Systematics of the Carex aquatilis and C. lenticularis lineages: Geographically and ecologically divergent sister clades of Carex section Phacocystis (Cyperaceae)

Julie A. Dragon and David S. Barrington

Plant Biology Department, 109 Carrigan Drive, University of Vermont, Burlington, Vermont 05405 USA

Carex aquatilis is a highly diverse and geographically widespread member of one of the largest genera of flowering plants, Carex, and is ideally suited for the study of the role of hybridization and niche partitioning in ecological speciation. Phylogenetic analyses of nuclear ITS and ETS 1f and chloroplast psbA-trnH DNA sequences support the monophyly of a broadly defined Carex aquatilis-Carex lenticularis lineage, which includes C. aquatilis and C. lenticularis and their allies within section Phacocystis. However, neither taxon is monophyletic as currently circumscribed. The C. aquatilis lineage includes C. aquatilis and four morphologically and molecularly distinct salt-tolerant maritime taxa with which C. aquatilis s.s. is reported to form stabilized homoploid hybrids. The C. lenticularis lineage includes a paraphyletic C. lenticularis and seven allied species from both the New and Old World. The data provided here allow recognition of four species within the North American endemic C. lenticularis and suggest a neotropical origin for the C. lenticularis lineage with subsequent radiation and divergence through northwestern North America to Asia and via northeastern North America to Europe and southern South America. Evolutionary rate analyses indicate an origin for the C. aquatilis-C. lenticularis group around 1.89 million years ago during the early Pleistocene.

Key words: biogeography; Carex; Cyperaceae; ecological speciation; hybridization; molecular phylogeny; rates analysis; systematics.

The genus Carex L. is one of the most widespread and ecologically important of all plant genera, with approximately 2000 species and a cosmopolitan distribution (Reznicek, 1990). Understanding how a single genus has become so diverse has challenged botanists for over a century, in part from a lack of understanding of evolutionary relationships among the taxa. Efforts to infer evolutionary lineages and identify the processes that produced them, thought to include rapid chromosome evolution, hybridization, and ecological isolation, have been hampere in part by morphological convergence and regional rather than global sampling of species. With the increasing availability of molecular data from around the world, however, sound phylogenetic hypotheses now allow the rigorous re-examination of patterns and processes of evolution within the genus despite the challenges it presents. Carex aquatilis and its allied species represent a widespread and ecologically important taxonomic group ideally suited for the study of the role of hybridization and niche partitioning in ecological speciation.

The largest section of Carex is the largely Asian and North American section Phacocystis Dumort., which includes approximately 90 taxa, of which about 50 are endemic to Asia and 25 to North America. Within section Phacocystis, we previously used ITS sequence data to identify a monophyletic group of taxa herein called the Carex aquatilis-Carex lenticularis group (Dragon and Barrington, 2008). This group includes two highly diverse taxa, the North American endemic C. lenticularis, with five varieties, and the circumboreal C. aquatilis, with four varieties, along with several allied species. Though placed in the same subsection on the basis of shared morphology in several treatments (Kükenthal, 1909; Mackenzie, 1935), C. lenticularis and C. aquatilis have more recently been ascribed to different groups based on anatomical, chromosomal, and morphological differences (Standley et al., 2002). Within the C. aquatilis-C. lenticularis group, the initial phylogeny that we inferred indicated that, while C. aquatilis was monophyletic, C. lenticularis was not (Dragon and Barrington, 2008). However, several maritime species suggested to form hybrids with C. aquatilis (Standley et al., 2002), including C. subspathacea, C. ramenskii, C. paleacea, and C. lyngbyei, were not sampled. Given the potentially close relationship between these maritime taxa and C. aquatilis, and the polyphyly of C. lenticularis, it was clear that broader sampling across Phacocystis would be necessary to provide taxonomic and evolutionary clarity for the C. aquatilis-C. lenticularis group.

Understanding evolutionary relationships and classification of organisms provides a context in which systematists can estimate divergence times and trace geographic distribution of taxa through time. The Carex aquatilis-Carex lenticularis group is largely New World, with infraspecific variation and areas of endemism concentrated in western North America. This pattern of diversity has been inferred from studies of several organisms to be the result of Pleistocene periglacial fragmentation and refugial isolation of populations in the heterogeneous mountainous terrain of western North America (e.g., Brumfield et al., 2001; Hewitt, 2003). In addition, the oscillating temperatures that marked the Pleistocene produced repeated interglacial periods when gene flow between western taxa was more likely, obscuring species boundaries and retarding lineage differentiation over time (Axelrod and Raven, 1985; Good and...
with ethidium bromide. Cycle sequencing used the external sequencing primers ITS5L and ITS4 (White et al., 1990) for ITS and the same primers as used in the PCR reactions for ETS 1F and psbA-trnH. Automated sequencing on an ABI Prism 3130 × 1 automated sequencer (Vermont Cancer Center, Burlington, Vermont, USA) was used to process the amplified templates. Sequence chromatograms were proofed by inspection and edited using the program Sequence Navigator 1.0 (Perkin-Elmer, Wellesley, Massachusetts, USA). Sequences were initially aligned by Sequence Navigator, then adjusted manually by visual inspection. The aligned sequence data were analyzed for ambiguities. Indels were coded as present or absent and incorporated into the sequence data as single nucleotide changes. The poly A tail at the 3' end of ITS 1 was excluded from phylogenetic analysis. Taxa with identical sequences were combined in the phylogenetic analyses. Sequences for all specimens are available on GenBank (Appendix 1).

Phylogenetic analysis—Sequence data for each marker were analyzed separately and collectively in the program Modeltest 3.06 (Posada and Crandall, 1998) to determine the most appropriate model of evolution using two statistical frameworks for model selection, hierarchical likelihood ratio tests (hLRT) and the Akaike information criterion (AIC). For ETS, hLRT picked the TRN+G model, while AIC picked the GTR+G model. Though GTR+G had a slightly better likelihood score, it included three additional parameters. We chose TRN+G to best represent evolution in the marker because it had fewer parameters and was a Bayesian analysis of the combined data of ETS, both hLRT and AIC picked the K81u+I+G model to best explain the distribution of the variation observed. For psbA-trnH, hLRT picked F81+I though F81+G+ had a slightly better likelihood score. AIC picked F81+G with an identical number of parameters and likelihood score. We chose to use F81+I+G to represent the evolution of this marker. In the combined analysis, hLRT chose K81u+I+G, while AIC indicated TVM+I+G to be the best model. Because K81u+I+G had two fewer parameters, we used this model in analyses for which we could not partition the markers and apply different models of evolution to each.

Bayesian inference—Separate Bayesian analyses were conducted on the combined data set using the program MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) under the appropriate evolutionary models. The Markov chain Monte Carlo permutation of tree parameters was conducted with a random starting tree and included four incrementally heated chains, at temp = 0.2, for two runs of 3,000,000 generations each with sampling every 100 generations. The resultant Bayesian inference (BI) trees were plotted against their log-likelihood scores to determine the point of stationarity. All trees prior to stationarity were discarded as the burn-in phase, and all subsequent trees were retained (trees 5000–30,001). These tree were imported into the program PAUP* 4.0b10 (Swoford, 2002) to construct a 50% majority-rule consensus tree with posterior probabilities (PP) for all resolved clades.

Maximum parsimony and likelihood—Maximum parsimony (MP) analyses were performed using PAUP* 4.0b10 (Swoford, 2002) on the combined data set. Heuristic searches were conducted with 1000 replicates and random addition of the taxa to search for islands of equally most parsimonious trees, with 10 trees held during tree-bisection-reconnection (TBR) branch swapping, saving all the trees at each step (MultTrees) and with ACCTRAN character-state optimization. Bootstrap values (BS) were determined for 1000 replicates under the same heuristic criteria to assess the support for the clades identified. Only variable characters were included in the BS analysis. Maximum likelihood (ML) analysis, with an approximate likelihood ratio test (aLRT) of all branches (Anisimova and Gascuel, 2006), was performed in the program PHYML v2.4.5 (Guindon and Gascuel, 2003) using the K2P, GTR, and TN93 models of evolution. Under the GTR model, the tv/ti ratio and proportion of invariable sites were optimized, with four possible substitution-rate categories allowed. The ML tree generated under each model was viewed in the program Mega 4.0 (Tamura et al., 2007).

Biogeographic and morphological analyses—Evolutionary rates—Rates of evolution implied by the combined rDNA data were calculated using the penalized likelihood algorithm and the truncated Newton method in the program r8s (Sanderson, 2003). Penalized likelihood is a semiparametric smoothing method that relaxes the assumption of clock-like evolution. Cross-validation of the branch-length data from the Bayesian tree with the highest likelihood score yielded an optimal smoothing value of 10. Using penalized likelihood and this smoothing value, we calculated rate estimates for each branch. Two age constraints were used to calibrate the estimated rates of evolution across the tree as both maximum and fixed ages. The constraints were derived from...
several Hawaiian Island archipelago endemics from section Phacocystis: (1) a date of 4.7 million years ago (mya) (date of the formation of Kaua‘i; Price 2004) was used to constrain the node representing the most recent common ancestor of Carex kauaiensis, a taxon endemic to the island of Kaua‘i, and its sister taxon Carex alligata, endemic to all the islands in the archipelago, and (2) a date of 1.2 mya (date of the formation of Maui Nui; Price and Elliot-Fisk, 2004) to constrain the node of Carex nealae, an endemic species of the islands of Maui and Hawaii. These represent the earliest dates when either taxon could have originated, assuming the species have not lost lineages to extinction on older islands or elsewhere in the world. The curvature of the likelihood surface around the estimated parameter was used to determine the 95% confidence intervals (cutoff = 4 units) for the major nodes following Cutler (2000). The parameter values at which the log likelihood drops by a larger amount (cutoff = 4 units) were determined to examine the robustness of the date estimates.

Morphology—We examined 157 specimens that fit recent species concepts for taxa of section Phacocystis and the Carex aquatilis-Carex lenticularis group (Egorova, 1999; Standley et al., 2002). Morphological data were assembled and overlaid onto the maximum likelihood tree to identify the synapomorphies that were congruent with the major clades of the molecular tree and to trace their morphological evolutionary transformations. In addition to morphological data, diploid chromosome numbers, also cited in Egorova (1999) and Standley et al. (2002) were overlaid onto the inferred phylogeny as well.

RESULTS

Nuclear sequence analysis—Alignment of the nuclear sequence data yielded 1030 characters; 234 were variable and 126 (12.16%) were phylogenetically informative (Table 1). Aligned ITS sequences were 453 base pairs (bp) long (excluding 5.8S), of which 108 nucleotides were variable, and 71 (15.6%) were phylogenetically informative. No variation was found in the 5.8S gene. Aligned ETS sequenced were 577 bp long; 126 were variable, and 55 (9.5%) were phylogenetically informative.

For several species, multiple accessions were found to have either identical nuclear sequences or sequences that differed by single uninformative nucleotides. Inclusion of these sequences made the analyses overly cumbersome. In such cases, we chose to keep only the most geographically central accession in the final analyses. The removal of the other accessions did not influence the structure of the phylogeny.

Chloroplast sequence analysis—The chloroplast marker psbA-trnH is among the most variable cpDNA regions in angiosperms (Kress et al., 2005) and was found to be highly variable across the Poaceae (Saltonstall, 2001). In this study, the aligned psbA-trnH spacer was 533 bp long and contained 32 variable characters, of which 15 (2.8%) were phylogenetically informative (Table 1). None of the variation was within the included rps19 gene.

Phylogenetic analysis—The maximum log-likelihood values for the 30001 Bayesian inference trees sampled reached station-

Table 1. Summary statistics for the Carex aquatilis lineage from ITS, ETS 1f, and psbA-trnH sequence data.

<table>
<thead>
<tr>
<th>Sequence data</th>
<th>ITS</th>
<th>ETS 1f</th>
<th>psbA-trnH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aligned length (bp)</strong></td>
<td>453</td>
<td>577</td>
<td>533</td>
</tr>
<tr>
<td><strong>Length range (bp)</strong></td>
<td>451–453</td>
<td>572–577</td>
<td>533</td>
</tr>
<tr>
<td><strong>Variable sites</strong></td>
<td>108</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td><strong>Phylogenetically informative sites</strong></td>
<td>71</td>
<td>55</td>
<td>15</td>
</tr>
<tr>
<td><strong>Autapomorphies</strong></td>
<td>37</td>
<td>71</td>
<td>17</td>
</tr>
<tr>
<td><strong>Indels</strong></td>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><strong>AIC model</strong></td>
<td>K81uf+I+G</td>
<td>GTR+G</td>
<td>F81+I</td>
</tr>
</tbody>
</table>

Biogeographic and morphological analyses—r8s analysis—Under a maximum-age constraint module, the rate analysis estimated the divergence of the Carex aquatilis-Carex lenticularis group from its sister lineage at ca. 2.55 mya. Similarly, our analysis estimated the divergence of the two lineages in our study group—the Carex aquatilis lineage (clade I) and the Carex lenticularis lineage (clade VII)—at between 1.89 and 1.07 mya (clade I, Table 2, Fig. 1). We estimated the basal divergence of the Carex aquatilis lineage (clade I) to lie between 0.76 and 0.64 mya and in the Carex lenticularis lineage (clade VII) between 1.50 and 1.11 mya. Estimated dates for several clades originating more recently are reported in Table 2. With an alternative, fixed-age model for estimation of ages, the rate analysis yielded a slightly older range of dates for the divergence of the Carex aquatilis-Carex lenticularis group (approximately 3.01–2.98 mya) and for the radiation of the two major lineages (1.23–1.20 mya for clade II and 2.40–2.36 mya for clade VII). Because we used island formation as an earliest possible date for the rate analysis, as opposed to a fossil of known age, we believe the constrained-age model to be more appropriate. Doubling the end points for assessment of confidence of the parameter estimate to 4.0 units of the log likelihood yielded the same estimates for the fixed age analysis and older for the maximum age analysis (i.e., an age of 1.89–1.29 mya for the most recent common ancestor for the Carex aquatilis-Carex lenticularis group).

Morphological analysis—Many morphological characters or suites of characters, as well as chromosome counts, were found to support the clades inferred from the molecular data.
Fig. 1. The majority rule consensus of 25002 Bayesian inference trees. Posterior probabilities greater than or equal to 50% are indicated above the branch. Roman numerals below the branches indicate clade numbers. Numbers in parentheses indicate the accession used in the final analysis. Geographic range or location is shown to the right of the taxa (NA = North America, SA = South America, CA = Central America). Taxonomic changes are indicated in margin boxes for the taxa in boldface (see Table 3). Estimated dates of divergence, in millions of years ago (mya), are indicated for certain nodes.
The major morphological apomorphies and chromosome numbers for the inferred ingroup clades are indicated on the ML tree in Fig. 2.

DISCUSSION

The monophyly of the Carex aquatilis-Carex lenticularis group—Emergent ecological and geographic trends—Our first objective was to test the phylogenetic integrity of the monophyletic group that we had previously identified (Dragon and Barrington, 2008) with additional molecular data and the inclusion of all varieties and putative allies of both *Carex aquatilis* and *C. lenticularis*. The analysis using the combined sequence data continued to retrieve the *Carex aquatilis-Carex lenticularis* group with inclusion of several additional species (Fig. 1, clade I), regardless of analytic algorithm. The need for a number of taxonomic changes is revealed in the phylogeny based on the analysis of both the molecular and morphological data (see Table 3, Figs. 1 and 2); the formal taxonomic revision will be published elsewhere. Within the *C. aquatilis-C. lenticularis* group, two distinct lineages emerge. The first includes the *C. aquatilis* lineage (i.e., clade II; a paraphyletic *C. aquatilis* s.s. plus the *C. subspathacea* sublineage), and the second includes the *C. lenticularis* lineage (clade VII: the polyphyletic *C. lenticularis* and its allies; see Fig. 1). These clades reveal novel trends of ecological and geographic variation not previously identified for the members of the *C. aquatilis-C. lenticularis* group.

*Carex aquatilis* lineage (clade II)—Several morphological characters support the *Carex aquatilis* lineage (see Fig. 2). However, while unique morphological characters distinguish each of the species within the lineage, the molecular characters examined do not resolve these morphological entities. For instance, *Carex aquatilis* s.s. is a widespread, polymorphic wetland species that is genetically little differentiated from the most recent common ancestor it shares with the *C. subspathacea* sublineage, a polymorphic clade of morphologically distinct halophytes supported by six molecular synapomorphies (clade V, Figs. 1 and 2). There are several possible explanations for the lack of genetic divergence encountered in *C. aquatilis* s.s.: (1) the molecular markers examined have evolved at a slower rate for these members of the study group, (2) *C. aquatilis* s.s. is a recently evolved polymorphic species, or (3) synapomorphies have been lost as a result of introgression from the *C. subspathacea* sublineage, with which hybridization has been well documented (Faulkner, 1973; Cayouette and Morisset, 1985, 1986; Standley et al., 2002).

The *Carex subspathacea* sublineage (Fig. 2) is a lineage of morphologically and ecologically distinct species, distinguished by a more complete ecological shift to a halophytic habitat than *C. aquatilis* s.s. but for which molecular apomorphies were not found. The possible explanations for this result also include lack of evolution of the molecular markers, recent divergence, survival of ancestral lineages, and introgression.

Two subclades of *Carex aquatilis* s.s. relate to geographic distribution and not to current morphological varietal classification, with one clade comprising the European accessions of *C. aquatilis* var. *aquatilis* (clade IV, Fig. 1) and the other the North American accessions of var. *aquatilis*, var. *substricta*, and var. *minor* (clade VI, Fig. 1). Two morphological characters were identified to support the North American and Eurasian subdivision: smaller culm width in the North American plants and narrower pistillate scales in the Eurasian plants. A third subclade includes the robust, pendulous-spiked *C. aquatilis* var. *dives* and is inferred to be monophyletic as currently circumscribed (clade III, Fig. 1). *Carex aquatilis* var. *dives* is a brackish water species in western North America, with the exception of a disjunct population of freshwater plants on a refuge montane bald in North Carolina. We postulate that while geographic isolation has played a greater role in the speciation of the freshwater lineages of *C. aquatilis* across its range, ecological isolation is likely to have allowed divergence of the salt-tolerant variety *dives* across the majority of its range.

*Carex lenticularis* lineage (clade VII)—The *C. lenticularis* lineage (Fig. 1) comprises a species-rich group of taxa traditionally associated with *C. lenticularis*. The lineage differs from the *C. aquatilis* lineage in its higher number of apomorphies associated with most taxa, perhaps indicative of longer isolation of its members from each other (Fig. 2).

The sublineage comprising typical *C. lenticularis*, *C. rufina*, and *C. decidua* combines species with diverse geographies (clade XIII, Fig. 1). The southern South American *C. decidua* has accumulated several molecular autapomorphies in isolation and is morphologically distinguished from its sister species, typical *C. lenticularis*, by longer anthers, longer perigynium stipe, and wider leaves. The amphi-Atlantic *C. rufina* differs in overall size from *C. lenticularis* and *C. decidua*, perhaps from phenotypic dwarfing in the Arctic habitat it occupies. Its sister clade comprises (1) the amphi-Pacific *C. eleusinoides* and (2) *C. plectocarpa* from a single mountain in Montana (clade XII, Fig. 1). Several morphological features, including red-brown basal leaf sheaths and gynaeccandrous terminal spikes, unite the *C. eleusinoides-plectocarpa* clade.

A weak polytomy within the *C. lenticularis* lineage includes *C. enanderi*, represented by populations from Alberta to Alaska (north of 54° latitude) formerly ascribed to *C. lenticularis* var. *dolia* (clade IX, Fig. 1). These populations are phylogenetically distant from the Montana populations of *C. plectocarpa* (formerly *C. lenticularis* var. *dolia*) and are morphologically distinct from them in basal sheath color, perigynium and beak length, and perigynium stipe length. The inferred sister relationship between *C. enanderi* and a specimen identified as *C. decidua* from Colombia is only supported by only a few synapomorphies from ITS, which was difficult to sequence from the Colombian taxon. Another Colombian accession of *C. decidua* has been recently sequenced and supports a closer relationship between the northern Andean populations and the other boreotropical species *C. hermannii* and *C. cuchumatancensis* in subsequent analyses (data not shown). Morphologically both Colombian accessions are most similar to *C. brehmeri* from Bolivia, which remains to be analyzed molecularly.

The remaining members in clade IX include the rest of the western varieties of *C. lenticularis* (formerly *C. lenticularis* varieties *lipocarpa*, *limnophila*, and *impressa*), herein ascribed to *C. kelloggii*. Because initial analyses indicated that there was minimal molecular divergence (none to only single apomorphies) in multiple accessions representing all three varieties, only four accessions were included in the final analysis and presented in Figs. 1 and 2. Based on the molecular markers analyzed, there is no congruence between the minimal molecular variation observed and varietal circumscription, and the varieties cannot be distinguished from the most recent common ancestor they are inferred to share with the other species in the
Fig. 2. The Carex aquatilis-Carex lenticularis group and its sister clade from the maximum likelihood tree. Salt-tolerant taxa are indicated in boldface. Supporting molecular and partial morphological data are shown along the branches for the ingroup. ETS 1f nucleotides are shown as squares, ITS as circles, psbA-trnH as triangles. If symbols are italicized or open, then the character is homoplastic within the ingroup.
clade. However, because the three currently circumscribed varieties can be distinguished from each other morphologically using the dimensions of pistillate spikes and the color of the perigynium and pistillate scale, we will maintain them as *C. kelloggii* var. *kelloggii* (formerly lipocarpa), *C. kelloggii* var. *limnophila*, and *C. kelloggii* var. *impressa*. The only sister pair resolved by the molecular data comprised populations of *C. kelloggii* var. *kelloggii* (formerly *C. lenticularis* var. *lipocarpa*) from Arizona and Mexico. These populations are morphologically slightly more robust than more northern populations of the species but will not be distinguished as a separate variety.

Several hypotheses to explain the lack of genetic variation between the ancestor to the sublineage and *C. kelloggii* are tenable. One possibility is that *C. kelloggii* represents the surviving ancestor to the rest of the clade, as evidenced by the absence of apomorphies in all but the southern populations of the species. Another possibility is that the rugged western North American topography combined with Pleistocene glacial–interglacial stages could have allowed for repeated periods of gene flow where populations merged at lower altitudes during warmer periods then again became isolated at higher altitudes during cool periods. Such cyclical changes could explain the paucity of molecular apomorphies for *C. kelloggii*. For species complexes in *Carex* section *Ovales*, Hipp et al. (2006) found the western North American taxa to be involved in most of the nonmonophyletic relationships and also cited “retention of ancestral polymorphisms or ongoing gene flow between close relatives” to explain the nonmonophyly of the identified clades.

**Section Phacocystis**—With approximately 48% of section *Phacocystis* represented, we find that the *Carex aquatilis-Carex lenticularis* group (Fig. 1, clade I) is derived within the section. The sister to the group remains unresolved despite our broad sampling from within the section. However, in our effort to resolve the position of the *C. aquatilis-C. lenticularis* group, we did confirm the paraphyly of section *Phacocystis* (see Fig. 1). Three of the North American members of section *Scitae* are nested within section *Phacocystis* Dumort. The morphological features used to define section *Phacocystis* (unisexual, bistigmatic flowers, sheathless proximal involucral bract, papillate perigynia that are backless or with only short teeth; $x = 27–48$) appear to be phylogenetically informative with the exception of stigma number, which is commonly used in artificial keys but appear to be phylogenetically homoplastic within several sections. The inclusion of the trisomic, amphi-Pacific section *Scitae*, which possess the other defining characters of section *Phacocystis*, would render *Phacocystis* monophyletic and is here recommended.

**Biogeographic pattern and rate of speciation**—*Carex aquatilis-Carex lenticularis* group—The *Carex aquatilis-Carex lenticularis* group shares its most recent common ancestor with a clade of largely northwestern North American montane species. It diverged from that ancestor as early as 2.55 mya during the end of the cool Pliocene (Fig. 1). As the ice ages of the Pleistocene began (1.89 mya), the group diverged into two distinct lineages, the *C. lenticularis* lineage and the *C. aquatilis* lineage.

The *Carex aquatilis* lineage is inferred to have radiated around 0.76 mya, during the Nebraskan ice age, a time marked by relatively short periods of extreme changes in climate. The data suggest that the divergence of *C. aquatilis* var. *dives* toward the end of the Nebraskan (0.65 mya) was due to ecological isolation in brackish waters from the widespread populations of freshwater *C. aquatilis* varieties. Disjunct freshwater populations of var. *dives* in the mountains of North Carolina are sister to the larger western North American populations; this geographic pattern is shared with numerous northern taxa that became isolated in the high altitudes of the Appalachians during the Pleistocene (White et al., 1984). The data further suggest that the *C. subspathacea* sublineage subsequently became ecologically isolated as well, but in even more saline habitats, possibly as advancing ice pushed some populations into even more saline habitats in North America and Eurasia, with additional freshwater varieties of *C. aquatilis* diverging in the isolation of the Kansan glacial period (ca. 0.30 mya). Similar patterns of ecological divergence are reflected in other taxonomic groups in which halophytes and glycophytes share a common ancestor (Rieseberg et al., 2003; Levin, 2004; Shepherd et al., 2004; O’Quinn and Hufford, 2005).

In contrast, the *C. lenticularis* lineage diverged much earlier, beginning in southwestern North America or Mexico, at least 1.50 mya. The lineage appears to have undergone an early tropical-alpine isolation during this pre-Nebraskan warm period, followed by dispersal to northwestern and northeastern North America shortly thereafter, with *C. kelloggii* and *C. enanderi* diverging from the ancestor of the lineage around 1.00 mya, just prior to the Nebraskan glacial age. The continued divergence of these new lineages may reflect a common pattern of postglacial expansion of distinct lineages in eastern and western North America (circa 0.60 mya) resulting from geographic isolation in regions with very different topographic and glacial histories (Hewitt and Ibrahim, 2001; Hewitt, 2003; Austin et al., 2004).

The most frequent haplotype of the three western North American varieties of *C. kelloggii* has its geographic center in southeastern Idaho. The remaining haplotype groups are inferred to result from radiation from this region to the northwest, the northeast, the southwest, and the south. Southern populations appear to be diverging in isolation, divided from the more northern populations by the desert of the southwestern United States. The low infraspecific morphological and genetic variation in northern populations of *C. kelloggii* reflects a pattern of variation observed in other organisms of western North American distribution: (1) infraspecific morphological variation but low genetic diversity and (2) higher endemism west of the Rocky Mountains (Hewitt and Ibrahim, 2001; Brunsfeld et al., 2001; Good and Sullivan, 2001). This pattern is thought to be a product of oscillating periods of montane isolation and lowland genetic exchange with Pleistocene climatic cycling in a region of high topographic and climatic diversity. Repeated episodes of gene flow and isolation could limit the segregation of genetic diversity among populations.

**Conclusion**—The genus *Carex* L. is one of the largest genera of flowering plants, occurring in nearly all habitat types, but particularly common in the extratropical wetlands of the world. Members of the genus are important taxa in wet meadows in Canada and Eurasia where they provide fodder and habitat for many wild and domesticated animals and filter water. *Carex aquatilis* s.l. represents a widespread and ecologically important taxon ideally suited for the study of the role of hybridization and niche partitioning in ecological speciation. This study clarifies the phylogenetic context in which evolutionary processes have acted on this group and includes the first attempt at dating speciation events within the genus in the absence of suitable fossil evidence.
The C. aquatilis-C. lenticularis group comprises two major lineages whose complex evolutionary histories have been characterized by ecological specialization as constrained by the recent geological history of North America. The C. aquatilis lineage evidences two recent transformations in habitat preference in the north, one leading to a distinctive monophyletic sublineage of coastal halophytic species (the C. subspathacea sublineage) and the other to brackish-water-tolerant populations of Carex aquatilis var. dives. The remainder of the clade comprises a widespread and morphologically diverse series of facultatively glycophytic populations of a paraphyletic Carex aquatilis. This paraphyly possibly reflects the survival of ancestral freshwater C. aquatilis alongside the C. subspathacea sublineage, which has undergone divergence isolated at least in part in saline environments. Contact zones between habitat types may be regions of hybrid formation and recurrent gene flow (Volkova et al., 2008).

### Table 2. Estimated ages of the basal divergence of the major clades of the Carex aquatilis-lenticularis group based on penalized-likelihood analysis. Confidence intervals were estimated only for clades in boldfaced type.

<table>
<thead>
<tr>
<th>Clade no.</th>
<th>Clade name</th>
<th>Estimated dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Carex aquatilis-lenticularis clade</td>
<td>1.89–1.07</td>
</tr>
<tr>
<td>II</td>
<td>Carex aquatilis sensu lato</td>
<td>0.76–0.64</td>
</tr>
<tr>
<td>III</td>
<td>Carex aquatilis var. dives clade</td>
<td>0.65</td>
</tr>
<tr>
<td>IV</td>
<td>Carex aquatilis, Eurasian clade</td>
<td>0.32</td>
</tr>
<tr>
<td>V</td>
<td>Carex subspathacea clade</td>
<td>0.25</td>
</tr>
<tr>
<td>VI</td>
<td>Carex aquatilis, No. American clade</td>
<td>0.14</td>
</tr>
<tr>
<td>VII</td>
<td>Carex lenticularis and allies</td>
<td>1.50–1.11</td>
</tr>
<tr>
<td>VIII</td>
<td>Carex hermannii plus clade IX</td>
<td>1.31</td>
</tr>
<tr>
<td>IX</td>
<td>Carex kelloggii-enanderi clade</td>
<td>1.00</td>
</tr>
<tr>
<td>X</td>
<td>Carex cuchumatanensis plus clade X</td>
<td>1.16</td>
</tr>
<tr>
<td>XI</td>
<td>Carex lenticularis sensu lato</td>
<td>0.99</td>
</tr>
<tr>
<td>XII</td>
<td>Carex eleasinoides-plectocarpa clade</td>
<td>0.60</td>
</tr>
<tr>
<td>XIII</td>
<td>Carex ryphina-lenticularis-decidua clade</td>
<td>0.38</td>
</tr>
</tbody>
</table>

The C. lenticularis clade includes two sublineages. In the monophyletic clade that includes typical C. lenticularis, the data suggest that an early northern-neotropical history of divergence subsequent to the origination of the volcanic mountains of Mexico was followed by a more recent history in temperate northern North America. In contrast, the clade that includes paraphyletic C. kelloggii presents no clear patterns of diversification.

The interest in species nonmonophyly is increasing (Syring et al., 2007) and has been identified in the genus Carex by several researchers (Roalson and Friar, 2004a, b; Hipp et al., 2006; King and Roalson, 2008). Potential sources of apparent nonmonophyly include methodological sources such as inadequate phylogenetic signal, amplification of paralogous loci, imperfect taxonomy, as well as biological sources including introgressive hybridization, incomplete lineage sorting, recombination of divergent alleles. While these phenomena complicate phylogenetic analyses, they can provide cases with which to examine species-level divergence processes. The data described here indicate that the C. aquatilis lineage, with its high level of diversity and its broad distribution, has the potential to provide a model lineage with which to examine speciation and processes that confound phylogenetic inquiry, including hybridization, chromosome change, rapid radiation, and ecological divergence. In light of global climate change, an improved understanding of the processes that lead to rapid speciation and habitat shift, particularly toward increased saline tolerance, is critical.

### LITERATURE CITED


Egorova, T. V. 1999. The sedges (Carex L.) of Russia and adjacent states (within the limits of the former USSR). Missouri Botanical Garden, St. Louis, Missouri, USA.


Appendix 1. Collection data and GenBank accession numbers for all specimens of Carex used in this study. Specimens without GenBank accession numbers were used for morphological analyses only. Where present, the number in parentheses after the taxon name is the number assigned to that accession for use in the molecular analyses involving more than one voucher of the same species. Of these, the specimens indicated in Table 3 and Fig. 1 as in need of taxonomic revision are presented as originally identified, but were submitted to GenBank with their annotated names. Among the GenBank accession numbers, N/A indicates where a sequence is not available for a particular specimen. Herbarium acronyms follow Index Herbariorum (http://sweetgum.nybg.org/IH/).

Taxon (accession no.); Origin; (Herbarium); GenBank accessions: ITS, ETS 1F, and psbA-trnH.