Systematic Botany (2006), 31(1): pp. 70–82

Phylogeny of *Carex* subg. *Vignea* (Cyperaceae) Based on Non-coding nrDNA Sequence Data

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Communicating Editor: Wendy B. Zomlefer

ABSTRACT. *Carex* subg. *Vignea* is characterized by sessile bisexual spikes, distigmatic flowers, and the lack of cladoprophylls. Phylogenies reconstructed using nrDNA internal and external transcribed spacer (ITS and ETS 1f) sequences for 100 vignean taxa support this subgenus as monophyletic. The atypical *C. gibba* is sister to all remaining taxa. Many clades in the remainder of the subgenus do not correspond to easily defined morphological groups, with species representative of several disparate sections frequently contained within a single clade. Many traditionally recognized sections are not supported, although others such as sects. *Ovales*, *Stellulatae*, and *Glareosae* are monophyletic. Tree topologies indicate that gynaecandry has evolved multiple times in subg. *Vignea*. Species of uncertain subgeneric affinity are variously placed in our analysis. *Carex fecunda*, previously linked to subg. *Vignea*, is positioned within the outgroup composed of species traditionally placed in subg. *Carex* and *Vigneastra*. While species with highly compound inflorescences are often regarded as ancestral in *Carex*, our results indicate that this inflorescence type has evolved multiple times and is derived within subg. *Vignea*.

Cyperaceae, with over 5000 species in 104 genera (Goetghebeur 1998), is one of the ten largest families of flowering plants. Tribe Cariceae Kunth ex Dumort. comprises five genera and over 2100 species and is distinguished from other tribes in the family by unisexual flowers with each carpellate flower fully to partially enclosed in a sac-like structure called a perigynium. *Carex* L., with approximately 2000 species, constitutes nearly half of the family and almost all the species in the Cariceae.

In the only comprehensive monograph of *Carex*, Kükenthal (1909) divided the genus into four subgenera: 1) *Psyllophora* (Degl.) Peterm. (= *Primocarex* Kük. in Engl.; solitary, terminal spikes); 2) *Carex* [mostly tristigmatic flowers, peduncled unisexual spikes, with the peduncle of at least the lowest spike subtended by a scale-like or ocreaform cladoprophyll (Reznicek 1990)]; 3) *Vigneastra* (Tuck.) Kük. [= *Indocarex* (Baill.) Kük. in Engl.; a mainly tropical group; decompound bisexual spikes with the peduncles of the primary axes subtended by cladoprophylls but with secondary and tertiary floral aggregations associated with perigynium-like inflorescence prophylls (Reznicek 1990)]; and 4) *Vignea* (P. Beauv. ex Lestib. f.) Perterm. (sessile bisexual spikes, usually distigmatic flowers, no prophylls, setaceous bracts). Subsequent authors have recognized three (*Carex*, *Vigneastra*, and *Vignea*) or sometimes two (*Carex* and *Vignea*) subgenera (Reznicek 1990 and papers cited therein). The segregation of a fifth subgenus, subg. *Kreczetoviczia* Egorova (Egorova 1999), a group of about 140 distigmatic subg. *Carex* species, has not been widely accepted (Ball and Reznicek 2002).

Traditionally subg. *Vigneastra*, with its highly compound inflorescences (defined here as one with spikes or ''primary branches'' containing secondary and tertiary axes comprised of staminate and/or carpellate flowers), has been hypothesized as ancestral for the genus. A reduction in the number of inflorescence branches may have resulted in the derivation of subg. *Carex*. A decrease in branching, stigma number, and peduncle length and loss of prophylls may have also led to the evolution of subg. *Vignea*. Subgenus *Psyllophora* is usually regarded as polyphyletic and comprised of taxa from the other three subgenera or possibly from other genera in the Cariceae (Reznicek 1990).

Views contrary to this hypothesis are few. However, Reznicek (1990) questioned that subg. *Vigneastra* is primitive and suggested that the least derived features (e.g., highly compound inflorescences) are found in species considered close to or part of subg. *Vignea*. These include *C. fecunda* and allies (sect. *Fecundae*), *C. crus-corvi* (sect. *Vulpinae*), and *C. decomposita* (sect. *Heleoglochin*) (Reznicek 1990). Subgenus *Carex* was hypothesized to have been derived from subg. *Vignea* by the reduction of bisexual spikes to single perigynia. The origin and relationship of subg. *Vigneastra* was uncertain.

Molecular phylogenetic studies by Starr et al. (1999), Yen and Olmstead (2000), Roalson et al. (2001), and Starr et al. (2004, in press) have challenged previous hypotheses regarding the evolution of *Carex*. These studies indicate that genera of Cariceae must be included within a more broadly circumscribed *Carex*. Within this expanded concept of the genus, three ma-

jor clades can be distinguished: 1) a compound clade, comprising primarily multispicate species traditionally placed in subg. *Vigneastra*, and portions of subg. *Psyllophora* and *Carex*; 2) a reduced clade comprising primarily unispicate species traditionally placed in portions of subg. *Carex*, *Psyllophora*, plus all other genera in the Cariceae (*Cymophyllous* Mack., *Kobresia* Willd., *Uncinia* Pers., *Schoenoxiphium* Nees); and 3) subg. *Vignea*.

Despite different hypotheses regarding classification of *Carex* and the Cariceae, subg. *Vignea*, with about 300 species in 28 sections (Ford and Naczi unpubl. data), appears to be monophyletic (Nannfeldt 1977; Reznicek 1990; Egorova 1999; Yen and Olmstead 2000; Roalson et al. 2001; Hendrichs et al. 2004b). This subgenus reaches its greatest taxonomic diversity in North America. The current distribution and ecology of the subgenus suggest that it may have evolved in cooler climates of the New World, but there is little evidence to support this hypothesis (Ball 1990). Evolutionary trends within subg. *Vignea* are poorly known, although species have been placed into one of two groups based upon whether they possess androgynous (staminate flowers borne distal to carpellate) or gynaecandrous (carpellate flowers borne distal to staminate) spikes. These two groups may represent major lineages within the subgenus, with some authors suggesting that gynaecandry is derived (e.g., Egorova 1999). Many sections may be monophyletic but the sectional affinities of some species (e.g., *C. sychnocephala*, *C. disperma*, *C. illota*, etc.) are in doubt. While recent phylogenetic studies of Cariceae have shown that unispicate tristigmatic species are not members of subg. *Vignea* (Starr et al. 1999, Starr et al. 2004), the phylogenetic position of unispicate distigmatic species, (e.g., *C. gynocrates*) has not been fully clarified.

The few phylogenetic studies that focus specifically on subg. *Vignea* are preliminary. A study of the relationships among the gynaecandrous species by Yelton and Naczi (2001) using morphological and anatomical characters indicated the following phylogenetic hypothesis: sects. (*Stellulatae* + (*C. seorsa* + ((*Glareosae* + *Deweyanae*) + (*Remotae* + (*Cyperoideae* + (*Ovales* + *Planatae*)))))). *Carex seorsa*, previously placed in sect. *Stellulatae*, was found to belong to a separate, undescribed section. *Carex laeviculmis*, which has been variously classified as a member of sect. *Stellulate* or *Deweyanae*, was placed within sect. *Glareosae*.

Molecular studies by Roalson et al. (2001), which included 16 species of subg. *Vignea*, did not support an androgynous/gynaecandrous split and failed to reveal any trends in character evolution. A larger study of subg. *Vignea* by Hendrichs et al. (2004b) based on nrDNA internal transcribed spacer (ITS) sequence data from 58 species, representing 20 sections, also failed to show strong patterns of relationship.

This study represents a twofold increase in the number of species sampled over previous studies and employs both ITS and external transcribed spacer (ETS 1f) nrDNA data. Our general goal was to gain a better understanding of the phylogenetic position of critical taxa often associated with subg. *Vignea* and to determine the phylogeny of its species. More specifically, we were interested in determining: (1) the evolutionary position of phylogenetically crucial species such as those with highly compound or unispicate inflorescences; (2) trends in morphological character evolution; and (3) whether traditionally recognized sections are monophyletic.

Materials and Methods

Taxon Selection. A worldwide list of subg. *Vignea* species was compiled from a search of monographs and floras (bibliography available from B. A. F. upon request). From this list we selected 100 taxa, representing 26 of the 28 currently recognized sections for DNA analysis (Appendix 1). An effort was made to select exemplars from each section plus species of controversial phylogenetic placement. Section *Ovales*, the largest section in subg. *Vignea* (ca. 85 species; Mastrogiuseppe et al. 2002), was sparsely sampled because the results of previous research have shown this section to be monophyletic (Hipp, in press). Sixteen other species representing the compound and reduced clades, plus species of uncertain subgeneric affinity, were also included in this study (Appendix 1).

Species from the compound clade were chosen as the outgroup for all analyses (Appendix 1). Previous studies have shown this clade to be monophyletic (BS > 85%) and distinct from subg. *Vignea* (Roalson et al. 2001; Starr et al. 2004). In addition, a recent study by Starr et al. (2004) shows this clade as sister to a clade comprising subg. *Vignea* and the reduced clade. The ingroup was comprised of three assemblages of species: 1) those traditionally placed in subg. *Vignea*; 2) those of uncertain subgeneric affinity; and 3) species placed in the reduced clade in earlier phylogenetic studies (Starr et al. 2003, 2004, in press). This mix of ingroup species was required to test the monophyly of subg. *Vignea* and to determine the placement of phylogenetically controversial species.

DNA Extraction, Amplification, Sequencing, and Alignment. DNA was isolated from ca. 20–25 mg of silica gel dried or herbarium specimen leaf tissue according to the protocols outlined in the Dneasy Plant Mini Kit (Qiagen 69106). Elution in the final steps was accomplished using a total of 100 μl of AE buffer instead of the recommended 200 μl. The internal transcribed spacer (ITS) region (from $^{3'}$18S—$^{5'}$26S, including 5.8S) was PCR amplified using the forward primer 17SE (Sun et al. 1994) and the reverse primer ITS-4 (White et al. 1990). A fragment of the 5′ external transcribed spacer region (ETS 1f) was amplified using the forward primer ETS-1F and the reverse primer 18S-R (Starr et al. 2003). Each reaction mixture contained the following: 5 μl of 10× PCR buffer; 4 μl of a 2.5 mM stock solution of all four dNTPs; 3 μl of a 50 mM stock solution of $MgCl_2$; 2 μl × 2 of a 10 pmol/μl concentration of each primer; 1 μl *Taq* (2–3 units); and 1 μl of template DNA (10–50 ng). All reactions were adjusted to a final volume of 50 μl using deionized water. Problematic sequences were repeated with the addition of 5 μl of a 5M concentration of betaine (Sigma # B-0300) to each reaction. PCR was undertaken using an MJ Research PTC-100 thermal cycler using the following parameters for each reaction: 1 cycle of 2 min at 94° C; 30 cycles of 30 sec at 94° C, 30 sec at 50° C, and 1 min 20 sec at 72° C. All PCR products were purified using Montage PCR Centrifugal Filter Devices (Millipore UFC7PCR50) and eluted to 25 μl using deionized water. Purified sequencing products were run on an ABI 377XL sequencer (University Core DNA Services, University of Calgary) using PCR primers.

Sequence Analysis. The boundaries of ITS-1, 5.8S, ITS-2, and ETS-1f were determined by comparison to the sequences generated by Starr et al. (1999, 2003, 2004). Forward and reverse sequences for each sample were assembled and edited in Sequencher 4.2 (Gene Codes Corporation, Inc.) before alignment in ClustalX (default settings; Thompson et al. 1997). This initial alignment was then subjected to a heuristic search using PAUP* 4.0b10 (Swofford 2002) to determine initial tree length. Further alignments were undertaken manually using the edit mode in PAUP* and the procedure outlined in Starr et al. (2004), which accepts or rejects manual adjustments on the basis of parsimony. The final matrix used for this study included the entire ITS-1, 5.8S, ITS-2, ETS-1f region with the exception of base pairs 17–22, 183–187 (ITS-1), 245–259 (ITS-1, 5′ end of 5.8S), 588–602 (ITS-2), and 786–789, 899–907 (ETS-1f), which were excluded due to the presence of repeated elements or alignment ambiguity. Aligned sequences for this study can be obtained from TreeBASE (study accession S1319). Individual sequences are available through GenBank (Appendix 1). The number of base pairs in each sequence and G/C content were determined using the BASEFREQ command in PAUP*, while the SHOWDIST command was used to calculate an uncorrected (''p'') distance matrix between all combinations of taxa. Indels were included in parsimony analyses and were coded using the ''simple gap coding method'' of Simmons and Ochoterena (2000) as implemented in GapCoder (Young and Healy 2003).

Phylogenetic Analyses. Heuristic parsimony searches in PAUP* 4.0b10 (Swofford 2002) were conducted using 2075 replicates of a random addition of taxa. Save all minimal trees (MULTREES), collapse all zero length branches (COLLAPSE), and tree-bisection-reconnection (TBR) commands were employed with branch swapping occurring on best trees only. In order to more effectively search through tree space, the NCHUCK and CHUCKSCORE (set arbitrarily at 100) options were used to limit the number of trees retained per replicate to 1000. Clade support was determined using bootstrap analysis (heuristic searches, 10,000 replicates, simple stepwise addition of taxa). During these searches, the COLLAPSE and TBR commands were employed with the MULTREES command turned off (DeBry and Olmstead 2000). Clade support was categorized as poor (<55%), weak (55%-64%), moderate (65%-74%), good (75%-84%), very good (85%-94%) or strong (95%-100%) (Hillis and Bull 1993; Huelsenbeck et al. 1996). Consistency (CI) and retention (RI) indices were used to evaluate homoplasy and overall clade support. The CSTATUS command was used to determine the total number of parsimony informative and uninformative characters.

Results from heuristic searches were compared with the most parsimonious trees recovered from a Parsimony Ratchet (Nixon 1999) analysis in PAUPRat (20 independent ratchet searches, 200 iterations: Sikes and Lewis 2001). The parsimony ratchet is ideal for large datasets because of its increased computational speed achieved through increased island visitation as opposed to thorough island searching.

An incongruence length (ILD) test (Farris et al. 1994) of ITS and ETS datasets (indels included) was accomplished using a heuristic search and a simple addition of taxa for 100 random partitions of the data in PAUP* 4.0b10 (Swofford 2002). As with previous phylogenetic studies of the Cariceae, our study showed an incongruence between ITS and ETS data sets ($P = 0.01$; Cunningham 1997; Starr et al. 2004). Many authors, including Yoder et al. (2001) and Dowton and Austin (2002) have questioned the use of incongruence tests. Since phylogenetic accuracy depends on many factors other than congruence between datasets (Hipp et al. 2004), and because of the importance of using all relevant data in a phylogenetic study (Kluge 1989; Nixon and Carpenter 1996), ITS and ETS datasets were combined for all phylogenetic analyses.

Heuristic maximum likelihood (ML) analysis (with indels excluded) was also used to generate phylogenetic hypotheses. TBR branch swapping and five random additions of taxa were employed. Modeltest 3.06 (Posada and Crandall 1998) was used to evaluate 56 nested evolutionary models and identify the model that best fits these data. A general-time-reversible (GTR) model incorporating a correction for rate of heterogeneity across sites (gamma distribution, G) and an estimate of the proportion of invariable sites was selected and used during all searches. To determine whether branch lengths on optimal trees were significantly greater than zero, a likelihood-ratio test was employed using the ZEROLENTEST command in PAUP* with ''full'' optimization. This procedure optimizes all branch lengths under the constraint that one of the branches is zero, for each branch in the tree.

A Shimodaira-Hasegawa (SH; Shimodaira and Hasegawa 1999) test was used to compare the optimal ML tree with six most parsimonious trees chosen at random. Comparisons were also made with constraint trees to evaluate hypotheses regarding the division of subg. *Vignea* into androgynous and gynaecandrous or tristigmatic and distigmatic clades. Optimal trees were estimated using the procedure outlined above where gynaecandrous and distigmatic taxa, respectively, were constrained to be monophyletic. One-tailed tests were generated from 10,000 bootstrap replicates using the re-sampling estimated log-likelihood method (RELL; Kishino et al. 1990) in PAUP*.

RESULTS

Sequence Statistics. Sequence statistics for the nrDNA dataset used in this analysis are presented in Table 1. ITS-1 and ITS-2 varied between 439 to 449 bp in length, while ETS-1f ranged between 589–598 bp. The 5.8S region was 166 bp. Sequence divergence values within subg. *Vignea* were usually lower than when species from the outgroup and reduced clade were included in comparisons (circumscription of subg. *Vignea* based on results of phylogenetic analyses, Figs. 1, 2). For the ITS region, the highest divergence was between *C. monostachya* from the reduced clade and *C. neurocarpa* from subg. *Vignea* (19.53%). A value of 17.83% was found between vignean species *C. neurocarpa* and *C. physodes*. For ETS-1f, the highest divergence was obtained in a comparison between *C. nardina* from the reduced clade and *C. appropinquata* from subg. *Vignea* (21.08%). Within subg. *Vignea*, the highest value was found in a comparison between *C. gibba* and *C. appropinquata* (12.75%). Divergence values between some vignean and non-vignean taxa were lower than between pairwise comparisons of some subg. *Vignea* species. For example, for ETS 1f the divergence between *C. gibba* and *C. appropinquata* (12.75%) is higher than the value obtained in a comparison between *C. nigra* (outgroup) and *C. gibba* (11.60%). Divergence values approaching zero were found between different accessions of the same species. However, some pairwise comparisons between different species, such as *C. occidentalis* vs. *C. hoodii* or *C. wiegandii* vs. *C. interior* yielded values close to zero. The G+C content ranged from 54.95 to 64.24% for the entire nrDNA dataset set, with the ITS region averaging higher than the 5.8S and ETS-1f regions. Two possible paralogous ITS sequences were detected during our study: one in *C. kobomugi*, the other in *C. capitata*. Paralogs were identified by their 5–10% lower G+C content, frequent mutations in the 5.8S region, and a pattern of all mutations (in comparison to closely related taxa) in the ITS regions being transitions. The addition of betaine allowed amplification of potential ITS orthologs.

TABLE 1. Sequence statistics for ITS and ETS 1f datasets used in the phylogenetic analysis of *Carex* subg. *Vignea* Circumscription of subg. *Vignea* is based on results from phylogenetic analyses (Figs. 1, 2).

	ITS-1 + ITS-2	5.8S	ETS 1f	All nrDNA regions combined
Number of base pairs—subg. *Vignea*	439–449	166	589–598	1197–1211
—including reduced clade and outgroup	434–449	166	588–600	1194–1211
G+C content (%)—subg. *Vignea*	60.86–74.93	53.62–56.02	50.57–59.15	54.95–64.24
—including reduced clade and outgroup	60.86–74.93	53.62–56.02	50.57–59.67	54.95–64.24
Sequence divergence (%)—subg. *Vignea*	0.00–17.83	0.00–2.41	0.00–12.75	0.00–12.55
—including reduced clade and outgroup	0.00–19.53	0.00–2.41	0.00–21.08	0.00–17.37
Potentially parsimony informative sites—subg. *Vignea*	173	3	234	410
—including reduced clade and outgroup	194	4	312	510
Constant sites—subg. *Vignea*	216	154	328	698
—including reduced clade and outgroup	193	153	252	598
Autapomorphic sites—subg. *Vignea*	62	4	73	139
—including reduced clade and outgroup	64	4	71	139
Total number of aligned sites	451	161	635	1247
Potentially parsimony informative indels—subg. *Vignea*	12	0	14	26
—including reduced clade and outgroup	19	0	42	61
Constant indels_esubg. *Vignea*	11	0	35	46
—including reduced clade and outgroup	0	0	0	0
Autapomorphic indels—subg. *Vignea*	20	0	22	42
—including reduced clade and outgroup	24	0	29	53
Total number of aligned indels	43	0	71	114

Parsimony Analysis. The matrix for all parsimony analyses included 1361 characters (1247 bp + 114 indels). A total of 571 characters were potentially parsimony informative, with 312 and 194 characters from the ETS-1f and ITS regions, respectively. The 5.8S region contributed only four characters, while indels provided 61 potentially parsimony informative characters (Table 1).

Heuristic parsimony searches based on 2075 replicates of a random addition of taxa found 1017 equally parsimonious trees of 2757 steps in length (CI = 0.40; RI = 0.74). Figure 1 is the strict consensus of these trees. The PAUPRat analysis produced 2280 most parsimonious trees of 2757 steps in length with the strict consensus of these trees topologically identical to the hypothesis depicted in Fig. 1 but in a fraction of the time (< 2 hours vs. >96 hours).

Bootstrap support was moderate to strong for many terminal as well as basal clades. The ingroup and reduced clade formed very well to strongly supported monophyletic groups (BS = 100 and 86%, respectively). Subgenus *Vignea* was strongly supported as monophyletic (BS = 96%) with *C. gibba* being sister to the rest of the subgenus (BS = 99%). Mid-level clades were inadequately resolved with poor bootstrap values. Species of uncertain subgeneric affinities were variously placed in this analysis with distigmatic androgynous unispicate species placed in the reduced clade (*C. nardina*, *C. capitata*) while gynaecandrous (*C. exilis*) or dioecious unispicate species (*C. gynocrates*, *C. dioica* L.) were found within subg. *Vignea*. *Carex fecunda* was firmly nested within the outgroup.

Maximum Likelihood Analysis. A Shimodaira-Hasegawa (SH) test comparing the optimal ML tree (Fig. 2) with six most parsimonious trees chosen at random indicated that cladograms produced using these two methods were not significantly different ($P > 0.05$: Table 2). Only seven branches in the ML tree were not significantly different from zero ($P > 0.05$). As in parsimony analyses, the ML tree supported the monophyly of subg. *Vignea* with *C. gibba* sister to all remaining taxa. A number of clades were identified within subg. *Vignea*, but these usually represented an eclectic assortment of taxa with very few traditionally recognized sections. For example, the uppermost clade in Fig. 2 (from *C. duriuscula* to *C. aggregata*, inclusive) contains species from eight sections (*Phaestoglochin*, *Vulpinae*, *Multiflorae*, *Heleoglochin*, *Inversae*, *Foetidae*, *Divisae*, *Deweyanae*). Although species from some sections are scattered throughout the cladogram (e.g., sects. *Phaestoglochin*, *Deweyanae*, and *Divisae*), not all traditionally recognized sections are polyphyletic. Sections *Stellulate* (including sect. *Elongatae*), *Glareosae* (with the noticeable absence of *C. arcta*), and *Ovales* (with the exception of *C. illota* and with the inclusion of *C. sychnocephala*) are monophyletic. Many smaller monophyletic assemblages are also evident, including sects. *Macrocephalae*, *Bracteosae*, *Holarrhenae*, *Phleoideae*, *Physoglochin*, and the *C. rosea* complex (*C. rosea* s. str., *C. radiata*, *C. socialis*, and *C. texensis*). Species of controversial sectional placement are variously positioned. For example, *C. disperma*, traditionally placed within its own monotypic section or within sect. *Glareosae* (Mackenzie 1935; Toivonen 2002), is found at the base of a clade with

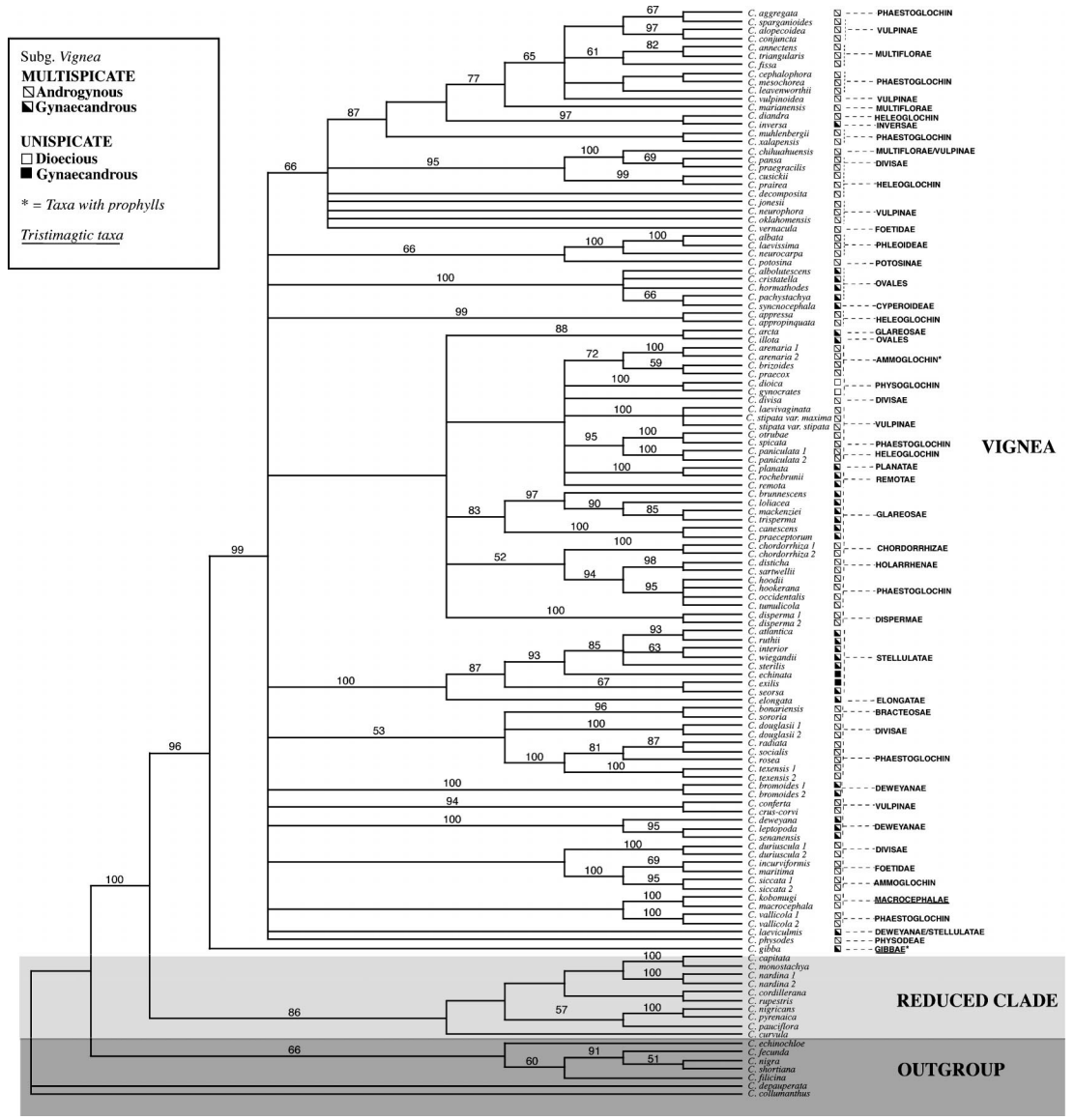

FIG. 1. Strict consensus of 1017 most parsimonious trees resulting from heuristic searches of a combined ITS and ETS 1f data set. Numbers above branches represent bootstrap values. Names on the right hand side of the cladogram represent the section to which each species is most commonly referred.

species from seven different sections. *Carex laeviculmis*, placed in sect. *Deweyanae*, *Stellulate*, or *Glareosae* (Reznicek and Ball 1980; Yelton and Naczi 2001; Naczi 2002), is sister to a large clade that includes species from nine different sections. Distinctive taxa such as *C. physodes* (inflated orange-brown perigynia) or *C. potosina* (highly tuberculate perigynia) are deeply nested within subg. *Vignea*.

The results from the ML analysis provide insights into the evolution of a number of characters commonly used in infrageneric classification (Fig. 2; Table 2). The SH test does not support the hypothesis of separate gynaecandrous/androgynous clades with the gynaecandrous condition evolving independently eight times. Similarly, the evolution of three stigmas appears to have occurred at least twice during the evolution of subg. *Vignea*, once in *C. gibba* and again in sect. *Macrocephalae*, although the SH test does not reject the possibility of a monophyletic distigmatic clade. Prophylls occur rarely in subg. *Vignea* but appear to have evolved twice during its evolution: in *C. gibba* and in the clade that includes *C. arenaria*, *C. brizoides*, and *C. praecox* (sect. *Ammoglochin*).

Unispicate distigmatic species are variously posi-

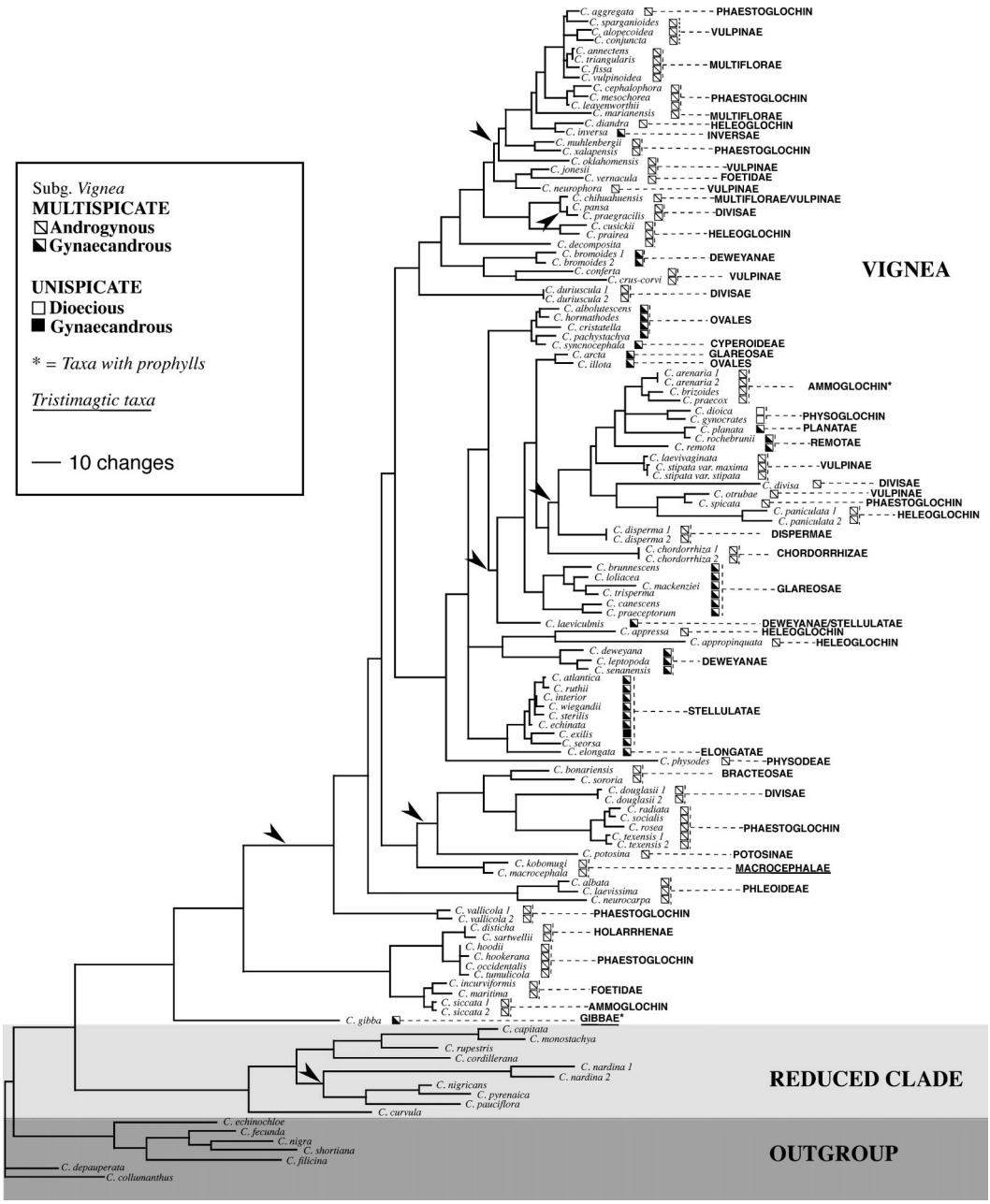

FIG. 2. Maximum likelihood tree based on five random additions of taxa using the combined ITS and ETS 1f data set and a GTR + G + *I* model of sequence evolution. Branch lengths not significantly different from zero ($P > 0.05$) are indicated by arrows. Names on the right hand side of the cladogram represent the section to which each species is most commonly referred.

tioned in this analysis (Fig. 2). Gynaecandrous and dioecious species (e.g., *C. exilis*, *C. gynocrates*, *C. dioica*) are placed within subg. *Vignea*, while androgynous taxa (*C. capitata*, *C. nardina*) are placed in the reduced clade with tristigmatic androgynous unispicate species. At the other end of the continuum are species with large compound inflorescences. *Carex crus-corvi*, *C. decomposita*, and *C. paniculata* occupy derived positions within subg. *Vignea*, while *C. fecunda* is placed with multispicate species in the outgroup.

DISCUSSION

Sequence Statistics. Sequence statistics for subg. *Vignea* are within the range reported by Starr et al. (1999, 2003, 2004) and are similar to those found in

TABLE 2. Shimodaira-Hasegawa (SH) test comparing optimal trees obtained from searches using maximum likelihood (1, GTR + G + *I*) and parsimony (2-7; trees chosen at random), and searches where selected morphological groups in *Carex* subg. *Vignea* were forced to be monophyletic using a constraint tree (8, distigmatic species; 9, gynaecandrous species). Tests are one-tailed and conducted assuming a GTR + G + *I* model of sequence evolution. Asterisk next to *P*-values indicates significance at the $\alpha = 0.05$ level.

Tree	-ln *L*	-ln *L* Difference	Steps	SH-test *P*-value
1 (GTR)	15640.68771	(best)	2595	—
2 (Par)	15681.90279	41.21508	2570	0.408948
3 (Par)	15671.31597	30.62825	2570	0.525415
4 (Par)	15676.63730	35.94958	2570	0.467321
5 (Par)	15677.12520	36.43748	2570	0.461088
6 (Par)	15708.67918	67.99146	2570	0.184261
7 (Par)	15675.87098	35.183260	2570	0.472719
8 (Distigmatic)	15670.76429	30.07658	2596	0.522147
9 (Gynaec)	16004.56191	363.87419	2685	0.000000*

much broader samplings that include species outside of the Cariceae. In this study, divergence values between some vignean and non-vignean taxa were lower than between pairwise comparisons that included subg. *Vignea* species only. This indicates that diversification within the three clades in the Cariceae has been substantial or that these clades have experienced different rates of molecular evolution.

Attempts to sequence the ITS region in *C. capitata* and *C. kobomugi* without the use of betaine resulted in the amplification of paralogs. Many authors have commented on problems associated with nrDNA polymorphism, paralogy, and pseudogenes (e.g., Álvarez and Wendel 2003; Bailey et al. 2003). Although the detection of nrDNA pseudogenes can be determined through fluorescent in situ hybridization (FISH) and genomic in situ hybridization (GISH) studies, these approaches are impractical given the context of most phylogenetic research (Álvarez and Wendel 2003). Our experience indicates that paralogous sequences can be detected through a careful review of raw sequence data and through alignment of suspect sequences with those of closely related species. This approach typically reveals a pattern of high sequence divergence due almost entirely to transitions from guanine to adenine and cytosine to thymine (= lower G+C content). Also, paralogous sequences typically possess mutations in the highly conserved 5.8S region (Buckler et al. 1997; pers. obs.). Given that Cariceae nrDNA sequences have some of the highest G+C content known in flowering plants (up to 74% G+C; Starr et al. 2003, 2004) and the fact that we detected two paralogous sequences in reactions lacking betaine, we highly recommend the use of either betaine or any other highly denaturing cosolvent (e.g., DMSO) in future studies involving Cariceae nrDNA. Difficulties with the nrDNA region highlight the need to develop new, low-copy nuclear sequences for phylogenetic research in the Cariceae (see Small et al. 2004).

Circumscription of Subg.* Vignea *and Placement of Taxa of Uncertain Subgeneric Affinity. Despite the controversy surrounding the circumscription of most traditionally recognized *Carex* subgenera, most authors regard subg. *Vignea* as monophyletic. This conclusion is reached regardless of the data (morphology—Kükenthal 1909; Smith and Faulkner 1976; Reznicek 1990; Egorova 1999; Ball and Reznicek 2002; smut fungi—Savile and Calder 1953; Nannfeldt 1977; or molecules—Yen and Olmstead 2000; Roalson et al. 2001; Starr et al. 2004).

While subg. *Vignea* is regarded as monophyletic, subgeneric limits are blurred by a few species that possess a combination of subgeneric traits. These transitional species fall into two groups: distigmatic unispicate species and distigmatic multispicate species centered on *C. fecunda* (sect. *Fecundae*). The results of this study, plus the findings of Starr et al. (2004), allow for a refined circumscription of subg. *Vignea* and well supported phylogenetic placement of these problematic taxa. In unispicate species the following pattern is evident: distigmatic gynaecandrous (e.g., *C. exilis*) or dioecious (e.g., *C. gynocrates*, *C. dioica*) species are members of subg. *Vignea*. Distigmatic androgynous species (e.g., *C. capitata*, *C. nardina*) are part of the reduced clade and are sister to tristigmatic androgynous unispicate species such as *C. nigricans*, *C. pyrenaica*, *C. monostachya*, and *C. pauciflora*. *Carex curvula*, sometimes regarded as a tristigmatic member of subg. *Vignea* (e.g., Chater 1980), and *C. cordillerana*, traditionally placed in subg. *Carex*, are also part of this clade (Starr et al. 2004).

Starr et al. (2004) have hypothesized that unispicate androgynous vs. gynaecandrous/dioecious inflorescence types are the result of different evolutionary processes. The unispicate dioecious and gynaecandrous condition in subg. *Vignea* may have arisen through a reduction in spike number and, in the case of dioecious individuals, possible sex changes within a spike (= digressive reduction sensu Kreczetovicz 1936). Androgynous unispicate inflorescences, on the other hand, may have evolved from the reduction of entire spikes to single spikelets (= individual flowers, =

transmutive reduction sensu Kreczetovicz 1936). Alternatively, the carpellate flowers may represent a reduction of lateral carpellate spikes whereas the terminal staminate portion represents a single, terminal staminate spike (= pseudomonostachyous sensu Kreczetovicz). This latter scenario is supported by recent interpretations regarding carpellate flowers as reduced spikelets but staminate flowers as single flowers (Egorova 1999; Ball and Reznicek 2002). Regardless of the mechanisms, the unispicate androgynous vs. unispicate gynaecandrous/dioecious condition is likely restricted to distinct lineages that diverged relatively early in the evolution of *Carex* (Starr et al. 2004).

Carex fecunda has one of the most highly branched (= compound) inflorescences in *Carex* (Reznicek 1990). While most authors place this taxon in subg. *Carex* (e.g., Kükenthal 1909; Barros 1935, 1947), Reznicek (1990) presents evidence that this species possesses features that are intermediate between subg. *Carex* and subg. *Vignea*. Furthermore, this and other species in sect. *Fecundae* are hypothesized to exemplify the ancestral inflorescence type in the genus (Reznicek 1990). Like members of subg. *Vignea*, *C. fecunda* is distigmatic, possesses small, androgynous tertiary spikes, and lacks cladoprophylls. However, the primary and secondary branches are often peduncled, and distal portions of the inflorescence are often characterized by single ranks of perigynia or staminate flowers—features more characteristic of subg. *Carex*. Our results show that *C. fecunda* is not transitional between subg. *Vignea* and *Carex* but is part of the outgroup composed of species from the compound clade. Furthermore, the nested position of this species indicates that it does not exemplify the primitive condition in *Carex* and that characters such as absence of prophylls, compound inflorescences, and two stigmas have been secondarily derived. Other members of sect. *Fecundae* with highly compound inflorescences such as *C. davıd-smithii* Reznicek and *C. catamarcensis* Kük. have been hypothesized as members of subg. *Vignea* (Smith and Reznicek 1992). Like *C. fecunda*, these species have uniform, small, androgynous tertiary spikes and lack cladoprophylls. However, the primary and secondary branches possess the short, stiff peduncles typical of subg. *Vignea*. The inclusion of these poorly known Andean species is critical in future phylogenetic studies of the genus.

***Evolutionary Significance of* C. gibba *and the Phylogenetic Position of Subg.* Vignea.** *Carex gibba* is the sole member of sect. *Gibbae*, a problematic taxon. Like many species in subg. *Vignea*, *C. gibba* has sessile gynaecandrous spikes. On the other hand, this species possesses cladoprophylls (Song-Wang 1994), three stigmas, and large foliaceous (vs. setaceous) bracts, features characteristic of subg. *Carex* and *Vigneastra*. The placement of *C. gibba* as sister to subg. *Vignea* supports two stigmas, a lack of cladoprophylls, and setaceous bracts as apomorphic in subg. *Vignea*. Three stigmas, prophylls, and leafy bracts are likely plesiomorphic for subg. *Vignea* and possibly for *Carex*.

The evolutionary significance of *C. gibba* is difficult to determine on the basis of this and other phylogenetic studies (Yen and Olmstead 2000; Roalson et al. 2001; Starr et al. 2004; Waterway and Starr in press). For example, scenarios placing subg. *Vignea* at the base of the Cariceae (Roalson et al. 2001) raise the possibility that *C. gibba* could represent an ancestral condition for the tribe. Other hypotheses place subg. *Vignea* in more derived positions, either as sister to the compound clade (Yen and Olmstead 2000) or in a transitory position between the reduced and the compound clade. Strategic taxon sampling and the use of multiple molecular markers may provide a greater understanding of the relationship between the three major clades in the Cariceae and a clearer understanding of the phylogenetic position of *C. gibba*.

Morphological Character Evolution. Characters such as floral arrangement, stigma number, and prophyll presence have figured prominently in the systematics of *Carex*. Gynaecandrous vs. androgynous spikes has often been used to divide subg. *Vignea* into two broad groups. Authors such as Yelton and Naczi (2001) have considered gynaecandrous species to be a probable clade, and Egorova (1999) proposed that gynaecandry was derived within subg. *Vignea*. Our results show that gynaecandry has evolved multiple times during the evolution of this subgenus.

Only three species in subg. *Vignea* are tristigmatic: *C. gibba*, *C. macrocephala*, and *C. kobomugi*. While the SH test does not reject the possibility of a monophyletic distigmatic clade, both parsimony and maximum likelihood trees show the retention of three stigmas in *C. gibba* with an independent evolution of this condition in *C. kobomugi* and *C. macrocephala*, the sole members of sect. *Macrocephalae*. Section *Macrocephalae* represents a distinct evolutionary offshoot of subg. *Vignea* characterized by being paradioecious (genets are monoecious but individual ramets are unisexual—Standley 1984) and tristigmatic.

As with stigma number, prophylls also appear twice during the evolution of subg. *Vignea*: in *C. gibba* (see above) and in the ancestor that gave rise to *C. arenaria*, *C. praecox*, and *C. brizoides* (sect. *Ammoglochin*). The latter three species are placed within subsect. *Herporrhizae* (O. Lang) Kük. in Engl. by Egorova (1999). Subsection *Herporrhizae* contains nine species and is the only taxon in subg. *Vignea*, other than *C. gibba*, with prophylls. Subsection *Herporrhizae* is distantly related to *C. siccata* (sect. *Ammoglochin* subsect. *Siccatae* J. Carey sensu Egorova 1999), a species lacking prophylls.

The anatomy and homology of prophylls with other inflorescence structures has been discussed at length

by numerous authors (e.g., Kukkonen 1986; Reznicek 1990; Timonen 1993). Reznicek (1990) has hypothesized that two types of prophylls occur in *Carex*: cladoprophylls, tubular structures that are thought to be homologous with the scales subtending the perigynia, and inflorescence prophylls, structures that are thought to be homologous with perigynia. Based on an examination of herbarium specimens two distinct prophyll types occur in subg. *Vignea*. In *C. gibba*, the prophylls are tubular and occasionally possess an abortive pistil in their axils (Ford and Naczi unpubl. data; Song-Wang 1994). The prophylls of *C. arenaria*, *C. praecox*, and *C. brizoides* have free margins, no vestiges of reproductive structures, and are similar to the pistillate scales (Ford and Naczi unpubl. data; Egorova 1999). Our observations are consistent with tree topologies indicating that these structures may not be homologous.

Are Highly Compound Inflorescences Ancestral? One assumption about character evolution in *Carex* is that the ancestral inflorescence type is the highly compound inflorescence found in subg. *Vigneastra* or in certain species in subg. *Vignea* (e.g., Smith and Faulkner 1976; Reznicek 1990). While the tree topologies of Starr et al. (2004) indicate that the ancestor of extant Cariceae may have possessed a multispicate inflorescence, our results indicate that species with highly compound inflorescences (e.g. *C. crus-corvi*, *C. decomposita*, and *C. paniculata*) have evolved multiple times and are derived.

Intra- and Infra-Sectional Relationships. Most sections in subg. *Vignea* are likely polyphyletic (with the notable exception of the gynaecandrous sects. *Ovales*, *Stellulate*, and *Glareosae*). Convergences and parallelisms are responsible for creating many of the species assemblages that we currently recognize as sections. This breakdown of sectional structure is supported by many molecular phylogenetic studies of *Carex* (Yen and Olmstead 2000; Roalson 2001; Hendrichs et al. 2004a, 2004b; Waterway and Starr in press; but see Starr et al. 1999), and in particular subg. *Vignea* (Hendrichs et al. 2004b; Waterway and Starr in press). In some instances these re-arrangements are supported morphologically. For example, the placement of *C. vernacula* (sect. *Foetidae*) in a clade with *C. jonesii* and *C. neurophora* (sect. *Vulpinae*) is consistent with the morphology (condensed globose inflorescences, flat leaves) and phytogeography of these species (restricted to western North America). However, in other cases the implied relationships are difficult to correlate with morphology. While we have not yet found morphological, ecological, or phytogeographical patterns associated with many of the clades we have identified, the phylogenetic pattern mirrors the morphological variation found within and between the sections themselves. For example, species-level taxonomic problems abound within the gynaecandrous sects. *Ovales*, *Stellulate*, and *Glareosae* due to the narrow differences between species yet these sections are relatively well defined morphologically (Reznicek and Ball 1980; Reznicek 1993; Mastrogiuseppe et al. 2002). These sections were found to be monophyletic in our study. In contrast, many androgynous sections (e.g., *Phaestoglochin*, *Foetidae*, *Multiflorae*, *Divisae*, *Vulpinae*, and *Heleoglochin*) are morphologically diverse and are not clearly distinguishable from one another (see Cochrane 2002; Standley 2002a, 2002b). This high level of morphological variation, coupled with an incomplete understanding of sectional limits, may account for the disparate placement of these taxa in our analysis.

Species complexes within many androgynous sections are monophyletic. For example, within sect. *Phaestoglochin* smaller species groupings are evident. The *C. mesochorea/leavenworthii/cephalophora* complex with its highly congested inflorescences and tight sheaths, lacking prominent cross veins, is in a different clade from *C. aggregata/sparganioides* with more elongate inflorescences and loose leaf sheaths with prominent cross veins. The eastern North American *C. rosea* complex, defined by widely spreading perigynia with conspicuously spongy thickened bases, is also monophyletic. A western North American assemblage of *C. hoodii/hookerana/occidentalis/tumulicola* is also evident.

The placement of some species of controversial sectional assignment (e.g., *C. laeviculmis*, *C. disperma*, *C. arcta*, *C. illota*) at the base of larger clades was frequently observed in our analyses. This placement suggests these taxa may be close to the ancestors that gave rise to some of the larger clades within subg. *Vignea*. Conflicting sectional taxonomies may be the result of emphasis on retained plesiomorphic characters in earlier classifications.

Our study forms the basis for reinterpretation of phylogenetic relationships within subg. *Vignea*. Additional chloroplast and nuclear markers as well as anatomical and micro- and macromorphological data would add to our understanding of this subgenus.

ACKNOWLEDGEMENTS. Funding for this project was provided by a Natural Sciences and Engineering Research Grant (NSERC) to BAF. A special thanks to Jim Solomon (MO) and A. A. Reznicek (MICH) for their hospitality and assistance during herbarium visits. Karen Wilson provided copies of floristic literature on Australian and New Zealand *Carex*. Thanks to Michele Piercey-Normore for advice on data analysis and Rod Lastra for assistance in preparing Figs. 1 and 2.

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APPENDIX 1

Voucher data for specimens used in molecular phylogenetic analyses of *Carex* subg. *Vignea*. Specimens are arranged in alphabetical order by section. GenBank numbers are in the order ITS, ETS 1f. Herbarium abbreviations follow Index Herbariorum (www.nybg.org/bsci/ih/ih.html).

Sect. *Ammoglochin* Dumort., ***Carex arenaria*** L., (1) UNITED KINGDOM: Scotland, Lunan Bay Sand Dunes, *Starr 98020 & Scott* (FHO, WIN) DQ115100, DQ115101; (2) U. S. A.: Delaware, Sussex Co., ca. 3 mi N of town of Fenwick Island, *Naczi 9460 & McAvoy* (DOV, WIN) DQ115102, DQ115103; ***Carex brizoides*** L., GERMANY: Bayern, ca. 55 km E of Nürnberg, ca. 3 km SW of Kirchenthumbach, *Spellenberg 11575 & Mahrt* (MICH) DQ115108, DQ115109; ***Carex praecox*** Schreb., RUSSIA: Kalmykia, ca. 15 km SW of Elista, *Skvortsov s.n. & Kostina* (MO) DQ115248, DQ115249; ***Carex siccata*** Dewey, (1) CANADA: Manitoba, ca. 1.5 km N of town of Falcon Lake, *Naczi 9862 & Ford* (DOV) DQ115274, DQ115275; (2) CANADA: Manitoba, Manitoba Model Forest, *Fuller 96-27, Kembel & Olson* (WIN) DQ115276, DQ115277.

Sect. *Bracteosae* Pax, ***Carex bonariensis*** Desf., BOLIVIA: Chapare, Cochabamba, Rio Corani Mayu, *Ritter 3372 & Wood* (MICH) DQ115106, DQ115107; ***Carex sororia*** Kunth, PARAGUAY: Cordillera, 17 km W of Arroyos y Esteros, *Zardini 22153 & Velázquez* (MICH) DQ115280, DQ115281.

Sect. *Capituligerae* Kük., ***Carex capitata*** L., CANADA: Manitoba, Twin Lakes, ca. 20 km E of Churchill, *Ford 02379 et al.* (WIN) DQ115118, DQ115119.

Sect. *Chordorrhizae* (Heuff.) Meinsh., ***Carex chordorrhiza*** Ehrh. ex L. f., (1) U. S. A.: Michigan, Montmorency Co., ca. 3 mi NE of Vienna, *Naczi 9454 et al.* (DOV) DQ115124, DQ115125; (2) CANADA: Manitoba, Thompson area, *Steinaur s.n.* (WIN) DQ115126, DQ115127.

Sect. *Curvulae* Tuck., ***Carex curvula*** All., FRANCE: Col du Galibier, *Playford 9803 et al.,* (FHO) AY242030, AY242031.

Sect. *Cyperoideae* Don in Loudon, ***Carex sychnocephala*** J. Carey, CANADA: Manitoba, Moose Lake, *Keleher 658* (WIN) DQ115292, DQ115293.

Sect. *Deweyanae* (Tuck. ex Mack.) Mack. in Britton et al., ***Carex bromoides*** Schkuhr ex Willd. ssp. ***bromoides,*** (1) U. S. A.: Alabama, Conecuh Co., ca. 5 mi. S of Evergreen, *Ford 0076 & Naczi* (WIN) DQ115110, DQ115111; (2) U. S. A.: Maryland, Cecil Co., 2.5 mi SSE of Conowingo, *Naczi 8064* (DOV, WIN) DQ115112, DQ115113; ***Carex deweyana*** Schwein. var. ***deweyana,*** CANADA: Manitoba, Whiteshell Provincial Park, ca. 6 km E of Falcon Lake town site, *Ford 0124 et al.* (WIN) DQ115142, DQ115143; ***Carex laeviculmis*** Meinsh., U.S.A.: California, Del Norte Co., ca. 12 mi NE of Gasquet, *Naczi 3263* (DOV) DQ115196, DQ115197; ***Carex leptopoda*** Mack. in Rydb., U. S. A.: Oregon, Lane Co., ca. 21 mi SE of Eugene, Willamette National Forest, *Yelton 31* (DOV, WIN) DQ115204, DQ115205; ***Carex senanensis*** Ohwi, JAPAN: Gifu, Ono-Gun, Asahi-mura, *Nagase 91535* (KYO) DQ115268, DQ115269.

Sect. *Dispermae* Ohwi, ***Carex disperma*** Dewey, (1) CANADA: Manitoba, Manitoba Model Forest, *Fuller & Olson 96-297* (WIN) DQ115148, DQ115149; (2) CANADA: Manitoba, Whiteshell Pro-

vincial Park, ca. 6 km E of Falcon Lake town site, *Ford 0116 et al.* (WIN) DQ115150, DQ115151.

Sect. *Divisae* H. Christ ex Kük. in Engl., ***Carex divisa*** Huds., U. S. A.: Maryland, St. Mary's Co., St. Inigoes, *Davis 2509* (MICH) DQ115154, DQ115155; (1) (2) ***Carex douglasii*** Boott in Hook., U. S. A.: Colorado, Park Co., Pike National Forest, Kenosha Pass, *Ford 99252 et al.* (WIN) DQ115156, DQ115157, DQ115158, DQ115159; ***Carex duriuscula*** C. A. Mey., (1) CANADA: Manitoba, Spruce Woods Provincial Park, Isputinaw Self-guiding Trail, *Ford 0125 et al.* (WIN) DQ115164, DQ115165; (2) CANADA: Manitoba, ca. 10 km N of Glenboro, Spruce Woods Provincial Park, *Naczi 9926 & Ford* (DOV) DQ115162, DQ115163; ***Carex pansa*** L.H. Bailey, U. S. A.: Oregon, Coos Co., North Spit, Coos Bay, *Zika 13144 & Wilson* (MICH) DQ115238, DQ115239; ***Carex praegracilis*** W. Boott, U. S. A.: Michigan, Washtenaw Co., Ann Arbor, *Naczi 8276* (DOV) DQ115250, DQ115251.

Sect. *Dornera* Heuff., ***Carex nigricans*** C. A. Meyer, CANADA: British Columbia, summit of Mount Revelstoke, *Ford 9720* (WIN) AY242042, AY242043; ***Carex pyrenaica*** Wahlenb., NEW ZEALAND: Fiordland, Southland Land District, *Ford 104/98* (FHO) AY244528, AY244529.

Sect. *Elongatae* (Kunth) Kük. in Engl., ***Carex elongata*** L., FINLAND: Tammela, Alva, *Alho & Laine s.n.* (WIN) DQ115166, DQ115167.

Sect. *Fecundae* Kük. in Engl., ***Carex fecunda*** Steud., BOLIVIA: La Paz, Inquisivi, 1–4 km SW of Quime, *Lewis 38074* (MICH) DQ115170, DQ115171.

Sect. *Foetidae* (Tuck. ex L.H. Bailey) Kük. in Engl., ***Carex incurviformis*** Mack. in Rydb., U. S. A.: Colorado, Park Co., Pike National Forest, Horseshoe Cirque area, *Tallent 517* (MICH) DQ115186, DQ115187; ***Carex maritima*** Gunnerus, CANADA: Manitoba, Wapusk National Park, North Bank of Owl River, *Punter 03-711 & Normore* (WIN) DQ115214, DQ115215; ***Carex vernacula*** L.H. Bailey, U. S. A.: California, Mono Co., Toiyabe National Forest, S of Sonora Pass, *Tallent 854* (MICH) DQ115306, DQ115307.

Sect. *Gibbae* Kük. in Engl., ***Carex gibba*** Wahlenb., CHINA: Hunan, Li Ling, Da Lin County, *Liu 6741* (MO) DQ115174, DQ115175.

Sect. *Glareosae* Don in Loudon, ***Carex arcta*** Boott, U. S. A.: Oregon, Douglas Co. Old Fairview, *Kuykendall, Newhouse & Wilson 10070* (MICH) DQ115098, DQ115099; ***Carex brunnescens*** (Pers.) Poir. in Lam. et al., CANADA: Manitoba, Whiteshell Provincial Park, ca. 6 km E of Falcon Lake town site, *Ford 0118 et al.* (WIN) DQ115114, DQ115115; ***Carex canescens*** L., CANADA: Ontario, N part of Lake of the Woods, mouth of Wiley Bay, *Ford 98178 et al.* (WIN) DQ115116, DQ115117; ***Carex loliacea*** L., FINLAND: Paltamo, *Kause & Seikkula s.n.* (WIN) DQ115206, DQ115207; ***Carex mackenziei*** V. I. Krecz. in Kom. et al., CANADA: Manitoba, Churchill area, *Zbiegniewicz 83-253* (WIN) DQ115208, DQ115209; ***Carex praeceptorum*** Mack. in Britton et al., U. S. A.: Oregon, Grant Co., High Lake, Strawberry Mountain Wilderness, Malheur National Forest, *Zika 12598* (MICH) DQ115246, DQ115247; ***Carex trisperma*** Dewey, U. S. A.: Pennsylvania, Bradford Co., 3 mi S of Leroy, *Naczi 8220* (DOV) DQ115298, DQ115299.

Sect. *Heleoglochin* Dumort., ***Carex appressa*** R. Br., NEW ZEALAND: Canterbury Dist., Coutts Island, *Punter & Fineran s.n.* (WIN) DQ115094, DQ115095; ***Carex appropinquata*** Schumach., ESTONIA: Hapsalu, W of Puhtu ornithology field station, *Klackenberg & Eriksson Nr 7* (MO) DQ115096, DQ115097; ***Carex cusickii*** Mack. ex Piper & Beattie, U. S. A.: Oregon, Clatsop Co., SW of Warrenton, *Sundberg & Word 3805* (MICH) DQ115138, DQ115139; ***Carex decomposita*** Muhl., U. S. A.: Delaware, New Castle Co., ca. 3 mi S of Middletown, *Naczi 9313 et al.* (DOV); DQ115140, DQ115141; ***Carex diandra*** Schrank, CANADA: Manitoba, Manitoba North Central Project site 36, *E. Punter s.n. & D. Punter* (WIN) DQ115144, DQ115145; ***Carex paniculata*** L., (1) UNITED KINGDOM: England, Oxfordshire, *Meagher s.n.* (MICH) DQ115234, DQ115235; (2) SPAIN: Almeria, Rio de Ohanes, *Pallares s.n.* (DOV) DQ115236, DQ115237; ***Carex prairea*** Dewey in Alph. Wood, U. S. A.: Ohio, Miami Co., 6.5 mi E of Tipp City, Silver Lake, *Naczi 8264 & Yelton* (WIN) DQ115252, DQ115253.

Sect. *Holarrhenae* (Döll) Pax in Engl. & Prantl, ***Carex disticha*** Huds., CANADA: Ontario, Simcoe Co., E side of Collingwood on S side of Hwy 26, *Catling 7061 & Brownell* (MICH) DQ115152, DQ115153; ***Carex sartwellii*** Dewey, CANADA: Manitoba, Gilbert Plains, *Parker 85-345* (WIN) DQ115266, DQ115267.

Sect. *Inversae* Kük. in Engl., ***Carex inversa*** R. Br., AUSTRALIA: Queensland, Moreton Dist., N of Ipswich near Pine Mountain, *Blake 20085* (MO) DQ115190, DQ115191.

Sect. *Leucoglochin* Dumort., ***Carex pauciflora*** Lightf., FRANCE: Col du Luitel, *Playford 9806 et al.* (FHO) AY242040, AY242041.

Sect. *Longespicatae* Kük. in Engl., ***Carex monostachya*** A. Rich., KENYA: *Muasya 1052* (K) AY241977, AY241978.

Sect. *Macrocephalae* Kük. in Engl., ***Carex kobomugi*** Ohwi, U. S. A.: Delaware, Sussex Co., ca. 2.5 mi S of Dewey Beach, *Naczi 9459 & McAvoy* (DOV, WIN) DQ115194, DQ115195; ***Carex macrocephala*** Willd. ex Spreng., CANADA: British Columbia, Tsawwassen, Boundary Bay Regional Park, *Ford 9715* (WIN) DQ115210, DQ115211.

Sect. *Multiflorae* (J. Carey) Kük. in Engl., ***Carex annectens*** (E. P. Bicknell) E. P. Bicknell, U. S. A.: Delaware, Kent Co., ca. 5 mi ENE of Smyrna, *Naczi 8119* (DOV) DQ115092, DQ115093; ***Carex chihuahuensis*** Mack., MEXICO: Sonora, Mpio. Yécora, 4.8 km SW of Puerto de la Cruz, *Steinmann 881 et al.* (MICH) DQ115122, DQ115123; ***Carex fissa*** Mack. in Britton var. ***aristata*** F. J. Herm., U. S. A.: Florida, Suwannee Co., ca. 4 mi W of White Springs, *Abbott 14202* (DOV) DQ115172, DQ115173; ***Carex marianensis*** Stacey, MEXICO: Durango, 10 km SW of El Salto, 1 km NE of Lecheria, *González & Reznicek 10314, Pinedo* (MICH) DQ115212, DQ115213; ***Carex triangularis*** Boeck., U. S. A.: Arkansas, Perry Co., Arkansas Hwy. 7 and Forest Service Rd. 83, *Hyatt 8047* (WIN) DQ115296, DQ115297; ***Carex vulpinoidea*** Michx., U. S. A.: Kentucky, Monroe Co., SE of Tomkinsville, along the W side of route 216, along McFarland Creek, *Ford 9872 & Naczi* (WIN) DQ115308, DQ115309.

Sect. *Nardinae* (Tuck.) Mack. in Britton et al., ***Carex nardina*** Fr., (1) CANADA: Manitoba, Wapusk National Park, *Ford 02230 et al.* (WIN) DQ115220, DQ115221; (2) U. S. A.: Wyoming, Big Horn Co., *Starr et al. WY96134* (FHO) AY241973, AY241974.

Sect. *Ovales* Kunth, ***Carex albolutescens*** Schwein., U. S. A.: Kentucky, Clinton Co., NW of Albany, along W side of route 639, *Ford 9849 & Naczi* (WIN) DQ115088, DQ115089; ***Carex cristatella*** Britton in Britton & A. Brown, U. S. A.: Michigan, Monroe Co., ca. 3 mi ENE of Petersburg, *Naczi 8277* (DOV) DQ115134, DQ115135; ***Carex hormathodes*** Fernald, U. S. A.: Delaware, Kent Co., ca. 5 mi ENE of Smyrna, *Naczi 8118* (DOV) DQ115182, DQ115183; ***Carex illota*** L. H. Bailey, U. S. A.: Colorado, Eagle Co., ca. 3 air mi SW of Gold Park, *Castaner 9481* (WIN) DQ115184, DQ115185; ***Carex pachystachya*** Cham. ex Steud., CANADA: British Columbia, summit of Mount Revelstoke, *Ford 9718* (WIN) DQ115232, DQ115233.

Sect. *Phaestoglochin* Dumort., ***Carex aggregata*** Mack., U. S. A.: Kentucky, Monroe Co., SE of Tomkinsville, along the W side of route 216, along McFarland Creek, 23 May 1998, *Ford 9874 & Naczi* (WIN) DQ115084, DQ115085; ***Carex cephalophora*** Muhl. ex Willd., U. S. A.: Kentucky, Clinton Co., NW of Albany, along E side of route 639, *Ford 9856 & Naczi* (WIN) DQ115120, DQ115121; ***Carex hoodii*** Boott in Hook., CANADA: Alberta, Castle Special Management Area of the Rocky Mountain Forest Reserve, N side of the Carbondale River, *Ford 00120 & Saarela* (WIN) DQ115178, DQ115179; ***Carex hookerana*** Dewey, CANADA: Manitoba, Souris Wildlife Management Area, W side of Hwy. 346 on N side of the Souris River, *Ford 0383 & Naczi* (WIN) DQ115180, DQ115181; ***Carex leavenworthii*** Dewey, U. S. A.: Kentucky, Monroe Co., SE of Tomkinsville, along the W side of route 216, along McFarland Creek, *Ford 9873 & Naczi* (WIN) DQ115202, DQ115203; ***Carex mesochorea*** Mack., U. S. A.: Kentucky, Campbell Co., Highland Heights, *Naczi 7835* (DOV, WIN) DQ115216, DQ115217; ***Carex***

muhlenbergii Schkuhr ex Willd., U. S. A.: Kentucky, Monroe Co., SE of Tomkinsville, along the W side of route 216, along McFarland Creek, *Ford 9893 & Naczi* (WIN) DQ115218, DQ115219; ***Carex occidentalis*** L. H. Bailey, U. S. A.: Colorado, Ouray Co., Uncompahgre National Forest, Hwy. 530, ca. 2 miles S of Ouray in the vicinity of Bear Creek Trailhead, *Ford 99279 et al.* (WIN) DQ115228, DQ115229; ***Carex radiata*** (Wahlenb.) Small, U. S. A.: Pennsylvania, Lackawanna Co., 4 mi E of Fleetville, *Naczi 8160* (DOV) DQ115254, DQ115255; ***Carex rosea*** Schkuhr ex Willd., U. S. A.: Kentucky, Clinton Co., NW of Albany, along E side of route 639, *Ford 9855 & Naczi* (WIN) DQ115262, DQ115263; ***Carex socialis*** Mohlenbr. & Schwegman, U.S.A.: Alabama, Jackson Co., 2.5 mi W of Fackler, *Naczi 9014* (DOV) DQ115278, DQ115279; ***Carex sparganioides*** Muhl. ex Willd., U. S. A.: Maryland, Cecil Co., 1 mi SE of Conowingo, *Naczi 8055* (DOV) DQ115282, DQ115283; ***Carex spicata*** Huds., U. S. A.: Delaware, Newcastle Co., Brandywine Creek State Park, ca. 2 mi NW of Talleyville, *Ford 0221 & Naczi* (WIN) DQ115284, DQ115285; ***Carex texensis*** (Torrey ex L. H. Bailey) L. H. Bailey, (1) U. S. A.: Tennessee, Franklin Co., S of Huntland, along E side of route 97, *Ford 98109 & Naczi* (WIN) DQ115258, DQ115259; (2) U. S. A.: Kentucky, Kenton Co., Fort Mitchell, *Naczi 7846* (DOV) DQ115294, DQ115295; ***Carex tumulicola*** Mack., U. S. A.: Oregon, Yamhill Co., Oak Ridge, *Wilson 5910* (MICH) DQ115300, DQ115301; ***Carex vallicola*** Dewey, (1) U. S. A.: Montana, Gallatin Co., 38 mi W West Yellowstone, *Morse 2245 & Jordon* (MICH) DQ115302, DQ115303; (2) U. S. A.: Utah, Washington Co., Santa Clara River, NW of Pine Valley, *Higgins 20294 et al.* (MO) DQ115304, DQ115305; ***Carex xalapensis*** Kunth, MEXICO: Hidalgo, Mpio. San Agustin Metzquititlan, Tuzanapa on road to Huayacocotla, *González & Reznicek 10745 et al.* (MICH) DQ115312, DQ115313.

Sect. *Phleoideae* Meinsh., ***Carex albata*** Boott ex Franch., Sav. JAPAN: Honshu, Toyama, Fukumitsu-cho, Nishi-tonami-gun, *Tsugaru 17287* (MO) DQ115086, DQ115087; ***Carex laevissima*** Nakai, RUSSIA: Primorskiy territory, suburb of Vladivostok, *Kharkevich s.n.* (MO) DQ115198, DQ115199; ***Carex neurocarpa*** Maxim., JAPAN: Honshuh, Hashimoto, Yawata-choh, Tsutsuki-gun, *Mitsuta 12747* (MICH) DQ115222, DQ115223.

Sect. *Phyllostachyae* Tuck. ex Kük. in Engl., ***Carex cordillerana*** Saarela & B. A. Ford, U. S. A.: Utah, Salt Lake Co., 12 mi SE of Salt Lake City, *Naczi 3433 & Thieret* (WIN) DQ115132, DQ115133.

Sect. *Physodeae* Meinsh., ***Carex physodes*** M. Bieb., TAJIKISTAN: Rhodzhor-Razjan Range, *Kochrareva 2579* (MO) DQ115240, DQ115241.

Sect. *Physoglochin* Dumort., ***Carex dioica*** L., UNITED KINGDOM: Scotland, Ben Lawers Visitors' Centre, *Starr 98015 & Scott* (FHO, WIN) DQ115146, DQ115147; ***Carex gynocrates*** Wormsk. ex Drejer, CANADA: Manitoba, Wapusk National Park, *Ford 02283 et al.* (WIN) DQ115176, DQ115177.

Sect. *Planatae* Akiyama, ***Carex planata*** Franch. & Sav., JAPAN: Honshu, Miyagi, Natori-shi, along the upper Masuda River, *Kurosawa 683 et al.* (MO) DQ115242, DQ115243.

Sect. *Potosinae* Mack., ***Carex postosina*** Hemsl., MEXICO: Coahuila, Mpio. Saltillo, Rancho Los Angeles, *González & Reznicek 10246, Pinedo* (MICH) DQ115244, DQ115245.

Sect. *Remotae* (Asch.) C. B. Clarke, ***Carex remota*** L., UNITED KINGDOM: England, Yorkshire Dales National Park, *Starr 98022 & Scott* (FHO, WIN) DQ115256, DQ115257; ***Carex rochebrunii*** Fr. & Sav., CHINA: Jiangxi, Jin Jiang (Nine River), Ma Wei Shui, *Lai & Shan 1468* (MO) DQ115260, DQ115261.

Sect. *Rupestres* (Tuck.) Meinsh. ***Carex rupestris*** All., FRANCE: Col du Galibier, *Playford 9801 et al.* (FHO) AY244521, AY244522.

Sect. *Stellulatae* Kunth, ***Carex atlantica*** L. H Bailey ssp. ***capillacea*** (L. H. Bailey) Reznicek, U. S. A.: Pennsylvania, Bradford Co., 5.5 mi SE of Canton, *Naczi 8212* (DOV) DQ115104, DQ115105; ***Carex echinata*** Murray, UNITED KINGDOM: Scotland, Sròn Dha Murchdi, *Starr 98009 & Scott* (FHO, WIN) DQ115160, DQ115161; ***Carex exilis*** Dewey, U. S. A.: Maine, Hancock Co., Corea Heath, ca. 1 mi NW of Corea, *Reznicek 9150* (MICH) DQ115168, DQ115169; ***Carex interior*** L. H. Bailey, CANADA: Manitoba, Whiteshell Provincial Park, ca. 6 km E of Falcon Lake town site, *Ford 0119 et al.* (WIN) DQ115188, DQ115189; ***Carex ruthii*** Mack. in Britton et al., U. S. A.: Georgia, Whitley Gap shelter, Appalachian Trail, *Noel & Zartman s.n.* (MICH) DQ115264, DQ115265; ***Carex seorsa*** Howe in Gordinier & Howe, U. S. A.: Kentucky, Clinton Co., NW of Albany, along W side of route 639, *Ford 9848 & Naczi* (WIN) DQ115270, DQ115271; ***Carex sterilis*** Willd., U. S. A.: Ohio, Miami Co., 6.5 mi E of Tipp City, Silver Lake, *Naczi 8260 & Yelton* (DOV, WIN) DQ115286, DQ115287; ***Carex wiegandii*** Mack. in Britton et al., U. S. A.: Maine: Hancock Co., Corea Heath, ca. 1 mi NW of Corea, *Reznicek 9152* (MICH) DQ115310, DQ115311.

Sect. *Vulpinae* (Heuff.) H. Christ, ***Carex alopecoidea*** Tuck., CANADA: Manitoba, NE of Hartney, *Keleher s.n.* (WIN) DQ115090, DQ115091; ***Carex conferta*** Hochst. ex A. Rich. var. ***lycurus*** (K. Schum.) K. A. Lye, ZIMBABWE: Vumba Mountains, Toozes swamp, *Browning 562* (MICH) DQ115128, DQ115129; ***Carex conjuncta*** Boott, U. S. A.: Kentucky, Campbell Co., Silver Grove, *Ford 98135 & Naczi* (WIN) DQ115130, DQ115131; ***Carex crus-corvi*** Shuttl. in Kunze, U. S. A.: Mississippi, Warren Co., 2.8 mi N Yazoo River Crossing of Hwy. 61, *Bryson 5877* (WIN) DQ115136, DQ115137; ***Carex jonesii*** L. H. Bailey, U. S. A.: Wyoming, Carbon Co., Sierra Madre Mountains, *Castaner 9413* (MO) DQ115192, DQ115193; ***Carex laevivaginata*** (Kük.) Mack. in Britton & A. Brown, U. S. A.: Ohio, Miami Co., 6.5 mi E of Tipp City, *Naczi 8252 & Yelton* (DOV) DQ115200, DQ115201; ***Carex neurophora*** Mack. in Abrams & R. S. Ferris, U. S. A.: Oregon, Baker Co., S side of Anthony Lake, Wallowa-Whitman National Forest, *Wilson 7476 et al.* (MICH) DQ115224, DQ115225; ***Carex oklahomensis*** Mack., U. S. A.: Delaware, Kent Co., 1.6 mi NW of Hartly, *Naczi 9373* (DOV) DQ115230, DQ115231; ***Carex otrubae*** Podp., UNITED KINGDOM: England, Oxfordshire, Oxford, *Starr 98023* (WIN) DQ115226, DQ115227; ***Carex stipata*** Muhl ex Willd. var. ***maxima*** Chapm. ex Boott, U. S. A.: Delaware, New Castle Co., ca. 0.5 mi NE of Granogue, *Naczi 8081* (DOV) DQ115288, DQ115289; ***Carex stipata*** Muhl. ex Willd. var. ***stipata,*** U. S. A.: Maryland, Cecil Co. Providence, *Naczi 8042* (DOV) DQ115290, DQ115291.

Outgroup

Sect. *Abditispicae* G. A. Wheeler, ***Carex collumanthus*** (Steyerm.) L. E. Mora, COLOMBIA: Arauca, Sierra Nevada del Cocuy, *Cleef 8875* (NY) AY241987, AY241988.

Sect. *Indicae* Tuck., ***Carex echinochloë*** Kunze, KENYA: *Muasya 1051* (K) AY241992, AY241993.

Sect. *Indicae* Tuck. ***Carex filicina*** Nees, TAIWAN: Yang Ming Shan National Park, Da Tun Shan, *Yen 0076* (WTU) AY241996, AY241997.

Sect. *Phacocystis* Dumort., ***Carex nigra*** (L.) Reichard, FRANCE: Col du Luitel, *Playford 9807 et al.* (FHO) AY241989, AY241990.

Sect. *Rhomboidales* Kük in Engl., ***Carex depauperata*** Curtis ex With., UNITED KINGDOM: England, Surrey, *Rich 01* (OXF) AY241984, AY241985.

Sect. *Shortianae* (L. H. Bailey) Mack., ***Carex shortiana*** Dewey, U. S. A.: Kentucky, Campbell Co., Silver Grove, *Ford 98134 & Naczi* (WIN) DQ115272, DQ115273.