American Journal of Botany 96(10): 1896–1906. 2009.

SYSTEMATICS OF THE *CAREX AQUATILIS* AND *C. LENTICULARIS* LINEAGES: GEOGRAPHICALLY AND ECOLOGICALLY DIVERGENT SISTER CLADES OF *CAREX* SECTION *PHACOCYSTIS* (CYPERACEAE)[1]

JULIE A. DRAGON[2] 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 hampered 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., Brumsfeld 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

[1] Manuscript received 4 December 2008; revision accepted 19 June 2009.

The authors thank all the scientists who assisted with this research, especially C. Paris, L. Standley, M. Waterway, S. Schmitz, J. Cayouette, staff at the herbaria that provided research materials, and numerous botanists whose generous efforts to collect specimens for this research helped to give it such wide geographic and taxonomic breadth.

[2] Author for correspondence (Julie.Dragon@uvm.edu); present address: Department of Biology, University of Vermont 109 Carrigan Dr., Burlington, VT 05405-0086 USA

doi:10.3732/ajb.0800404

Sullivan, 2001; Hewitt and Ibrahim, 2001). The complex history of the *C. aquatilis-C. lenticularis* group is likely the result of these same processes.

In this study, additional molecular and morphological data from extensive sampling of section *Phacocystis* were analyzed with the specific goals of (1) elucidating the broader phylogenetic history of the *Carex aquatilis-Carex lenticularis* group, (2) recircumscribing the species and varieties within the lineages as monophyletic taxa where practicable, and (3) inferring the biogeographic history of the lineages in relation to Pleistocene events in North America. Our hypotheses were (1) that the *C. aquatilis-C. lenticularis* group is monophyletic but (2) that it includes several other species, including the maritime species with which *C. aquatilis* is suggested to hybridize, (3) that it is sister to *C. lenticularis* and its allies, and (4) that Pleistocene glacial events in western North America affected patterns of speciation within the group.

MATERIALS AND METHODS

Taxon sampling—We examined 157 specimens representing 42 species and five varieties. Of these, 107 specimens were used in the molecular analysis. This sample represents approximately 48% of section *Phacocystis*, including nearly all of the North American taxa and all of the major recognized species groups, including species potentially a part of the *Carex aquatilis-Carex lenticularis* group (sensu Standley et al., 2002; Dragon and Barrington, 2008). The sample also includes relevant outgroup taxa from sections that we previously determined to be candidate members of section *Phacocystis* or closely related to it (Dragon and Barrington, 2008). A complete list of taxa used in this study, including GenBank accession numbers and voucher information, is presented in Appendix 1.

DNA extraction, amplification, and sequencing—Total genomic DNA was extracted from fresh material using the DNEasy Plant Mini Kit (Qiagen, Valencia, California, USA) and from silica-dried material with a CTAB buffer following the protocol of Doyle and Doyle (1987). Using the Techne TC-312 thermocycler (Techne, Duxford, UK) and the polymerase chain reaction (PCR), we used to isolate templates of double-stranded PCR product for two nuclear and one chloroplast marker. PCR of the internal transcribed spacer region (ITS) and the included 5.8S rDNA used the primers ITS4 (White et al., 1990) and ABI1 (Sun et al., 1994) in the following 25 μL reaction mixture: 1× reaction buffer, 3 μM $MgCl_2$, 5% betaine, 200 μM of each dNTP, 1 μM of both primers, 1 U *TaKaRa Ex Taq* DNA polymerase (TaKaRa Bio, Shiga, Japan), and 50–100 ng of genomic DNA template. Amplification of the DNA employed the following thermocycling protocols: 94°C initial denaturing for 2 min; followed by 32 cycles of 96°C denaturing for 1 min, 50°C annealing for 1 min, and 72°C extension for 1 min; followed by a final extension at 72°C for 7 min. PCR of one of the external transcribed spacer fragments (ETS 1f) employed the primers ETSF and 18S (Starr et al., 2003) in the following 25 μL reaction mixture: 1× reaction buffer (50 μM KCl, 10 μM Tris-HCl, 2.5 μM $MgCl_2$,) 1× betaine, 200 μM of each dNTP, 1 μM of both primers, 1 U *TaKaRa Ex Taq* DNA polymerase (TaKaRa Bio), and 50–100 ng of genomic DNA template. Amplification followed the thermocycling protocols of Starr et al. (2003). PCR of the *psbA-trnH* spacer was completed using primers psbA and trnH (GUG) of Saltonstall (2001) and two internal primers that we designed from the *rps19* gene (which in some monocots, including *Carex*, lies within the *psbA-trnH* spacer); these were psbAR (5′ CAA TGG TTG GCC ATA CAA TCG 3′) and psbAF (5′ CGA TTG TAT GGC CAA CCA TTG 3′). While some accessions were successfully amplified using just the two external primers, most were amplified in two parts using the two internal primers. The PCR was conducted in the following 25 μL reaction mixture: 1× reaction buffer, 200 μM of each dNTP, 1 μM of both primers, 1 U *TaKaRa Ex Taq* DNA polymerase (TaKaRa Bio), and 50–100 ng of genomic DNA template. Amplification of the DNA employed the following thermocycling protocols: 94°C initial denaturing for 1 min; followed by 30 cycles of 94°C denaturing for 1 min, 57.5°C annealing for 1 min, and 72°C extension for 2 min; followed by a final extension at 72°C for 10 min.

Sequence alignment and coding—The PCR products were electrophoresed on 1% agarose gels in 1× Tris-borate-EDTA (TBE) buffer (pH 8.0) and stained with ethidium bromide. Cycle sequencing used the external sequencing primers ITSL and ITS4 (White et al., 1990) for ITS and the same primers as were 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 the Bayesian analysis setting for both models is identical. For ITS, both hLRT and AIC picked the K81uf+I+G model to best explain the distribution of the variation observed. For *psbA-trnH*, hLRT picked F81+I though F81+I+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 K81uf+I+G, while AIC indicated TVM+I+G to be the best model. Because K81uf+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 (Swofford, 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 (Swofford, 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 (MulTrees) and with ACCTRAN character-state optimization. Bootstrap values (BS) were determined for 1700 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 *C. 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 *C. 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 = 2 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 30 001 Bayesian inference trees sampled reached stationary at around tree 5000 in the first run and tree 2600 in the second run. The majority-rule consensus tree was produced from the remaining trees of each run separately. The consensus trees from the two runs converged on roughly the same posterior probabilities for all nodes; we chose the tree from the first run to represent the relationships among the taxa (Fig. 1). Strong support (95% CI; Zander, 2004) was found for the sister relationship of the *Carex aquatilis*-*Carex lenticularis* group (clade I, PP 100%), as well as the *C. aquatilis* lineage (clade II, PP 99%) and the *C. lenticularis* lineage (clade VII, PP 100%). Within the *C. aquatilis* lineage, strong support was also found for three clades of a paraphyletic *C. aquatilis* s.s.: (1) the North American *C. aquatlis* (clade VI, PP 96%), (2) the Eurasian *C. aquatilis* (clade IV, PP 97%), and (3) the *C. subspathacea* sublineage (clade V, PP 100%). Within the *C. lenticularis* lineage, nearly all the relationships inferred were supported by a 97–100% PP.

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

Sequence data	ITS	ETS 1f	*psbA-trnH*
Aligned length (bp)	453	577	533
Length range (bp)	451–453	572–577	533
Variable sites	108	126	32
Phylogenetically informative sites	71	55	15
Autapomorphies	37	71	17
Indels	1	6	0
AIC model	K81uf+I+G	GTR+G	F81+I

The three maximum likelihood trees generated under the three different evolutionary models were topologically identical, with only minor differences in aLRT values and branch lengths. The GTR ML tree (Fig. 2) and the BI consensus tree suggested that different taxa were sister to the *Carex aquatilis*-*Carex lenticularis* group, though neither was strongly supported. Within the *C. aquatilis*-*C. lenticularis* group, topological differences in the ML tree largely involved greater resolution of polytomies inferred in the BI tree; however, these relationships were also too weakly support to be reliable. The maximum parsimony analysis yielded eight most parsimonious trees of 461 steps (CI = 0.616, RI = 0.783, HI = 0.384). In the ingroup, differences in the eight trees involved the same poorly supported clades (IX) as were found in the Bayesian analysis. The consensus MP tree (not shown) was topologically most similar to the ML tree, with greater clade resolution than the BI tree, but it also lacked support for those clades.

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 *C. aquatilis* lineage (clade II) and the *C. 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 *C. aquatilis* lineage (clade II) to lie between 0.76 and 0.64 mya and in the *C. 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 *C. aquatilis*-*C. 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 *C. aquatilis*-*C. 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.

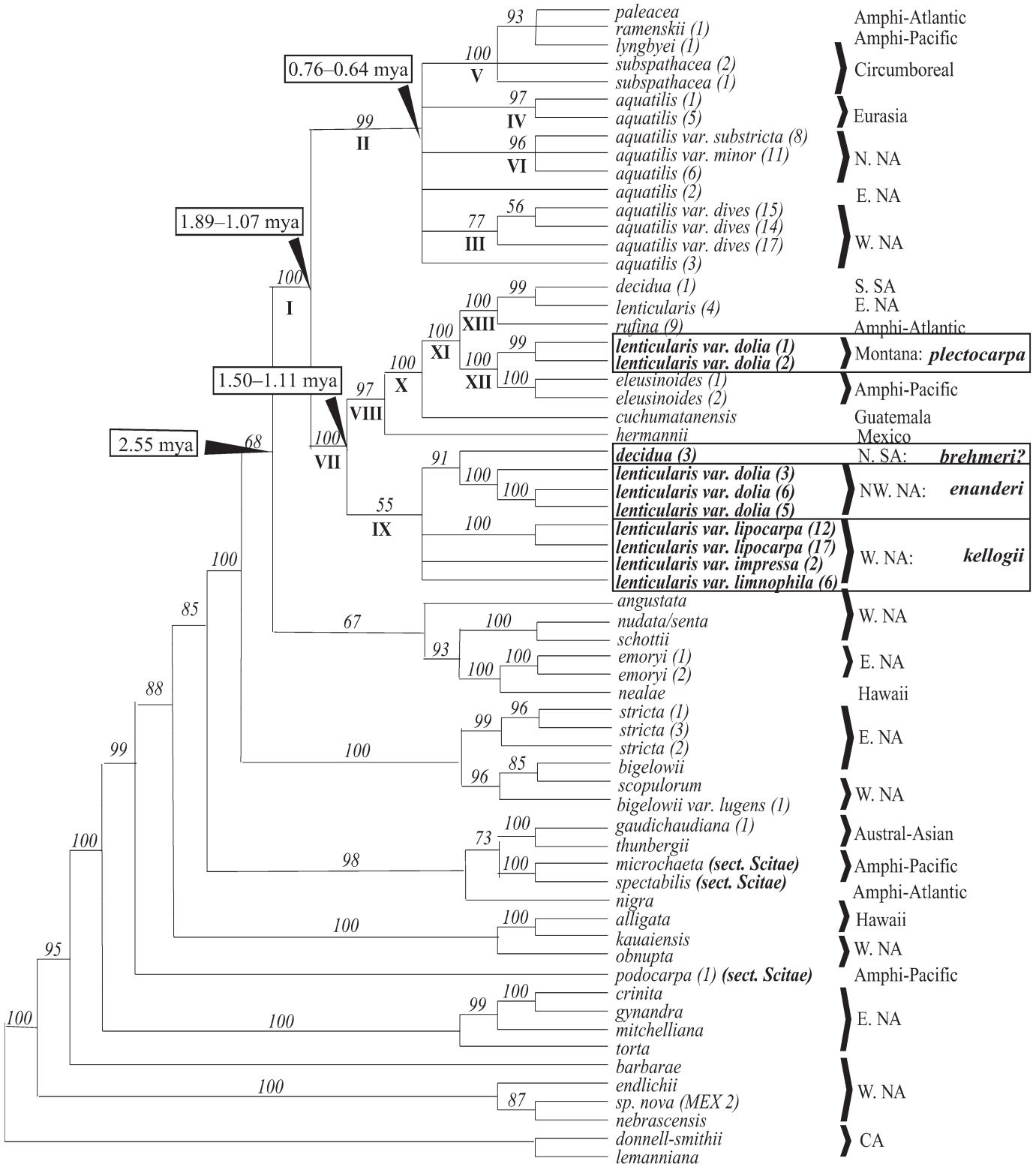

Fig. 1. The majority rule consensus of 25 002 Bayesian inference trees. Posterior probabilities greater than or equal to 50% are indicated above the branch. Roman numerals below the branches indicate clade numbers. Numbers in parentheses indicate the accession used in the final analysis. Geographic range 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 refugial 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 gynaecandrous 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 synapomories 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. cuchumatanensis* 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

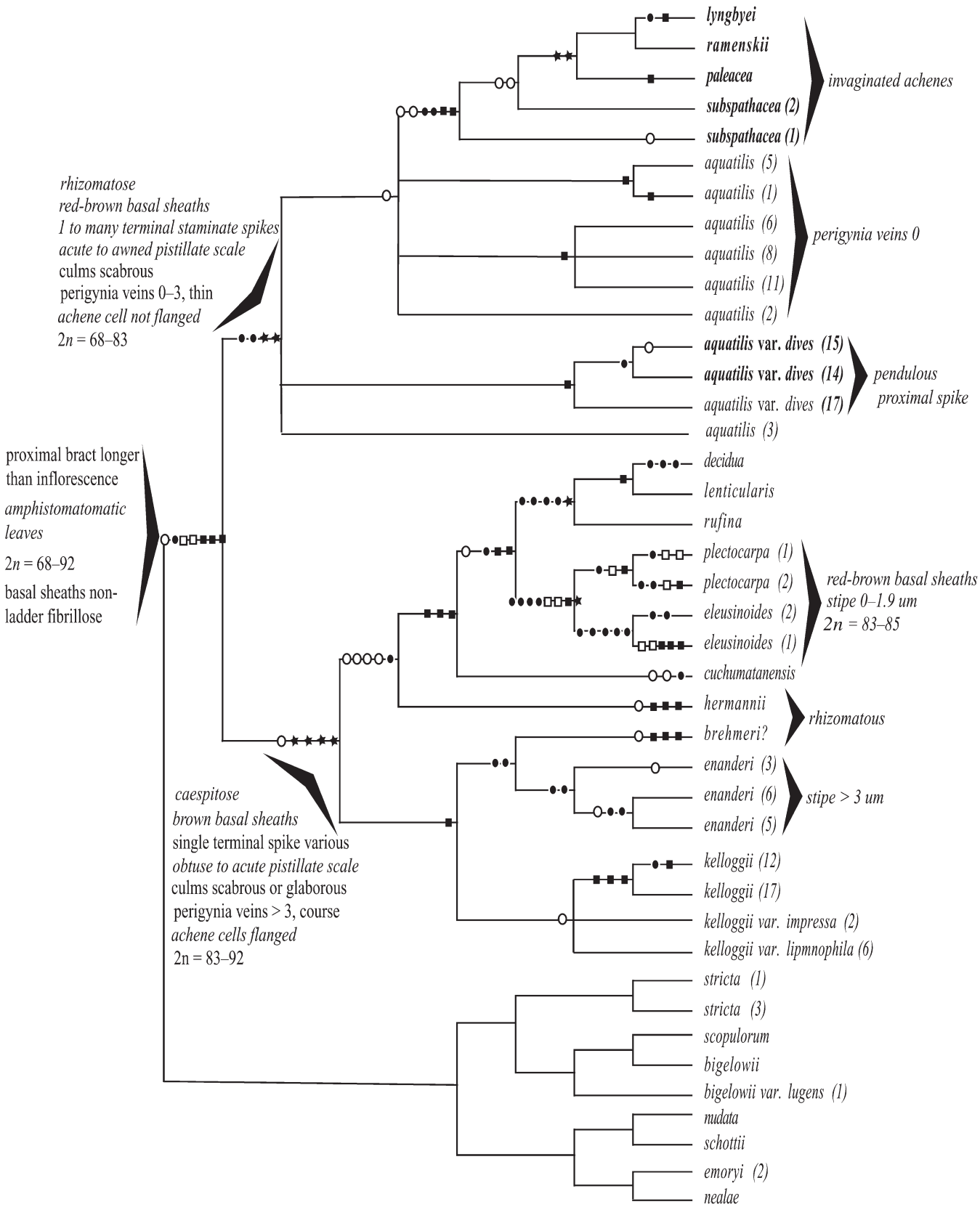

Fig. 2. The *Carex aquatilis-Carex lenticularis* group and its sister clade from the maximum likelihood tree. Salt-tolerant taxa are indicated in boldface. Supporting molecular and partial morphological data are shown along the branches for the ingroup. ETS 1f nucleotides are shown as squares, ITS as 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, papillose perigynia that are beakless 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 homoplastic within several sections. The inclusion of the tristigmatic, 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.

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.

Clade no.	Clade name	Estimated dates
I	***Carex aquatilis-lenticularis* clade**	**1.89–1.07**
II	***Carex aquatilis* sensu lato**	**0.76–0.64**
III	*Carex aquatilis* var. *dives* clade	0.65
IV	*Carex aquatilis,* Eurasian clade	0.32
V	*Carex subspathacea* clade	0.25
VI	*Carex aquatilis,* No. American clade	0.14
VII	***Carex lenticularis* and allies**	**1.50–1.11**
VIII	*Carex hermannii* plus clade IX	1.31
IX	*Carex kelloggii-enanderi* clade	1.00
X	*Carex cuchumatanenesis* plus clade X	1.16
XI	*Carex lenticularis* sensu lato	0.99
XII	*Carex eleusinoides-plectocarpa* clade	0.60
XIII	*Carex rufina-lenticularis-decidua* clade	0.38

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 3. The members of the *Carex aquatilis-Carex lenticularis* group as named in the most recent treatments of the species (Standley, 1987; Standley et al., 2002) and as we choose to recognize them based on this and previous work (Dragon, 2006).

Clade	Previously recognized species name	Taxonomic revision based on present study
III	*C. subspathacea*	No change
III	*C. lyngbyei*	No change
III	*C. ramenskii*	No change
III	*C. paleacea*	No change
IV	*C. aquatilis* var. *dives*	No change
V	C. *aquatilis* var. *minor*	No change pending broader sampling
V	*C. aquatilis* var. *substricta*	No change pending broader sampling
VI	*C. aquatilis*	No change pending broader sampling
XIII	*C. lenticularis* var. *lipocarpa*	*C. kelloggii* Boott
XIII	*C. lenticularis* var. *impressa*	*C. kelloggii* var. *impressa* (L. H. Bailey) L. A. Standley
XIII	*C. lenticularis* var. *limnophila*	*C. kelloggii* var. *limnophila* (T. Holm) Cronquist
XIV	*C. lenticularis* var. *dolia*	*C. enanderi* Hultén
VIII	*C. hermannii*	No change
VIII	*C. decidua*	*C. brehmeri* Boeck. (tentatively)
VIII	*C. cuchumatanensis*	No change
XI	*C. rufina*	No change
XI	*C. lenticularis*	No change
XI	*C. decidua*	No change
XII	*C. lenticularis* var. *dolia*	*C. plectocarpa* F. J. Hermann
XII	*C. eleusinoides*	No change

The *C. lenticularis* lineage 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 increased understanding of the processes that lead to rapid speciation and habitat shift, particularly toward increased saline tolerance, is critical.

LITERATURE CITED

ANISIMOVA, M., AND O. GASCUEL. 2006. Approximate likelihood ratio test for branches: A fast, accurate and powerful alternative. *Systematic Biology* 55: 539–552.

AUSTIN, J. D., S. C. LOUGHHEED, AND P. T. BOAG. 2004. Discordant temporal and geographic patterns in maternal lineages of eastern North American frogs, *Rana catesbeiana* (Ranidae) and *Pseudacris crucifer* (Hylidae). *Molecular Phylogenetics and Evolution* 32: 799–816.

AXELROD, D. I., AND P. H. RAVEN. 1985. Origins of the Cordilleran flora. *Journal of Biogeography* 12: 21–47.

BRUNSFELD, S. J., J. SULLIVAN, D. E. SOLTIS, AND P. S. SOLTIS. 2001. Comparative phylogeography of northwestern North America: A synthesis. *In* J. Silvertown and J. Antonovics [eds.], Integrating ecology and evolution in a spatial context, 319–339. Blackwell Science, Oxford, UK.

CAYOUETTE, J., AND P. MORISSET. 1985. Chromosome studies on natural hybrids of *Carex* (sections *Phacocystis* and *Cryptocarpae*) in northeastern North America, and their taxonomic implications. *Canadian Journal of Botany* 63: 1957–1982.

CAYOUETTE, J., AND P. MORISSET. 1986. Chromosome studies on *Carex paleacea* Wahl., *C. nigra* (L.) Reichard, and *C. aquatilis* Wahl. in northeastern North America. *Cytologia* 51: 817–856.

CUTLER, D. J. 2000. Estimating divergence times in the presence of an overdispersed molecular clock. *Molecular Biology and Evolution* 17: 1647–1660.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

DRAGON, J. A. 2006. Molecular phylogeny and systematics of the *Carex aquatilis* group section *Phacocystis* (Cyperaceae). Ph.D. dissertation, University of Vermont, Burlington, Vermont, USA.

DRAGON, J. A., AND D. S. BARRINGTON. 2008. East vs. West: Monophyletic clades within the paraphyletic *Carex acuta* complex, section *Phacocystis* (Cyperaceae). *In* R. F. C. Naczi and B. A. Ford [eds.], Sedges: Uses, diversity, and systematics of the Cyperaceae, 215–226. Monographs in Systematic Botany no. 108. Missouri Botanical Garden Press, St. Louis, Missouri, USA.

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.

FAULKNER, J. S. 1973. Experimental hybridization of north-west European species in *Carex* section *Acutae* (Cyperaceae). *Botanical Journal of the Linnean Society* 67: 233–253.

GOOD, J. M., AND J. SULLIVAN. 2001. Biogeography of the red-tailed chipmunk (*Tamias rufiicaudus*), a northern Rocky Mountain endemic. *Molecular Ecology* 10: 2683–2695.

GUINDON, S., AND O. GASCUEL. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology* 52: 696–704.

HEWITT, G. M. 2003. Ice ages, species distributions, and evolution. *In* L. J. Rothschild and A. Lister [eds.], Evolution on planet earth: The impact on the physical environment, 339–361. Academic Press, Boston, Massachusetts, USA.

HEWITT, G. M., AND K. M. IBRAHIM. 2001. Inferring glacial refugia and historical migrations with molecular phylogenies. *In* J. Silvertown and J. Antonovics [eds.], Integrating ecology and evolution in a spatial context, 271–294. Blackwell Sciences, Oxford, UK.

HIPP, A. L., A. A. REZNICEK, P. E. ROTHROCK, AND J. A. WEBER. 2006. Phylogeny and classification of *Carex* section *Ovales* (Cyperaceae). *International Journal of Plant Sciences* 167: 1029–1048.

KING, M. G., AND E. H. ROALSON. 2008. Exploring evolutionary dynamics of nrDNA in *Carex* subgenus *Vignea* (Cyperaceae). *Systematic Botany* 33: 514–524.

KRESS, W. J., K. J. WURDACK, E. A. ZIMMER, L. A. WEIGT, AND D. H. JANZEN. 2005. Use of DNA barcodes to identify flowering plants. *Proceedings of the National Academy of Sciences, USA* 102: 8369–8374.

KÜKENTHAL, G. 1909. Cyperaceae–Caricoideae. *In* A. Engler [ed.], Das Pflanzenreich IV, 20, Heft 38. Englemann, Leipzig, Germany.

LEVIN, D. A. 2004. Ecological speciation: Crossing the divide. *Systematic Botany* 29: 807–816.

MACKENZIE, K. K. 1935. Cyperaceae–Cariceae. *North American Flora* 18: 169–478.

O'QUINN, R., AND L. HUFFORD. 2005. Molecular systematics of Montieae (Portulacaceae): Implications for taxonomy, biogeography, and ecology. *Systematic Botany* 30: 314–331.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: Testing the model of DNA substitutions. *Bioinformatics* 14: 817–818.

PRICE, J. P. 2004. Floristic biogeography of the Hawaiian Islands: Influences of area, environment and paleogeography. *Journal of Biogeography* 31: 487–500.

RIESEBERG, L. H., O. RAYMOND, D. M. ROSENTHAL, Z. LAI, K. LIVINGSTONE, T. NAKAZATO, J. L. DURPHY, ET AL. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* 301: 1211–1216.

REZNICEK, A. A. 1990. Evolution in sedges (*Carex*, Cyperaceae). *Canadian Journal of Botany* 68: 1409–1432.

ROALSON, E. H., AND E. A. FRIAR. 2004a. Phylogenetic analysis of the nuclear alcohol dehydrogenase (*Adh*) gene family in *Carex* section *Acrocystis* (Cyperaceae) and combined analyses of *Adh* and nuclear ribosomal ITS and ETS sequences for inferring species relationships. *Molecular Phylogenetics and Evolution* 33: 671–686.

ROALSON, E. H., AND E. A. FRIAR. 2004b. Phylogenetic relationships and biogeographic patterns in *Carex* section *Acrocystis* (Cyperaceae) using nrDNA ITS and ETS sequence data. *Plant Systematics and Evolution* 243: 175–187.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

SALTONSTALL, K. 2001. A set of primers for amplification of noncoding regions of chloroplast DNA in the grasses. *Molecular Ecology Notes* 1: 76–78.

SANDERSON, M. J. 2003. r8s: Inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics* 19: 301–302.

SHEPHERD, K. A., M. WAYCOT, AND A. CALLADINE. 2004. Radiation of the Australian Salicornioideae (Chenopodiaceae)—Based on evidence from nuclear and chloroplast DNA sequences. *American Journal of Botany* 91: 1387–1397.

STANDLEY, L. A. 1987. Anatomical studies of *Carex cuchumatanensis, C. decidua, C. hermannii* (Cyperaceae) and comparisons with North American taxa of the *C. acuta* complex. *Brittonia* 39: 11–19.

STANDLEY, L. A., J. CAYOUETTE, AND L. BRUEDERLE. 2002. *Carex* sect. *Phacocystis* Dumortier. *In* Flora of North America Editorial Committee [eds.], Flora of North America, north of Mexico, vol. 23, 379–401. Oxford University Press, New York, New York, USA.

STARR, J. R., S. A. HARRIS, AND D. A. SIMPSON. 2003. Potential of the 5′ and 3′ ends of the intergenic spacer (IGS) of rDNA in the Cyperaceae: New sequences for lower-level phylogenies in sedges with an example from *Uncinia* Pers. *International Journal of Plant Sciences* 164: 213–227.

SUN, Y., D. Z. SKINNER, G. H. LIANG, AND S. H. HULBERT. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 29–32.

SWOFFORD, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, Massachusetts, USA.

SYRING, J., K. FARRELL, R. BUSINSKY, R. CRONN, AND A. LISTON. 2007. Widespread genealogical nonmonophyly in species of *Pinus* subgenus *Strobus. Systematic Biology* 56: 163–181.

TAMURA, K., J. DUDLEY, M. NEI, AND S. KUMAR. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596–1599.

VOLKOVA, P. A., A. B. SHIPUNOV, R. ELVEN, AND C. BROCHMANN. 2008. The seashore sedges of the Russian Kola Peninsula: How many species? *Flora* 203: 523–533.

WHITE, P. S., R. I. MILLER, AND G. S. RAMSEUR. 1984. The species–area relationship of the Southern Appalachian high peaks: Vascular plant richness and rare plant distributions. *Castanea* 49: 47–61.

WHITE, T. J., T. D. BRUNS, S. B. LEE, AND J. W. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White [eds.], PCR protocols: A guide to methods and applications, 315–322. Academic Press, San Diego, California, 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 under 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, *Voucher*; (Herbarium); GenBank accessions: ITS, ETS 1f, and *psbA-trnH*.

Carex acuta* var. *pallida Boott; USA, Oregon (type), *Lyall s. n. 1861*; (GH). ***C. alligata*** Boott; USA, Hawaii, Maua'i, *CS04-012*; (VT); GQ223562, GQ223477, GQ223642. ***C. angustata*** Boott; USA, California, *L. Janeway 7823*; (VT); GQ223563, GQ223479, GQ223644. ***C. aquatilis*** Wahl. (1); Finland, *Kukonen 12828*; (RSA); AF284892, AY770435, GQ223645. ***C. aquatilis*** Wahl. (2); USA, Alaska, *J. Dragon 03-82*; (VT); GQ223564, GQ223480, GQ223646. ***C. aquatilis*** Wahl. (3); USA, Arizona, *Carl-Eric Granfelt 70-75*; (ARIZ); GQ223572, GQ223488, GQ223652. ***C. aquatilis*** Wahl. (5); Russia, Siberia, *M. Fsor s. n.*, Aug. 2, 1980; (US); N/A, GQ223492, N/A. ***C. aquatilis*** Wahl. (6); USA, Utah, *Maguire et al. 16485*; (VT); GQ223576, GQ223493, GQ223654. ***C. aquatilis*** Wahl.; Lapland (type), *Wahlenberg s. n.*; (UPS). ***C. aquatilis*** Wahl.; Finland, South Häme, *P. Uotila 24209 and S. Vuokko*; (LAS). ***C. aquatilis*** Wahl.; Finland, Larsmo, *C. Cedercreutz s. n.*; (VT). ***C. aquatilis*** Wahl.; USA, Colorado, *L. A. Standley 453*; (LAS). ***C. aquatilis*** Wahl.; USA, Washington, *L. A. Standley 390*; (LAS). ***C. aquatilis*** Wahl.; Canada, Yukon, *S. G. Aiken 87226*; (LAS). ***C. aquatilis*** Wahl.; USA, Montana, *P. P. Lowry 2646*; (LAS). ***C. aquatilis* var. *dives*** (T. Holm) Kük. (14); USA, California, *L. Janeway 7935*; (VT); GQ223571, GQ223487, GQ223651. ***C. aquatilis* var. *dives*** (T. Holm) Kük. (15); USA, Washington, *L. A. Standley 83*; (LAS); GQ223575, GQ223491, GQ223653. ***C. aquatilis* var. *dives*** (T. Holm) Kük. (16); USA, Alaska, *Brad Krieckhaus 139*; (VT); GQ223569, GQ223485, N/A. ***C. aquatilis* var. *dives*** (T. Holm) Kük. (17); USA, North Carolina, *L. A. Standley s. n.*; (VT); GQ223573, GQ223489, N/A. ***C. aquatilis* var. *dives*** (T. Holm) Kük.; USA, Oregon (type), *Henderson s. n., 1883*; (US). ***C. aquatilis* var. *dives*** (T. Holm) Kük.; USA, Oregon, *L. A. Standley 226*; (LAS). ***C. aquatilis* var. *minor*** Boott (11); USA, Alaska, *B. Bennett 02-048*; (B. A. Bennett Herbarium); GQ223568, GQ223484, N/A. ***C. aquatilis* var. *minor*** Boott (12); Canada, Ellesmere Island, *B. Bennett 00-1015*; (B. A. Bennett Herbarium); GQ223567, GQ223483, GQ223649. ***C. aquatilis* var. *minor*** Boott (13); USA, Alaska, *M. K. Raynolds s. n.*, Aug. 2003; (VT); GQ223570, GQ223486, GQ223650. ***C. aquatilis var. minor*** Boott; Greenland, Godhavn Disco, *G. Kleist s. n.*, 1905; (VT); N/A, GQ223496, N/A. ***C. aquatilis var. minor*** Boott; Magadan Oblast, Chukotka, *C. L. Parker 4671*; (ALA); GQ223578, GQ223495, GQ223656. ***C. aquatilis* var. *substricta*** Kük.; USA, New York (type), *Sartwell 56*; (GH). ***C. aquatilis* var. *substricta*** Kük. (7); USA, Montana, *P. Lesica 7531*; (LAS); GQ223574, GQ223490, N/A. ***C. aquatilis* var. *substricta*** Kük. (8); USA, Vermont, *J. Dragon 02-70*; (VT); AY770467, AY770436, GQ223647. ***C. aquatilis* var. *substricta*** Kük. (9); USA, Vermont, *J. Dragon 02-69*; (VT); GQ223565, GQ223481, N/A. ***C. aquatilis* var. *substricta*** Kük.; USA, Vermont, *L. A. Standley 941*; (LAS); ***C. aquatilis* var. *substricta*** Kük.; Canada, Alberta, *G. H. Turner 1941*; (VT). ***C. aquatilis* var. *substricta*** Kük.; USA, New York, *B. W. Tinker 3197*; (VT).

C. barbarae Dewey; USA, California, *L. Janeway 8242*; (VT); GQ223579, GQ223497, GQ223657. ***C. bigelowii*** Torr.; USA, Vermont, *J. Dragon 02-1b*; (VT); AY770469, AY770438, GQ223658. ***C. bigelowii* var. *lugens*** (T. Holm) T. V. Egorova, (1); USA, Alaska, *J. Dragon 03-91*; (VT); GQ223580, GQ223498, GQ223659. ***C. bigelowii* var. *lugens*** (T. Holm) T. V. Egorova, (2); USA, Alaska, *B. Bennett 03-527*; (B. A. Bennett Herbarium); GQ223581, GQ223499, GQ223660.

C. crinita Lam.; Canada, Quebec, *Waterway 99.002* (MTMG); AY757650, AY757589, N/A. ***C. cuchumatanensis*** Standl. & Steyerm.; Guatemala, Sierra de los Cuchumatanes, *Beaman 3897* (US); GQ223582, GQ223500, GQ223661. ***C. cuchumatanensis*** Standl. & Steyerm.; Guatemala, Nucá, *J. Steyermark 49769*; (F) and (US). ***C. cuchumatanensis*** Standl. & Steyerm.; Guatemala, Tunimá (type), *J. Steyermark 48347*; (F) and (US). ***C. cuchumatanensis*** Standl. & Steyerm.; Guatemala, Chiantla, *D. Smith 175*; (F).

C. decidua Boott (1); Argentina, Tierra del Fuego, *M. Robson s. n.*, Feb. 2000; (VT); GQ223584, GQ223502, GQ223663. ***C. decidua*** Boott (2); Argentina, Peninsula Brunswick, *O. Dollenz 1008*, Jan. 14, 1982; (GH); GQ223585, GQ223503, GQ223664. ***C. decidua*** Boott (3); Columbia, Sierra Madre del Cocuy, *Grubb et al. 306*; (US); N/A, GQ223504, N/A. ***C. decidua*** Boott; Argentina, Gobernación del Rio Negro, *M. Barros 2320*; (F). ***C. decidua*** Boott; ARGENTINA, Sta. Cruz, *H. Sleumer 1299*; (US). ***C. decidua*** Boott; Argentina, Tierra Del Fuego (type), *Banks and Solander s. n.*; (GH). ***C. decidua*** Boott; Chile, Prov. of O'Higgins, *F. W. Pennell 12311*; (US). ***C. decidua*** Boott; Chile, Valeau Lamin, *C. Joseph 5796*; (US). ***C. donnell-smithii*** L.H. Bailey; Mexico, Chiapas, *Shilom Ton 8199*; (RSA); AF285005, AY770439, N/A.

C. eleusinoides Turcz. ex Kunth (1); Russia, Buryatskaya, *Elias 12025*; (RSA); AF285006, AY770441, GQ223671. ***C. eleusinoides*** Turcz. ex Kunth (2); USA, Alaska, *B. Bennett 02-242*; (B. A. Bennett Herbarium); GQ223594, GQ223513, GQ223673. ***C. eleusinoides*** Turcz. ex Kunth (3); Russia, Magadan Oblast, *C. L. Parker 4497*; (ALA); GQ223595, GQ223514, N/A. ***C. eleusinoides*** Turcz. ex Kunth (4); Canada, Yukon, *B. Bennett 99-171*; (B. A. Bennett Herbarium); GQ223596, 7GQ223515, N/A. ***C. emoryi*** Dewey (1); USA, Texas, *L. A. Standley 1307*; (LAS); GQ223597, GQ223516, GQ223674. ***C. emoryi*** Dewey (2); USA, Colorado, *D. J. Cooper 2329*; (COLO); GQ223598, GQ223517, N/A. ***C. enanderi*** Hultén; USA, Alaska (type), *S. J. Enander s. n.*, 1913?; (S). ***C. endlichii*** Kük.; USA, Arizona, *Fritts 94-6*; (ARIZ); GQ223599, GQ223518, N/A. ***C. eurystachya*** Hermann; Canada, Alberta (type), *F. J. Hermann 13529*; (GH).

C. gaudichaudiana Kunth (1); New Zealand, Waiuta, *K. A. Ford 28/98*; (VT); AY770472, AY770443, GQ223675. ***C. gaudichaudiana*** Kunth (2); New Zealand, Otago, *K. A. Ford 23/01*; (VT); GQ223600, GQ223519, N/A; ***C. gynandra*** Schwein.; Canada, Quebec, *Waterway 99*; (MTMG); DQ998920, DQ998867, N/A.

C. hermannii Cochrane; Mexico, Volcán Ixtaccíhuatl, *M. Sundue 388*; (VT); AY770473, AY770444, GQ223676. ***C. hermannii*** Cochrane; Mexico, Volcán Ixtaccíhuatl, *P. F. Zika 15391*; (VT). ***C. hermannii*** Cochrane; Mexico, Volcán Ixtaccíhuatl, *Rzedowski 36660-a*; (WIS). ***C. hermannii*** Cochrane; Mexico, Volcán Ixtaccíhuatl (type), *T. S. Cochrane and B. A. Cochrane 8565*; (WIS).

C. kauaiesis R. Krauss; USA, Hawaii, Kaua'i, *W. L. Wagner et al. 5050* acc. 1983.374; (BISH); GQ223604, GQ223523, GQ223680. ***C. kelloggii*** Boott; USA, California (type), *Kellogg s. n.*; (GH).

C. lemanniana Boott; Costa Rica, San Jose, *M. Sundue et al. 389*; (VT); GQ223605, AY770446, N/A. ***C. lenticularis*** Michx. (3); USA, Vermont, *J. Dragon 00-1c*; (VT); AY770475, AY770447, GQ223681. ***C. lenticularis*** Michx. (4); USA, Michigan, *D. Henson 1788*; (WIS); GQ223606, GQ223524, GQ223682. ***C. lenticularis*** Michx. (5); USA, Michigan, *A. Arez et al. 8265*; (WIS); GQ223607, GQ223525, N/A. ***C. lenticularis*** Michx. (6); USA, Wisconsin, *Judziewicz 9132*; (WIS); GQ223608, GQ223526, N/A. ***C. lenticularis*** Michx. (7); Canada, Alberta, *K. Vujnovic et al. s. n.*, July 18, 2001; (ALTA); GQ223609, GQ223527, N/A. ***C. lenticularis*** Michx. (8); Canada, Newfoundland, *L. A. Standley 1486*; (LAS); GQ223610, GQ223528, N/A. ***C. lenticularis*** Michx.; Canada, Alberta, *C. Wallis s. n.*, July 3, 1983; (ALTA). ***C. lenticularis*** Michx.; Canada, Quebec, *L. A. Standley 1433*; (LAS). ***C. lenticularis*** Michx.; USA, Minnesota, *L. A. Standley 1367*; (LAS). ***C. lenticularis*** Michx.; Canada, Ontario, *L. A. Standley 1406*; (LAS). ***C. lenticularis*** Michx.; Canada, Ontario, *L. A. Standley 1411*; (LAS). ***C. lenticularis*** Michx.; Canada, Saskatchewan, *G. F. Ledingham 49-365*; (ALTA). ***C. lenticularis* var. *dolia*** (Jones) Standley; USA, Montana (type), *M. E. Jones s. n.*, Aug. 26, 1909; (POM). ***C. lenticularis* var. *dolia*** (Jones) Standley (1)]; USA,

Montana, *P. Lesica 8196*; (MONTU); GQ223587, GQ223587, GQ223665. ***C. lenticularis* var. *dolia*** (Jones) Standley (2); USA, Montana, *P. Lesica 8307*; (MONTU); GQ223592, GQ223511, GQ223669. ***C. lenticularis* var. *dolia*** (Jones) Standley (3); USA, Alaska, *J. Dragon 03-83*; (VT); GQ223588, GQ223507, GQ223666. ***C. lenticularis* var. *dolia*** (Jones) Standley (4); Canada, British Columbia, *B. Bennett 97-685*; (B. A. Bennett Herbarium); GQ223589, GQ223508, N/A. ***C. lenticularis* var. *dolia*** (Jones) Standley (5); Canada, Yukon, *B. Bennett 03-955*; (B. A. Bennett Herbarium); GQ223591, GQ223510, GQ223668. ***C. lenticularis* var. *dolia*** (Jones) Standley (6); Canada, Alberta, *P. J. Cotterill 00071004*; (ALTA); GQ223590, GQ223509, GQ223667. ***C. lenticularis* var. *dolia*** (Jones) Standley (7); Canada, British Columbia, *D. Vitt s. n.*, July 28–30, 1983; (ALTA); GQ223593, GQ223512, N/A. ***C. lenticularis* var. *impressa*** (L. H. Bailey) L. A. Standley (1); USA, California, *Ertter 10424*; (WSP); AY770474, AY770445, GQ223677. ***C. lenticularis* var. *impressa*** (L. H. Bailey) L. A. Standley (2); USA, Oregon, *L. Janeway 7950*; (VT); GQ223601, GQ223520, N/A. ***C. lenticularis* var. *impressa*** (L. H. Bailey) L. A. Standley (3); USA, Washington, *L. A. Standley 347* (LAS); GQ223602, GQ223521, GQ223678. ***C. lenticularis* var. *impressa*** (L. H. Bailey) L. A. Standley (4); USA, Idaho, *C. Johnson 1726*; (ID); GQ223603, GQ223522, GQ223679. ***C. lenticularis* var. *impressa*** (L. H. Bailey) L. A. Standley; USA, California (type), *Kellogg s. n.*; (BH). ***C. lenticularis* var. *limnophila*** (T. Holm) Cronquist (5); USA, Alaska, *Peter F. Zika 16999*; (VT); GQ223611, GQ223529, GQ223683. ***C. lenticularis* var. *limnophila*** (T. Holm) Cronquist (6); USA, Alaska, *J. Dragon 03-87*; (VT); GQ223612, GQ223530, N/A. ***C. lenticularis* var. *limnophila*** (T. Holm) Cronquist (7); Canada, British Columbia, *P. Zika 13558*; (WTU); GQ223615, GQ223533, N/A. ***C. lenticularis* var. *limnophila*** (T. Holm) Cronquist (8); USA, Oregon, *P. Zika 13143*; (WTU); GQ223614, GQ223532, GQ223684. ***C. lenticularis* var. *limnophila*** (T. Holm) Cronquist; USA, Alaska (type), *Macoun 16613*; (US). ***C. lenticularis* var. *lipocarpa*** (T. Holm) L. A. Standley (9); USA, Oregon, *Thompson 95154*; (VT); AY770476, AY770448, GQ223685. ***C. lenticularis* var. *lipocarpa*** (T. Holm) L. A. Standley (10); USA, Washington, *D. Barrington 2091*; (VT); AY770477, AY770449, GQ223686. ***C. lenticularis* var. *lipocarpa*** (T. Holm) L. A. Standley (11); USA, California, *L. Janeway 7875*; (VT); GQ223616, GQ223534, GQ223687. ***C. lenticularis* var. *lipocarpa*** (T. Holm) L. A. Standley (12); USA, Arizona, *McLaughlin 6946*; (ARIZ); GQ223617, GQ223535, GQ223688. ***C. lenticularis* var. *lipocarpa*** (T. Holm) L. A. Standley (13); USA, Arizona, *Baker 13529*; (ARIZ); GQ223618, GQ223536, N/A. ***C. lenticularis* var. *lipocarpa*** (T. Holm) L. A. Standley (14); Canada, Alberta, *L. Allen and V. Loewen s. n*., June 24, 1986; (ATLA); GQ223619, GQ223537, N/A. ***C. lenticularis* var. *lipocarpa*** (T. Holm) L. A. Standley (15); USA, Wyoming, *D. Castaner 9419*; (LAS); GQ223620, GQ223538, GQ223689. ***C. lenticularis* var. *lipocarpa*** (T. Holm) L. A. Standley (16); USA, Colorado, *Nan Lederer 490*; (COLO); GQ223621, GQ223539, GQ223690. ***C. lenticularis* var. *lipocarpa*** (T. Holm) L. A. Standley (17); Mexico, Pueblo Nuevo, *A. A. Reznicek & S. González 11147*; (MICH); AY770478, AY770450, GQ223692. ***C. lenticularis* var. *lipocarpa*** (T. Holm) L. A. Standley; Canada, British Columbia (type), *Macoun 33360*; (US). ***C. lyngbyei*** Hornem. (1); USA, Alaska, *J. Dragon 03-103*; (VT); GQ223622, GQ223540, GQ223691. ***C. lyngbyei*** Hornem. (2); Japan; (MTMG); GQ223623, GQ223541, N/A. ***C. lyngbyei*** Hornem.; Canada, British Columbia, E. *Brainerd 34*; (VT). ***C. lyngbyei*** Hornem.; Russia, Kurile Islands, *K. Kondo 6936*; (GH). ***C. lyngbyei*** Hornem.; Japan, Kitami, *N. Furuse 26458*; (GH). ***C. lyngbyei*** Hornem.; Russia, Kurile Islands, *K. Kondo 299*; (GH). ***C. lyngbyei*** Hornem.; Japan, Takahoko, *K. Yonkekura 6901*; (GH). ***C. lyngbyei*** Hornem.; Faroes Islands (type), *Lyngbyei 1817*; (C).

C. microchaeta T. Holm; USA, Alaska, *J. Dragon 03-85*; (VT); GQ223624, GQ223542, GQ223694. ***C. mitchelliana*** M. A. Curtis; USA, Georgia, *R. F. C. Naczi 8402 and B. Ford*, acc. 1199417; (MICH); GQ223625, GQ223625, N/A.

C. nealae R. Krauss; USA, Hawaii, *D. Herbst 5929*; (BISH); GQ223626, GQ223626, GQ223695. ***C. nebrascensis*** Dewey; USA, California, *L. Janeway 8316*; (VT); GQ223627, GQ223546, GQ223696. ***C. nigra*** (L.) Reich.; USA, Maine, *Dibble 9800*; (VT); AY770480, AY770452, GQ223697. ***C. nudata*** Boott; USA, California, *L. Janeway 7789*; (VT); GQ223629, GQ223548, GQ223700.

C. obnupta L. H. Bailey; USA, California, *L. Janeway 8321*; (VT); GQ223630, GQ223549, N/A.

C. paleacea Schreber ex Wahlenberg; USA, Maine, *S. Hill 30390*; (MICH); GQ223631, GQ223550, GQ223701. ***C. paleacea*** Schreber ex Wahlenberg; Finland, Keski-Pohjanmaa, *H. Toivonen and P. Uotila 23488*; (VT). ***C. paleacea*** Schreber ex Wahlenberg; Canada, Quebec, *J. Dragon 00-29*; (VT); GQ223632, GQ223632, GQ223702. ***C. paleacea*** Schreber ex Wahlenberg; Canada, Quebec, *E. J. Marshall 134*; (VT). ***C. plectocarpa*** Hermann; USA, Montana (type), *F. J. Hermann 18120*; (MONTU). ***C. podocarpa*** R. Br. (1); USA, Alaska, *J. Dragon 03-84*; (VT); GQ223633, GQ223552, GQ223704. ***C. podocarpa*** R. Br. (2); Russia, Magadan Oblast, *Elias 11380*; (RSA); AF284998, AY770457, GQ223703.

C. ramenskii Kom. (1); USA, Alaska, *A. A. Reznicek 11510*; (VT); GQ223634, GQ223553, GQ223705. ***C. ramenskii*** Kom. (2); Japan; (MTMG). ***C. ramenskii*** Kom.; USA, Alaska, *T. Kelso 81-298*; (VT). ***C. recta*** Boott, (4); UK, Scotland, *M. Dean s. n*., July 2003; (VT); GQ223577, GQ223494, GQ223494. ***C. rufina*** Drejer (9); Iceland, Leirdalsheiði, *H. Kristinsson s. n*., Sept. 2001; (VT); AY770485, AY770453, GQ223706. ***C. rufina*** Drejer (10); Sweden, Lappland, Torne Lappmark, Kopparåsen, *A. Anderberg 2548*; (S); GQ223635, GQ223554, N/A. ***C. rufina*** Drejer; Greenland (type), *Wahlenberg s. n*.; (C). ***C. rufina*** Drejer; Greenland, Disko, *S. Laegaard s. n.*, Aug. 9, 1963; (VT).

C. schottii Dewey; USA, California; (RSA); AF285037, GQ223555, GQ223707. ***C. scopulorum*** Holm **var. *scopulorum***; USA, Nevada, *Holmgren & Reveal 1601*; (RSA); AF285056, AY770459, GQ223708. ***C. senta*** Boott; USA, California, *L. Janeway 8063*; (VT); GQ223636, GQ223556, GQ223709. ***C. stricta*** Lam. (1); USA, Vermont, *J. Dragon 02-73*; (VT); AY770486, AY770460, GQ223711. ***C. stricta*** Lam. (2); USA, New Hampshire, *D. Barrington s. n*., Aug. 2002; (VT); AY770487, AY770461, GQ223712. ***C. stricta*** Lam. (3); USA, Vermont, *Thompson 2132*; (VT); GQ223638, GQ223558, GQ223713. ***Carex* sp.** (MEX 2); MEXICO, Pueblo Nuevo, *A.A. Reznicek & S. González 11141*; (MICH); AY770479, AY770451, GQ223693. ***C. spectabilis*** Dewey; USA, Alaska, *J. Dragon 03-86*; (VT); GQ223637, GQ223557, GQ223710. ***C. subspathacea*** Wormskjold ex Hornem. (1); USA, Alaska, *Reznicek 11509*; (VT); GQ223639, GQ223559, GQ223714. ***C. subspathacea*** Wormskjold ex Hornem. (2); Greenland, West Pamiagdluk, *N. Jacobsen 516*; (VT); GQ223640, GQ223560, GQ223715. ***C. subspathacea*** Wormskjold ex Hornem.; Greenland, Pamiagdluk, *N. Jacobsen s. n*., July 30, 1970; (VT). ***C. subspathacea*** Wormskjold ex Hornem.; Japan, Honshu, *K. Yonkekura 6920*; (GH); GQ223641, GQ223561, GQ223716. ***C. subspathacea*** Wormskjold ex Hornem.; Japan, Hokkaido, *M. Amano and H. Ikeda 89070605*; (GH). ***C. subspathacea*** Wormskjold ex Hornem.; Russia, Medveji Islands, *Maximova s. n.*, Aug. 24, 1971; (GH).

C. thunbergii Steud.; Russia, Kuril Islands, *B. Semsrott 0193*; (WTU); AY770488, AY770462, GQ223717. ***C. torta*** Boott; USA, Vermont, *J. Dragon 1a*; (VT); AY770490, AY770464, GQ223718.