach, using a much wider survey of taxa. However, the potential of this region, and of DNA characters in general, for helping to resolve many long-standing phylogenetic and taxonomic problems in this genus has been demonstrated.

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