Chapter 10

East vs. West: Monophyletic Clades Within the Paraphyletic *Carex acuta* Complex, Section *Phacocystis* (Cyperaceae)

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ABSTRACT The *Carex acuta* L. complex, as defined by Standley, comprises 12 species from section *Phacocystis* Dumort., one of the largest sections within the genus *Carex* L. The species of the complex were originally distinguished by their nerved, stipitate perigynia with torulose bases adnate to iridescent achenes. Internal transcribed spacer (ITS) and external transcribed spacer fragment (ETS 1f) sequence data were used to test the phylogenetic integrity of the *C. acuta* complex, examine infraspecific variation within two of its polymorphic species, *C. nigra* (L.) Reichard and *C. lenticularis* Michx., and identify biogeographic patterns indicated by the phylogeny. The *C. acuta* complex was found to be paraphyletic as circumscribed. The newly discerned monophyletic groups comprise four clades: (1) an Austral–East Asian group; (2) a Eurasian clade; (3) a *C. bigelowii* Torr. ex Schwein.–*C. scopulorum* T. Holm–*C. stricta* Lam. clade; and (4) a clade of North American species, plus *C. aquatilis* Wahlenb. from Finland. Within this latter clade, western and eastern North American taxa are further divided into monophyletic groups. The two western varieties of *C. lenticularis* examined were more closely related to two Mexican taxa and the circumboreal *C. aquatilis* than they were to the eastern variety of *C. lenticularis*, which formed a clade with the amphi-Atlantic *C. rufina* Drejer. In contrast, *C. nigra* was found to be monophyletic, with little sequence divergence among American and European accessions of *C. nigra* or between them and their European allies. High infraspecific variation was found within *C. eleusinoides* Turcz. ex Kunth and *C. stricta*. Poor species circumscription and hybridization were considered as possible causes of this pattern.

KEY WORDS Biogeography, *Carex acuta* complex, ETS, ITS, phylogeny, polymorphic species, reticulate evolution, section *Phacocystis*.

The genus *Carex* L. is one of the most widespread and ecologically important of all plant genera (Reznicek, 1990). The diversity represented by this genus of approximately 2000 species has challenged systematists for over a century. Convergence and homoplasy, likely a consequence of extreme floral reduction, have hampered phylogenetic analysis and have complicated taxonomic circumscription. Given the challenges presented by morphological characters in *Carex,* systematists have increasingly turned to molecular data for increased phylogenetic resolution. The few molecular analyses completed to date have shed new light on subgeneric, sectional, and species-level relationships within *Carex* (Starr et al., 1999; Yen & Olmstead, 2000; Roalson et al., 2001). This research addresses problems of species phylogeny and delimitation in a widespread and problematic group, the *C. acuta* L. complex of section *Phacocystis* Dumort.

The *Carex acuta* complex was distinguished by Standley (1987a, b) based on the shared characters of conspicuously nerved stipitate perigynia with swollen torulose bases adnate to whitish iridescent achenes and chromosome numbers ranging from n = 42 to 46, unique within section *Phacocystis*. Roalson et al. (2001), in their analysis of sections and subgenera of *Carex* using internal transcribed spacer (ITS) and chloroplast DNA (cpDNA) sequences, included nine taxa of *Phacocystis*, three of which were members of the *C. acuta* complex. Although they found section *Phacocystis* to be paraphyletic, they provided preliminary molecular evidence that the *C. acuta* complex was monophyletic.

One of the key barriers to developing strong phylogenetic hypotheses for carices is the problem of species circumscription. In the *Carex acuta* complex, two species are prominent in this regard: *C. lenticularis* Michx. and *C. nigra* (L.) Reichard. Both taxa are highly variable morphologically depending on their habitat, elevation, and latitude. Where morphs overlap, intermediate populations are often found. While no confirmed hybrids involving *C. lenticularis* have been identified, *C. nigra* has been shown to produce fertile hybrids with a number of species of section *Phacocystis*, including *C. acuta*, *C. elata* All., *C. bigelowii* Torr. ex Schwein., *C. stricta* Lam., and *C. aquatilis* Wahlenb. (Faulkner, 1972, 1973). Thus both phenotypic variation and hybridization are possible explanations for the taxonomic complexity of these species.

The goal of the research described here was to use cladistic analysis of the ITS region and an external transcribed spacer fragment (ETS 1f) of ribosomal DNA (rDNA) to elucidate the phylogeny of the species placed in the *Carex acuta* complex. Our specific objectives were to (1) test the monophyly of the complex, (2) assess the infraspecific variation within the highly polymorphic species *C. nigra* and *C. lenticularis*, and (3) interpret biogeographic history in the light of the retrieved phylogeny.

MATERIALS AND METHODS

SAMPLING

Forty-one accessions representing 23 species were included in the analysis. Voucher data and GenBank accession numbers for all accessions are provided in Table 1. All the members of Standley's *Carex acuta* complex were sampled, with the exception of *C. decidua* Boott from South America and *C. cuchumatanensis* Standl. & Steyerm. from Guatemala, for a total of 10 species.

Because the choice of outgroup taxa was problematic, we chose a diversity of taxa that might be expected to be sister groups to the *Carex acuta* complex. The outgroup taxa we sampled fall into three groups. First, we sampled five taxa from section *Phacocystis*. Two additional unnamed Mexican taxa that share morphological features with the members of section *Phacocystis* and the *C. acuta* complex were also included in this first group. Second, following the suggestion of Standley (1987a) that the taxa of section *Bicolores* Tuck. were sister to those of the *C. acuta* complex, *C. bicolor* All. and *C. aurea* Nutt. were included as outgroup taxa. Third, taxa of other sections that share morphological features with members of section *Phacocystis* were also sampled. These include (1) *C. lemanniana* Boott and *C. donnell-smithii* L. H. Bailey (Central America) of section *Fecundae* Kük., and (2) the North American *C. bella* L. H. Bailey of section *Racemosae* G. Don and the amphi-Pacific *C. podocarpa* R. Br. of section *Scitae* Kük. *Carex podocarpa* was especially important to include in this study because Roalson et al. (2001) found it to be inserted within section *Phacocystis*.

DNA EXTRACTION AND SEQUENCING

Total genomic DNA was extracted from fresh material using the DNEasy Plant Mini Kit (Qiagen, Valencia, California), and from silica-dried material with a CTAB buffer following the protocol of Doyle and Doyle (1987).

The polymerase chain reaction (PCR) was used to amplify ITS and 5.8S rDNA using a 1:1 ratio of primers ITS-4 (White et al., 1990) and AB101 (Sun et al., 1994) (Table 3). Each 25-µl reaction mixture contained 2.5 µl of 10× reaction buffer, 5 µl of 5× Q-solution (Qiagen, Valencia, California), 0.5 µl dNTPs (10 mM each), 1.25 µl of both primers (10 µM concentrations), 0.125 µl *Taq* (5 units/µl), 23.75 µl ddH_2O, and 10–50 ng of DNA template. Double-stranded PCR products were produced in an Amplitron II Thermolyne thermocycler (Barnstead, Dubuque, Iowa). The DNA was pretreated at 96°C for two minutes followed by 32 cycles of DNA denaturation at 96°C for one minute, primer annealing at 50°C for one minute, and DNA strand extension by *Taq* DNA polymerase at 72°C for two minutes. The PCR was terminated by a final extension of 72°C for seven minutes.

PCR was also used to amplify the 3′ end of the ETS-1f using a 1:1 ratio of primers ETS-1F and 18S-R (Starr et al., 2003; see Table 3). Each 25-µl reaction mixture contained 1.25 µl of 100% glycerol, 11.5 µl of 100 mM KCl, 2.3 µl of 100 mM Tris HCl, 2.3 µl of 25 mM $MgCl_2$, 3.8 µl of 5× Q-solution (Qiagen, Valencia, California), 0.575 µl dNTPs (10 mM each), 0.575 µl of both primers (20 µM concentrations), 0.125 µl *Taq* (5 units/µl), and 10–50 ng of DNA template. Double-stranded PCR products were produced on an Amplitron II Thermolyne thermocycler (Barnstead, Dubuque, Iowa). The DNA was pretreated at 97°C for one minute followed by 40 cycles of DNA denaturation at 97°C for 10 seconds, primer annealing at 55°C for 30 seconds, and DNA strand extension by *Taq* DNA polymerase at 72°C for 20 seconds plus four additional seconds per cycle. The PCR was terminated by a final extension of 72°C for eight minutes.

The PCR products were electrophoresed on 1% agarose gels in 1× TBE buffer (pH 8.0). The gels were then stained with ethidium bromide and the DNA excised from the gels. A Qiaquick PCR Purification Kit (Qiagen, Valencia, California) was used to elute the DNA from the gels. Dye-terminator reaction mixtures of 20 µl were made with the purified DNA templates. The reactions were pretreated in the thermocycler for five minutes at 96°C. Cycle sequencing of the reactions comprised 25 cycles of DNA denaturation at 96°C for 10 seconds, primer annealing at 50°C for five seconds, and DNA strand extension by *Taq* DNA polymerase at 60°C for four minutes. Cycle sequencing of ITS 1 used the external sequencing primers ITS-L and internal sequencing primer ITS-2, while cycle sequencing of ITS 2 used external sequencing primer ITS-4 and ITS-3 (Table 3) (White et al., 1990; Hsiao et al., 1994). Cycle sequencing of ETS 1f used the same primers used in PCR. Automated sequencing was used to process the amplified templates (Vermont Cancer Center, Burlington, Vermont). Sequence chromatograms were proofed by inspection and edited using Sequence Navigator 1.0 (Perkin-Elmer, Wellesley, Massachusetts). Sequences were initially aligned by Sequence Navigator, followed by additional alignment by inspection. The aligned sequence data were analyzed for biases and ambiguities. Indels were coded as present or absent and incorporated into the sequence data. The poly-A tail at the 3′ end of ITS 1 was excluded from pairwise sequence-divergence and phylogenetic analysis. A single potentially informative site from the 5′ end of 5.8S was included in the analyses. Ambiguous nucleotides were scored as such in the data set.

PHYLOGENETIC ANALYSIS

Maximum parsimony was used to conduct a heuristic search for the shortest trees using PAUP 4.0beta8a (Swofford, 1998). We conducted this search by simple addition of 29 taxa for 500 replicates to search for islands of equally most parsimonious trees. Using tree bisection-reconnection (TBR) branch swapping, the resulting trees were swapped to completion, holding 10 trees at each stepwise addition to a maximum of 100,000 trees. Bootstrap values (BS) were determined for 1500 replicates (heuristic searches, TBR branch swapping, simple taxon addition) to assess the support for the clades identified. A separate analysis of each marker was conducted to test the level of incongruence between them. Incongruence was assessed using a partition homogeneity test and by directly comparing the

Table 1. Accession vouchers with taxon, locality, voucher information, and GenBank accession number.

Taxon	Origin	Voucher information	GenBank accession no. ITS/ETS
Carex acuta* complex sensu Standley (1987a, Standley et al., 2003), section *Phacocystis			
C. acuta L.	U.S.S.R., Siberia	*Elias et al. 8254* (RSA)	AF284892, AY770434
C. elata All.	SWEDEN, Uppland	*L. Thorán 1363* (S)	AY770470, AY770440
C. eleusinoides Turcz. ex Kunth (SIB1)	U.S.S.R., Buryatskaya	*Elias 12025* (RSA)	AF285006, AY770441
C. eleusinoides Turcz. ex Kunth (MONG1)	MONGOLIA	*S. Young s.n.* (VT)	AY770471, AY770442
C. hermannii Cochrane	MEXICO, Volcán Ixtaccíhuatl	*M. Sundue 388* (VT)	AY770473, AY770444
C. gaudichaudiana Kunth	NEW ZEALAND, Waiuta	*K. A. Ford 28/98* (VT)	AY770472, AY770443
C. lenticularis Michx. var. *lenticularis* (VT1)	U.S.A., Vermont	*J. Dragon 00-1c* (VT)	AY770475, AY770447
C. lenticularis Michx. var. *lenticularis* (MI1)	U.S.A., Michigan	*D. Henson 1788* (WIS)	
C. lenticularis Michx. (MI2)	U.S.A., Michigan	*A. Arez et al. 8265* (WIS)	
C. lenticularis Michx. var. *lipocarpa* (T. Holm) L. A. Standl. (OR1)	U.S.A., Oregon	*M. Thompson 95154* (VT)	AY770476, AY770448
C. lenticularis Michx. var. *lipocarpa* (T. Holm) L. A. Standl. (WA1)	U.S.A., Washington	*D. Barrington 2091* (VT)	AY770477, AY770449
C. lenticularis Michx. var. *impressa* (L. H. Bailey) L. A. Standl.	U.S.A., California	*B. Ertter 10424* (WSP)	AY770474, AY770445
C. nigra (L.) Reichard (MI1)	U.S.A., Michigan	*A. Reznicek s.n.* (VT)	
C. nigra (L.) Reichard (ME1)	U.S.A., Maine	*A. Dibble 9800* (VT)	AY770480, AY770452
C. nigra (L.) Reichard (IC1)	ICELAND, Akureyri	*H. Kristinnsson s.n.*	AY770481, AY770453
C. nigra (L.) Reichard (SU1)	SWEDEN, Södermanland	*F. Lind s.n.* (S)	AY770482, AY770454
C. nigra (L.) Reichard (SU2)	SWEDEN, Uppland	*L. Thorán 2426* (S)	AY770483, AY770455
C. nigra (L.) Reichard subsp. *juncella* (Fries) Á. Löve & D. Löve	SWEDEN, Lappland	*A. Anderberg 2092* (S)	AY770484, AY770456
C. rufina Drejer (IC1)	ICELAND, Leirdalsheiði	*H. Kristinnsson s.n.*	AY770485, AY770458

C. rufina Drejer (SU1)		SWEDEN, Lappland	*A. Anderberg 2548* (S)	
C. thunbergii Steud. (KI2)		RUSSIA, Kharimhotan	*B. Semsrott 0193* (WTU)	AY770488, AY770462
C. thunbergii Steud. (KI3)		RUSSIA, Makamushi	*B. Semsrott 1561* (WTU)	AY770489, AY770463
C. thunbergii Steud. (KI6)		RUSSIA, Paramushi	*S. Gage 4333* (WTU)	
C. trinervis Degl.		BELGIUM, De Panne	*R. Viane s.n.* (VT)	AY770491, AY770465
Outgroup taxa, section *Phacocystis* Dumort.				
C. aquatilis Wahlenb. (FI1)		FINLAND	*Kukonen 12828* (RSA)	AF284892, AY770435
C. aquatilis Wahlenb. var. *substricta* Kük. (VT1)		U.S.A., Vermont	*J. Dragon 02-70* (VT)	AY770467, AY770436
C. bigelowii Torr. ex Schwein.		U.S.A., Vermont	*J. Dragon 02-1b* (VT)	AY770469, AY770438
C. scopulorum T. Holm var. *scopulorum*		U.S.A., Nevada	*Holmgren & Reveal 1601* (RSA)	AF285056, AY770459
C. sp. (MX1)		MEXICO, Pueblo Nuevo	*A. A. Reznicek & S. González 11147* (VT)	AY770478, AY770450
C. sp. (MX2)		MEXICO, Pueblo Nuevo	*A. A. Reznicek & S. González 11141* (VT)	AY770479, AY770451
C. stricta Lam. (VT1)		U.S.A., Vermont	*J. Dragon 02-73* (VT)	AY770486, AY770460
C. stricta Lam. (NH1)		U.S.A., New Hampshire	*Barrington s.n.* (VT)	AY770487, AY770461
C. torta Boott		U.S.A., Vermont	*J. Dragon 1a* (VT)	AY770490, AY770464
Outgroup taxa, other sections				
C. bella L. H. Bailey	section *Racemosae*	U.S.A., New Mexico	*Roalson 823* (NMCR)	AF284966 (ITS only)
C. podocarpa R. Br.	section *Scitae*	U.S.S.R., Magadan Oblast	*Elias 11380* (RSA)	AF284998, AY770457
C. aurea Nutt.	section *Bicolores*	U.S.A., California	*DeDecker 5606* (RSA)	AF285062 (ITS only)
C. bicolor All. (JBQ1)	section *Bicolores*	CANADA, Québec, James Bay	*J. Dragon 00-56* (VT)	AY770468, AY770437
C. bicolor All. (IC1)	section *Bicolores*	ICELAND, Hólsfjöll	*Kristinnsson 21663* (AMNH)	
C. bicolor All. (SU1)	section *Bicolores*	SWEDEN, Lappland	*B. & K. Bremer 2169* (S)	
C. donnell-smithii L. H. Bailey	section *Fecundae*	MEXICO, Chiapas	*Shilom Ton 8199* (RSA)	AF285005, AY770439
C. lemanniana Boott	section *Fecundae*	COSTA RICA, Páramo de las Vuettas	*M. Sundue et al. 389* (VT)	AY770446 (ITS only)

topological differences between the ITS and ETS 1f trees.

In addition to maximum parsimony, maximum likelihood was also employed. The HKY + I + G model of evolution was found by Modeltest 3.06 (Posada & Crandall, 1998) to best explain the data. This model accounts for the unequal distribution of transitions to transversions [transition/transversion ratio = 3.1156 (kappa = 6.3930346)] and base frequencies (A = 0.17210, C = 0.29780, G = 0.29170, T = 0.23840), and a continuous (gamma) distribution of among-site rate variation (proportion of invariable sites = 0.5513, shape parameter alpha = 0.7323) within the data. This model was used to generate corrected distances and a maximum likelihood tree. Bootstrap analysis (heuristic searches, TBR, simple taxon addition) of 200 replicates was used to determine the statistical support for the branches of the maximum likelihood tree.

RESULTS

SEQUENCE ANALYSIS

The ranges and average lengths of ITS 1, ITS 2, and ETS 1f from the data set were similar to those found in other carices (Starr et al., 1999, 2003, 2008; Table 2). Twenty-six of 446 positions in the ITS sequence data had double nucleotides at a single site. These were inferred to be heterozygous nucleotides. Seven of these heterozygous nucleotides occurred at phylogenetically informative positions. In the ETS 1f sequences, 28 of 560 positions were inferred to be heterozygous. Of those, six occurred at phylogenetically informative positions. Of the 13 phylogenetically informative heterozygous nucleotides in the combined data set, four belonged to *Carex stricta* (VT1) (one from ETS 1f and three from ITS) and four to *C. bigelowii* (two from each marker). The remainder were distributed singly among five other taxa (three from ETS 1f and two from ITS).

PHYLOGENETIC ANALYSIS

Alignment of the sequence data yielded 1023 characters, 447 characters from ITS, and 567 from ETS 1f. ITS yielded 70 variable characters, 38 of which were phylogenetically informative (29 transitions; eight transversions; one indel), and 32 of which were autapomorphies. ETS 1f sequence data yielded 561 characters, of which 80 were variable, with 37 phylogenetically informative (26 transitions and 10 transversions; one indel), and 43 autapomorphies. See Table 2 for a summary of the ITS and ETS 1f sequence data.

Initial analysis of ITS sequence data indicated that among the outgroup taxa, the members of section *Bicolores* and *Carex bella* of section *Racemosae* were the most divergent. They therefore were omitted from further data collection and analysis. Among the remaining outgroup taxa, the two accessions of section *Fecundae* (*C. donnell-smithii* and *C. lemanniana*) and one of the unnamed Mexican taxa (which we called MX2) were the most divergent from the ingroup taxa; we used these three taxa to root all subsequent trees.

Two most parsimonious trees, of 215 steps (consistency index [CI] = 0.730, retention index [RI] = 0.808, homoplasy index [HI] = 0.270), were identified. The strict consensus of the two trees reveals the *Carex acuta* complex to be paraphyletic, with its members divided into two major clades, both of which include additional taxa from section *Phacocystis*, including *C. aquatilis*, *C. stricta*, *C. bigelowii*, and *C. scopulorum* T. Holm (Fig. 1). Sister to these two clades is *C. podocarpa* currently of section *Scitae*. The analysis did, however, identify the following four clades that can be characterized on the basis of the geographic distribution of their taxa: (1) a Eurasian clade, consisting of the Eurasian taxa of the *C. acuta* complex, including the amphi-Atlantic *C. nigra* (100% BS); (2) a *C. bigelowii–stricta* clade, comprising the circumpolar *C. bigelowii*, its close Pacific North American relative *C. scopulorum*, and the eastern North American *C. stricta* (99% BS); (3) a North American clade, including the American taxa of the complex plus the circumpolar *C. aquatilis* (65% BS); and (4) an Austral–East Asian clade, a clade of three taxa from the complex, two of which are from East Asia and one from New Zealand (< 50% BS). Within the North American clade, several smaller clades were resolved, including (1) the lenticularis–rufina clade, a clade of *C. lenticularis* var. *lenticularis* and *C. rufina* Drejer (100% BS); (2) the lipocarpa–impressa clade, including *C. lenticularis* var. *lipocarpa* (T. Holm) L. A. Standl., *C. lenticularis* var. *impressa* (L. H. Bailey) L. A. Standl., and MX1 (64% BS); and (3) a *C. aquatilis* clade, comprising *C. aquatilis* and *C. aquatilis* var. *substricta* Kük. (95% BS). Variation

Table 2. Summary statistics for ITS 1, ITS 2, and ETS 1f spacer regions. TI = transitions; TV = transversions.

	ITS 1	ITS 2	ETS 1f
Aligned length (bp)	219	227	560
Length range (bp)	216–219	223–227	553–560
Length average (bp)	217.4	225.9	557.9
GC content range	135–143	151–161	307–324
GC content average	138.78 (63%)	156.13 (69%)	317.36 (57%)
No. of variable sites	30	40	80
No. of phylogenetically informative sites	13 (10 TI; 2 TV; 1 indel)	15 (19 TI; 6 TV)	37 (26 TI; 10 TV; 1 indel)
No. of autapomorphies	17	25	43

between the two most parsimonious trees is due entirely to the unresolved relationship between three of the accessions of *C. nigra*. The maximum likelihood tree shows slightly stronger support for the sister relationship of the Eurasian and *C. bigelowii* clades (54%) found in the maximum-parsimony consensus tree, but slightly weaker support for the North American clade (68%). There were no topological differences between the maximum parsimony consensus tree and the maximum likelihood tree.

The data provided by the two DNA markers were found to be significantly incongruent. Analyzing ETS 1f alone, the same tree topology as that of the combined data set was found. ITS alone alternates the placement of the Austral–East Asian and *Carex bigelowii–stricta* clades, and places *C. gaudichaudiana* Kunth basal to the North American clade and *C. stricta* in a more derived position sister to *C. aquatilis* (data not shown).

INFRASPECIFIC VARIATION

Total pairwise sequence divergence in the taxa examined ranged from 0.10% to 5.58% under the HKY + I + G model for estimating the number of nucleotide substitutions. Low infraspecific sequence divergence was found among six accessions of *Carex nigra* (0.10% to 0.51%), although even less divergence was found between some accessions of *C. nigra* and three European taxa (0.10% to 0.30%). While overall divergence was higher among the four accessions of *C. lenticularis* (0.20% to 1.71%), greater sequence divergence was found among some accessions of *C. lenticularis* than between them and their nearest allies (0.20% to 1.94%), just as in *C. nigra*.

Multiple accessions of a few taxa had identical ITS and ETS 1f sequences, including three accessions of *Carex lenticularis* var. *lenticularis*, two North American accessions of *C. nigra* (ITS only), two accessions of *C. rufina*, and two accessions of *C. thunbergii* Steud. from the Kuril Islands (KI3 and KI6). Other taxa for which multiple accessions were sequenced did not have identical ITS and ETS 1f sequences, including accessions of *C. eleusinoides* Turcz. ex Kunth with 1.15% sequence divergence, *C. lenticularis* var. *lipocarpa* with 0.20% sequence divergence, *C. thunbergii* with 0.20% sequence divergence, and *C. stricta* with 1.39% sequence divergence. It is important to note that different accessions of *C. eleusinoides* and *C. stricta* created topologically different trees (data not presented). While only one accession of each of these species was included in this study, the implications of including these divergent individuals in phylogenetic analyses are discussed in more detail below.

DISCUSSION

MONOPHYLETIC GROUPS AND SISTER RELATIONSHIPS WITHIN SECTION *PHACOCYSTIS*

Our first objective was to test the phylogenetic integrity of the *Carex acuta* complex. The evidence provided by ITS and ETS 1f sequence data shows that the *C. acuta* complex, as circumscribed by Standley (1987a, b) is paraphyletic. The larger clade

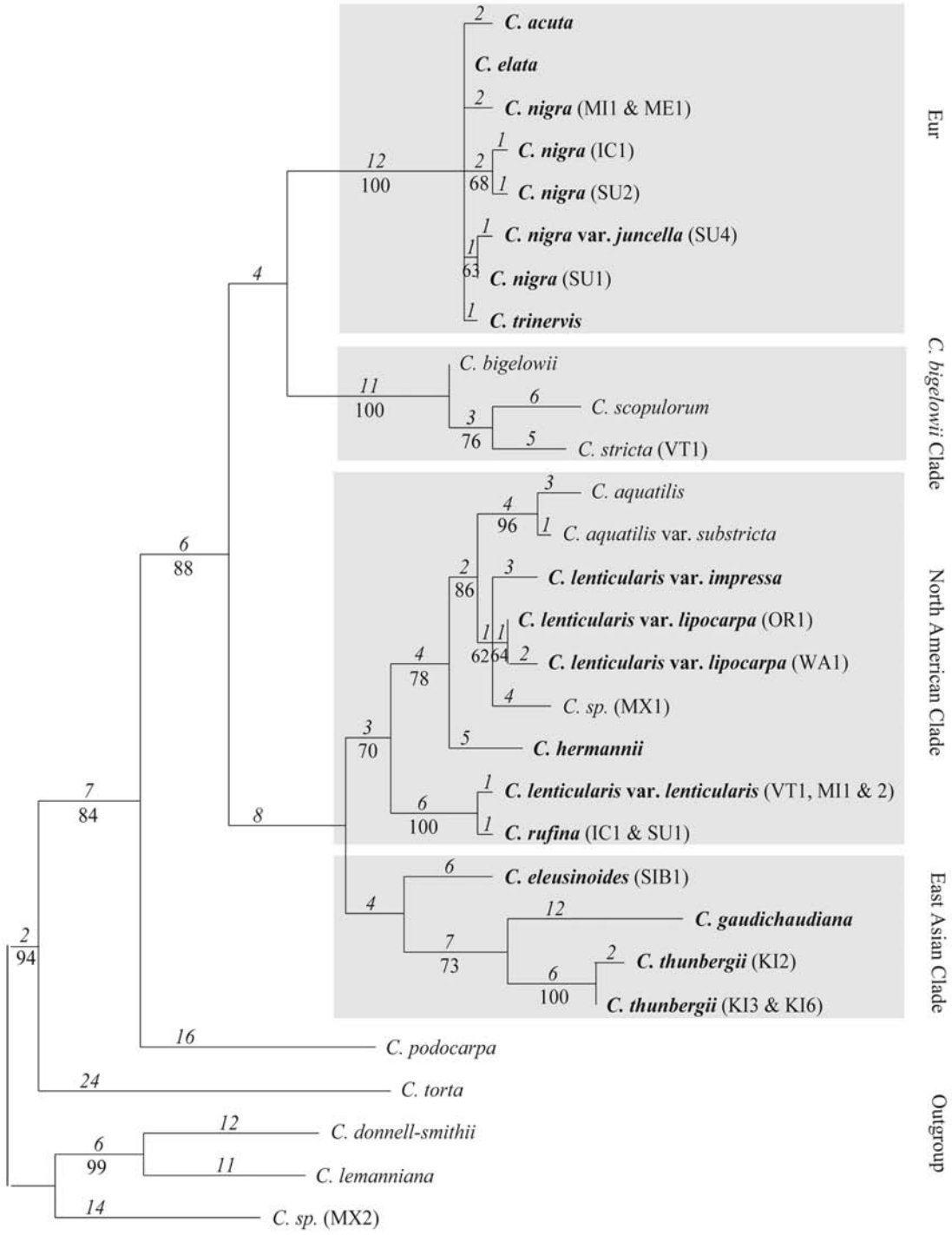

Figure 1. One of the two most parsimonious trees (length = 214; CI = 0.73; RI = 0.81; HI = 0.27). Numerals above the branches are branch lengths. Numerals below the branches are > 50% bootstrap estimates. Clade names are written vertically to the right of the corresponding clades. Taxa of the *Carex acuta* complex are in bold.

Table 3. Primer sequences used to amplify the rDNA internal transcribed spacers (ITS 1 and ITS 2) and a portion of the ETS between the tandem repeats of the 18S and 26S genes (ETS 1f).

Primer reference	Primer sequence
Forward primer ITS-L (Hsiao et al., 1994)	5′-TCGTAACAAGGTTTCCGTAGGTG-3′
Forward primer AB101 (Sun et al., 1994)	5′-ACGAATTCATGGTCCGGTGAAGTGTTCG-3′
Reverse primer ITS-4 (White et al., 1990)	5′-TCCTCCGCTTATTGATATGC-3′
Internal forward primer ITS-3 (White et al., 1990)	5′-GCATCGATGAAGAACGCAGC-3′
Internal reverse primer ITS-2 (White et al., 1990)	5′-GCTGCGTTCTTCATCGATGC-3′
Forward primer ETS-1F (Starr et al., 2003)	5′-CTGTGGCGTCGCATGAGTTG-3′
Reverse primer 18S-R (Starr et al., 2003)	5′-AGACAAGCATATGACTACTGGCAGG-3′

that includes all of the taxa of the *C. acuta* complex also includes four taxa from elsewhere in section *Phacocystis* (*C. aquatilis*, *C. bigelowii*, *C. scopulorum* var. *scopulorum*, and *C. stricta*; Fig. 1). Sister to this clade is *C. podocarpa* of section *Scitae*. The placement of *C. podocarpa* basal to this clade separates *C. torta* Boott from the rest of the members of section *Phacocystis*. While our data set includes only 20% of the species of section *Phacocystis*, our results suggest that either *C. torta* has been wrongly placed in section *Phacocystis* or that *Phacocystis* should include *C. podocarpa*. Species traditionally placed in the *C. acuta* complex are divided among four monophyletic groups. Three of these putative clades can be distinguished by their geographic distribution: Eurasian, Austral–East Asian, and North American (minus *C. bigelowii*, *C. scopulorum*, and *C. stricta*). The North American clade can be further subdivided into two clades, one with taxa of western distribution, and the other with taxa of eastern affinities. The geographic integrity of the majority of the clades identified thus far provides support for the phylogenetic history inferred from our study.

INFRASPECIFIC VARIATION

Our second objective was to examine infraspecific variation within two of the polymorphic taxa of the ingroup, *Carex lenticularis* and *C. nigra*. In the case of *C. lenticularis* and *C. nigra* and their allies, a recasting of species lineages may be in order. In addition, high infraspecific divergence was found among accessions of *C. eleusinoides* and *C. stricta*. In their case, the variation suggests a more complicated phylogenetic history.

Carex lenticularis

The molecular divergence between three of the varieties of the North American *Carex lenticularis* is consistent with their taxonomic recognition. However, the eastern variety *lenticularis* forms a well-supported clade with the amphi-Atlantic *C. rufina*, while the two western taxa, varieties *lipocarpa* and *impressa*, form a well-supported clade with one of the unnamed Mexican accessions (MX1). These results suggest that the western and eastern varieties of *C. lenticularis* either pertain to different species or are part of a much more broadly circumscribed *C. lenticularis*. The biogeographic pattern implied by the phylogeny suggests that there are isolated lineages in the western cordillera and Mexico and in eastern North America, a pattern also detected by Hipp (2008) in *Carex* sect. *Ovales* Kunth.

Carex nigra

In North America, populations of *Carex nigra* display some morphological variation, but are nonetheless interpreted as one species (Standley, 1987a). In the present study, the two North American accessions of *C. nigra* (from Maine and from a disjunct population in Michigan) share identical ITS and ETS 1f sequences, supporting the integrity of the species in North America. In contrast, we found varying amounts of sequence divergence when we considered the North American material in comparison with the Icelandic and European accessions (0.2% to 0.4%). Across Europe and Asia, the group of *C. nigra* is highly variable, leading to the recognition of closely allied segregate species and subspecies. Despite their morphological variation and

taxonomic status as species, the European taxa *C. acuta*, *C. trinervis* Degl., *C. nigra*, and *C. elata* exhibit little sequence divergence from one another (0.1% to 0.3%), and less variation than is found within *C. nigra*. The clade including *C. nigra* and its allies is genetically and morphologically more diverse in Europe than in eastern North America. It may be that the assemblage is a single variable species whose biogeographic history is fundamentally European, and that the North American populations are recent arrivals by long-distance dispersal or human introduction.

Carex eleusinoides

The two accessions of *Carex eleusinoides*, both from the tundra in the mountains near Lake Baikal, diverge by 1.15%. Although the two are phenetically most similar to one another in our analysis, separate phylogenetic analyses including the two accessions yield different results. The Siberian accession (SIB1) is sister to a clade that includes *C. gaudichaudiana* and *C. thunbergii* (Fig. 1), while the Mongolian accession (MONG1) is sister to *C. lenticularis* (data not shown). A phylogenetic analysis that included both accessions yielded a tree with decreased resolution and lower CI and RI values (data not shown). These observations suggest that *C. eleusinoides*, as currently circumscribed, may be polyphyletic.

Carex stricta

The sequences from the two accessions of *Carex stricta* differ by 1.39%, one of the largest distances so far encountered for accessions of a single species of *Carex*. When analyzed separately within the data set, the accession from ca. 200 m in Vermont is sister to *C. scopulorum* var. *scopulorum* (Fig. 1) from Nevada, while the accession from ca. 800 m in New Hampshire forms a weakly supported clade with eastern North American *C. aquatilis* var. *substricta* (data not shown). When both are included in the parsimony analysis they form a weak clade with each other, sister to the *C. bigelowii–scopulorum* clade, but support for all nodes in the North American clade is greatly reduced (data not shown).

RETICULATE EVOLUTION AND INFRASPECIFIC VARIATION

Considering the data on infraspecific variation our samples fall into two sets. On the one hand, *Carex nigra* and *C. lenticularis* appear to harbor substantial variation but, with adjustment of taxonomic species limits, may be possible to circumscribe as natural species. In contrast, *C. eleusinoides* and *C. stricta* each comprise a pair of accessions that lie distant in separate phylogenetic analyses and decrease measures of tree resolution and support in combined analyses (data not shown). These accessions are also among the richest in ambiguous (two-nucleotide) sites in the data. Thus, hybridization, the presence of paralogous sequences, and variation between copies of ITS and ETS 1f need to be explored as possible explanations of our results. *Carex stricta* is reported to hybridize with *C. aquatilis* and *C. nigra* based on observations of anomalous morphology within some sympatric populations (Standley, 1989; Standley et al., 2003). Similar evidence suggests that the formation of hybrids is frequent in section *Phacocystis*, particularly in salt marshes and along brackish waterways (Cayouette & Morisset, 1985; Standley et al., 2003). In our work, as in most molecular phylogenetic work, the habit of collecting accessions in mature fruit for morphological determination precludes the assembly of meiotic and pollen material critical in hybrid analysis. A more intensive approach, involving either (1) the cultivation of plants to allow for both cytological and molecular work, or (2) sequencing of clonal DNA derived from candidate-hybrid accessions, would yield the evidence necessary to discern hybrids in the data set. At the same time, the latter approach would distinguish ambiguous nucleotides in different genomes (as a result of hybridization) from those in different copies of ITS or ETS in the same genome (from a failure of concerted evolution in these sequences).

FUTURE RESEARCH

Future research into the systematics of section *Phacocystis* will include additional sampling within the section in order to test the monophyly of the clades identified in this study, and to increase understanding of the relationship among taxa within the section as a whole. Particular emphasis will be placed on the North American clade, which includes *Carex lenticularis* and its allies, and *C. aquatilis*, and will include an examination of the morphological characters that are common to both taxa, and further examination of the biogeographic patterns presented by the data.

The role of reticulation in the diversification of section *Phacocystis* requires additional attention.

For this study ITS and ETS markers will require cloning, and a comparison of nuclear to chloroplast markers would be useful. Additional crossing experiments and chromosome analyses, such as those conducted by Faulkner (1972, 1973) and Cayouette and Morisset (1985) could also provide further witness to the relationships among taxa and the role hybridization has played in the diversification of this morphologically complex group.

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