Amplified fragment length polymorphism analysis reveals three distinct taxa in *Carex digitalis* sect. *Careyanae* (Cyperaceae)

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Abstract: Unweighted pair-group (UPGMA) cluster, neighbor-joining (NJ), and parsimony analyses using amplified fragment length polymorphism (AFLP) data revealed the presence of three taxa within *Carex digitalis* Willd. (var. *digitalis*, var. *floridana*, var. *macropoda*). Even when taxa occur syntopically, genetic distinctiveness is maintained (Hertford Co., North Carolina populations of var. *macropoda* and var. *floridana*). Clades or clusters corresponding with vars. *digitalis* and *floridana* were well supported in all analyses. However, the var. *macropoda* clade was poorly supported on most trees. Despite our inability to fully resolve taxon relationships, AFLP data substantiate ongoing morphological and phytogeographic studies that show the presence of additional species diversity within sect. *Careyanae* and the eastern North American *Carex* flora.

Key words: Carex, sect. *Careyanae*, taxonomy, AFLP analysis, hidden species diversity.

Résumé : Les analyses de parcimonie, ainsi que des algorithmes UPGMA et neighbor-joining, appliquées au polymorphisme de la longueur des fragments amplifiés (AFLP) révèlent la présence de trois taxons au sein du complexe *Carex digitalis* Willd. (var. *digitalis*, var. *floridana*, var. *macropoda*). La distinction génétique se maintient, même lorsque que les taxons sont syntopiques (populations du var. *floridana* et du var. *macropoda* du comté d'Hertford, Caroline du Nord). Dans toutes les analyses, les clades ou regroupements correspondant aux var. *digitalis* et *floridana* sont bien supportés. Cependant, le clade du var. *macropoda* est mal supporté dans la plupart des dendrogrammes. En dépit de l'incapacité des auteurs à résoudre complètement les relations entre taxons, les données AFLP supportent les études morphologiques et phytogéographiques en cours, qui montrent la présence d'une diversité d'espèces additionnelles dans la sect. *Careyanae* de la flore de l'est de l'Amérique du Nord.

Mots clés : *Carex*, sect. *Careyanae*, taxonomie, analyses AFLP, diversité cryptique d'espèces.

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Introduction

Carex sect. *Careyanae* Tuck. ex Kük. is a group of eight species confined to deciduous forests in eastern North America (Bryson and Naczi 2002). Traditionally, members of this section were placed in a more broadly circumscribed sect. *Laxiflorae* (Kunth) Mack. However, molecular (Starr et al. 1999) and morphological evidence (e.g., perigynia acutely triangular in cross section, perigynia with usually >40 longitudinal nerves) clearly distinguish this section from the *Laxiflorae* s.s. (Naczi 1992; Bryson and Naczi 2002).

Carex digitalis Willd. is one of the most widespread species in sect. *Careyanae,* occurring from southern Ontario and Nova Scotia south to Florida and Texas. White or light brown basal sheaths, narrow leaves (<5.3 mm wide), flowering culms subequal in height to the vegetative shoots, proximal scales of the lateral spikes subtending perigynia, and relatively long (>3.6 mm), acute staminate scales distinguish this taxon from all other species in the section (Bryson and Naczi 2002). Fernald (1938, 1941, 1950) was the first to recognize three varieties of *C. digitalis*: *C. digitalis* var. *digitalis*; *C. digitalis* var. *floridana* (L. H. Bailey) Naczi and Bryson (= *C. digitalis* var. *asymetrica* Fernald); and *C. digitalis* var. *macropoda* Fernald. Variety *digitalis* and var. *floridana* are morphologically similar but distinguishable on the basis of perigynium size (2.5–3.3 mm vs. 3.2–4.2 mm) and curvature of the perigynium beak (barely excurved vs. noticeably excurved). These two taxa also have different geographical affinities. Variety *digitalis* is found throughout much of the range of the *C. digitalis* complex, with a noticeable absence from most of the Atlantic and Gulf Coastal Plains. On the other hand, var. *floridana* is found chiefly in the Atlantic and Gulf Coastal Plains from Virginia to Arkansas south to Texas and Florida (Bryson and Naczi 2002). Variety *macropoda* is the most distinctive member of this group and can be recognized by terminal spikes surpassing the bract blades of distal lateral spikes, long peduncled staminate spikes, and relatively narrow leaves (Bryson and Naczi 2002). This taxon is also widely distributed but is absent from the most northerly portions of this complex's range (Bryson and Naczi 2002). Despite these morphological and geographical trends, much of the

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variation in the *C. digitalis* complex has been dismissed as being of minor significance. As a result, most authors of floras and floristic atlases published since Fernald (1950) recognize no varietal taxa (e.g., Radford et al. 1968; Gleason and Cronquist 1991; Chester et al. 1993; Smith 1994) or suggest that these varieties are poorly defined and intergrade (e.g., Gleason 1952; Yatskievych 1999).

Taxonomic studies of North American plants over the last few decades have revealed the presence of a large number of undetected or undescribed species (Hartman and Nelson 1998; Naczi et al. 1998; Ertter 2000). This pattern of discovery is particular strong in *Carex* (e.g., Naczi et al. 1998; Ertter 2000; Naczi et al. 2001, 2002; Naczi and Ford 2001; Saarela and Ford 2001; Ford et al. in press) where an average of two new taxa have been described per year over the past 20 years (Hartman and Nelson 1998; Ertter 2000; Ford et al. in press). Molecular and (or) isozyme studies have been important in confirming the taxonomic status of many of these species (e.g., Ford et al. 1998*a*, 1998*b*; Starr et al. 1999; Ford and Naczi 2001; Ford et al. in press).

Carex digitalis provides further evidence of the taxonomic richness that exists within North American *Carex*. Fieldwork and detailed morphological studies indicate that substantial variation exists within *C. digitalis* and that this variation can be partitioned according to Fernald's (1938, 1941, 1950) taxonomy. Furthermore, a preliminary analysis of morphological character discontinuities and phytogeographical data suggest that these taxa should be recognized at the species level (R.F.C. Naczi and C.T. Bryson, data not shown). Amplified fragment length polymorphism (AFLP) data have proven to be highly useful in taxonomic studies focused on the circumscription of species complexes (e.g., Beismann et al. 1997; Anamthawat-Jónsson et al. 1999; Koopman et al. 2001; Parsons and Shaw 2001; Gobert et al. 2002; Ishida et al. 2003; Saarela et al. 2003; Koopman 2005). In this paper we present molecular evidence in support of a revised taxonomy of *C. digitalis*.

Materials and methods

Taxon sampling

Fifty-one individuals of *C. digitalis* s.l. were collected from 39 populations (16 var. *digitalis*, 15 var. *floridana*, 8 var. *macropoda*) from throughout the range of this complex (Table 1). In addition, two individuals of *Carex abscondita* Mack., a morphologically similar species also placed in sect. *Careyanae* (Naczi et al. 2001), were sampled (Table 1). In most instances, only one individual per population was collected. However, to assess intrapopulation variation and intergradation between taxa, two or three individuals per population were sampled in three populations of var. *digitalis*, one population of var. *macropoda*, and one population of var. *floridana*. Three individuals each of var. *macropoda* and var. *floridana* were analyzed from a mixed population in Hertford Co., North Carolina (Table 1).

Amplified fragment length polymorphism analysis

DNA was isolated from ca. 20–25 mg of silica gel dried leaf tissue according to the protocols outlined in the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). Elution in the final steps was accomplished using a total of 100 μL of AE buffer instead of the recommended 200 μL. AFLP analysis follows that of Vos et al. (1995). Initially, 250 ng of genomic DNA were digested with *Eco*RI and *Mse*I restriction endonucleases (1.3 units/μL each in 10.0 mmol/L Tris–HCl pH 7.4, 50.0 mmol/L NaCl, 0.1 mmol/L EDTA, 1.0 mmol/L dithiothreitol (DTT), 0.1 mg/mL BSA, 50% glycerol (*v*/*v*), 0.1% Triton X-100) and a 5× restriction digestion buffer (50.0 mmol/L Tris–HCl pH 7.4, 50.0 mmol/L Mg-acetate, 250.0 mmol/L K-acetate). Reactions were adjusted to a final volume of 25 μL using deionized water and incubated for 2 h at 37 °C. This mixture was incubated at 70 °C for 15 min and then rapidly cooled to 4 °C. Adapters were ligated to cohesive ends of restriction fragments by adding 25 μL of digested DNA to an adapter–ligation solution (30.0 pmol each of 3′ and 5′ *Mse*I adapter, 3.0 pmol each of 3′ and 5′ *Eco*RI adapter, 0.4 mmol/L ATP, 10.0 mmol/L Tris–HCl pH 7.5, 10.0 mmol/L Mg-acetate, 50.0 mmol/L K-acetate) and T4 DNA ligase (1.0 unit/μL in 10.0 mmol/L Tris–HCl pH 7.5, 1.0 mmol/L DTT, 50.0 mmol/L KCl, 50% (*v*/*v*) glycerol) in a total volume of 50 μL. Reactions were incubated at room temperature (approx. 20 °C) for 2 h and then diluted 10× using TE buffer (10.0 mmol/L Tris–HCl pH 8.0, 0.1 mmol/L EDTA). Preselective amplification of restriction fragments was performed using primers with one selective base at the 3′ end (*Eco*RI-A, *Mse*I-C) complementary to the core of the adapter sequence. Five microlitres of diluted DNA was combined with preamplification primer-mix (36.5 pmol/μL *Eco*RI-A primer, 32.7 pmol/μL *Mse*I-C primer, 4.9 mmol/L $MgCl_2$), 10× polymerase chain reaction (PCR) buffer, and 1.0 unit *Taq* in a total volume of 51 μL. PCR was undertaken using an MJ Research PTC-100 thermal cycler programmed for 20 cycles of 94 °C for 30 s, 56 °C for 1 min, and 72 °C for 1 min. PCR products were diluted between 30× and 50× with TE buffer depending on product concentration. Preamplified fragments were selectively amplified with *Eco*RI and *Mse*I primers with three selective nucleotides at the 3′ end. Initially nine primer-pair combinations were chosen on the basis of recommendations for monocot crops as outlined in AFLP protocol manuals (Applied Biosystems 2000; Life Technologies 2003). Eight of these combination produced clear polymorphic bands: 1, E-ACC + M-CAA; 2, E-ACC + M-CTA; 3, E-ACC + M-CTG; 4, E-ACA + M-CAA; 5, E-ACA + M-CTA; 6, E-ACA + M-CTG; 7, E-AAC + M-CTA; 8, E-AAC + M-CTG. For selective amplification, 4 μL of diluted preamplified PCR product was combined with PCR buffer (33.3 mmol/L Tris–HCl pH 8.4, 83.3 mmol/L KCl), 6.3 mmol/L $MgCl_2$, 1.3 mmol/L deoxynucleoside triphosphates (dNTPs), 0.1 pmol/μL *Eco*RI selective primer, 0.4 pmol/μL *Mse*I selective primer, and 0.1 unit *Taq*. Reactions were adjusted to a final volume of 10 μL using deionized water. The PCR amplification program was: 1 cycle of 94 °C for 30 s, 65 °C for 30 s, 72 °C for 1 min, followed by 12 cycles reducing the annealing temperature by 0.7 °C each cycle (65–56 °C), followed by 23 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min. *Eco*RI primers used in selective amplification were fluorescently labeled (6-FAM, HEX, or NED, GibcoBRL Life Technologies, San Diego, California) and analyzed using an a 3730 genetic analyzer and GeneScan 3.1.2 (Applied Biosystems, Foster City, California). GeneScan-500 LIZ labeled size standards were used in all analyses. DNA fragments of

Table 1. Collection data for *Carex digitalis* s.l. plus the outgroup species *Carex abscondita*.

Species	Collection data
Carex abscondita Mack.	**USA DELAWARE. Kent Co.:** 0.5 mi. (1 mi. = 1.609km) NNW of Dinahs Corner, *Naczi 9278 & Ford.* **GEORGIA. Upson Co.:** 8 mi. SSW of Thomaston, *Naczi 9186*.
Carex digitalis Willd. var. *digitalis*	**USA ALABAMA. Jackson Co.:** ca. 3.5 mi. NW of Carns, *Naczi 9040*. **ARKANSAS. Howard Co.:** ca. 8 mi. W of Umpire, W side of Cossatot River, *Naczi 9678 & Ford*. **Montgomery Co.:** 11 mi. W of Hopper, *Naczi 9684 & Ford*. **Pike Co.:** ca. 2.3 mi. N of Langley, *Naczi 9687 & Ford.* **DELAWARE. New Castle Co.:** 4 mi. N of Newark, *Naczi 9253*. **GEORGIA. Rabun Co.:** 12.5 mi. W of Clayton, *Naczi 9721*. **Union Co.:** 9 mi. ESE of Blairsville, *Naczi 9715*. **KENTUCKY. Estill Co.:** ca. 8.5 mi. SW of Irvine, *Naczi 7778*. **MAINE. Waldo Co.:** E side of route 7, 2.8 mi. S of junction with route 9/202 at Dixmont, *Reznicek 11339 & Reznicek*. **MASSACHUSETTS. Franklin Co.:** Sunderland, Mount Toby, *Zebryk 6251*. **MISSOURI. Pulaski Co.:** ca. 5 mi. SE of Richland, *Naczi 9624 & Ford* (2). **Warren Co.:** 7 mi. SW of Jonesburg, *Naczi 9617 & Ford* (2). **NEW YORK. Greene Co.:** 2.5 mi. SSE of Hunter, *Naczi 10441*. **PENNSYLVANIA. Pike Co.:** 5 mi. E of Greentown, *Naczi 9422*. **Somerset Co.:** 0.5 mi. S of Salisbury, *Naczi 9602* (3). **TENNESSEE. Van Buren Co.:** Fall Creek Falls State Park, *Fleming FCF-308 et al*. **VERMONT. Addison Co.:** ca. 1 mi. E of village of Lake Dunmore, *Naczi 9997*.
Carex digitalis var. *floridana* (L. H. Bailey) Naczi and Bryson	**USA ALABAMA. Coosa Co.:** ca. 6 mi. SW of Unity, *Naczi 8504 & Ford*. **Covington Co.:** 7.5 mi. NW of Lockhart, *Naczi 8467 & Ford*. **Cullman Co.:** 3.8 mi. SW of Bug Tussle (Wilburn), 22 April 2002, *Naczi 9091*. **Elmore Co.:** ca. 1.8 mi. SE of Titus, *Naczi 9111*. **Henry Co.:** ca. 3.5 mi. NE of Shorterville, *Naczi 5224*. **Winston Co.:** ca. 4.4 mi. SW of Addison, *Naczi 5429 & Bryson*. **ARKANSAS. Ouachita Co.:** Reader vicinity, immediately below White Oak Dam, *Hyatt 8515*. **FLORIDA. Gadsden Co.:** ca. 2.5 mi. SW of Chattahoochee, *Anderson 20665*. **GEORGIA. Chattahoochee Co.:** ca. 8.5 mi. SW of Cusseta, *Naczi 9149*. **Columbia Co.:** 2.7 mi. NNE of Appling, *Naczi 9536* (2). **Upson Co.:** 6.5 mi. SSE of Thomaston, *Naczi 9713*. **LOUISIANA. Vernon Parish:** Kisatchie National Forest, *Hyatt 10847*. **MISSISSIPPI. Simpson Co.:** Simpson Legion Lake, *Bryson 19247*. **Tishomingo Co.:** 4 mi. SE of Tishomingo, *Naczi 9698 et al*. **NORTH CAROLINA. Hertford Co.:** 3 mi. SE of Mapleton, *Naczi 9574* (3) (syntopic with *C. digitalis* var. *digitalis Naczi 9573*). **TEXAS. Jasper Co.:** ca. 12 mi. WSW of Jasper, *Naczi 1832*.
Carex digitalis var. *macropoda* Fernald	**USA ALABAMA. Jackson Co.:** ca. 2.7 mi. N of center of Skyline, *Naczi 9048*. **ARKANSAS. Sevier Co.:** 8 mi. E of DeQueen, *Naczi 9659 & Ford* (coastal plain); ca. 5 mi. W of Gillham, *Naczi 9663 & Ford* (ouachitas). **Georgia. Upson Co.:** 8 mi. SSW of Thomaston, *Naczi 9191*. **MARYLAND. Worcester Co.:** 0.5 mi. N of Ironshire, *McAvoy 4386*. **NORTH CAROLINA. Chatham Co.:** 10 mi. S of Pittsboro, *Naczi 9558* (2). **Hertford Co.:** 3 mi. SE of Mapleton, *Naczi 9573* (3) (syntopic with *C. digitalis* var. *macropoda Naczi 9574*). **SOUTH CAROLINA. Edgefield Co.:** ca. 8 mi. SW of Edgefield, *Nelson 18039*.

Note: The number of individuals sampled per population, when greater than one, is noted parenthetically following the citation. Vouchers are deposited in DOV.

50–500 base pairs were visualized for each primer-pair combination using Genographer 1.6.0 (Benham 2001). The presence (1) or absence (0) of strongly marked bands (= alleles) at each locus was scored to produce a final data matrix (TreeBASE study accession S1563). Closely spaced loci or loci characterized by weak bands were not scored for this analysis.

Data analysis

Genetic relationships among individuals were investigated using NEILI (restriction-site distance of Nei and Li 1979) and MEAN (mean number of pairwise character differences = simple matching coefficient for binary data) matrices and UPGMA (unweighted pair-group method) and NJ (neighbor-joining) methods in PAUP* v.4.0b10 (Swofford 2002). For NJ analyses, branch lengths were constrained to be nonnegative (Felsenstein 1993, as cited in Swofford et al. 1996), and trees were rooted using two individuals of *C. abscondita*. Branch support for all analyses was determined using bootstrap analysis (10 000 replicates) and was categorized as poor (<55%), weak (55%–64%), moderate (65%–74%), good (75%–84%), very good (or very well) (85%–94%), or strong (95%–100%) (Hillis and Bull 1993; Huelsenbeck et al. 1996). Trees from NJ and UPGMA analyses were compared with results from a heuristic NEILI analysis using default settings in PAUP*.

Heuristic parsimony searches in PAUP* were conducted using 10 000 replicates of a random addition of taxa. MULTREES (save all minimal trees), COLLAPSE (collapse all zero length branches), and TBR (tree-bisection-reconnection) commands were employed with branch swapping occurring on best trees only. Trees were rooted using two accessions of *C. abscondita.* Clade support was determined using bootstrap analysis (heuristic searches, 10 000 replicates, simple stepwise addition of taxa, COLLAPSE and TBR commands turned on, MULTREES turned off) and categorized using the criteria outlined above. DeBry and Olmstead (2000) have shown that the MULTRESS option "off" quickly generates bootstrap proportions that are indistinguishable from values produced by TBR when this option is turned "on".

Results

A total of 326 bands, ranging in size from 76 to 465 base pairs were scored for the eight primer combinations, with 248 of these being polymorphic for *C. digitalis*. Many more bands were observed using these primers, but inconsistencies in band intensity prevented their use in this

Table 2. Total number of high-frequency alleles (i.e., bands were present in one or two taxa with a frequency of >50%) for all pairwise comparisons of the three varieties in the *Carex digitalis* complex (*N*, number of individuals sampled per taxon).

Variety	*N*	var. *digitalis*	var. *floridana*	var. *macropoda*
var. *digitalis*	21	20		
var. *floridana*	19	4	65	
var. *macropoda*	11	28	11	24

Fig. 1. Unweighted pair-group analysis (UPGMA) of three varieties of *Carex digitalis* using amplified fragment length polymorphism (AFLP) data and a Nei Li (NEILI) association matrix. An asterisk (*) marks individuals collected from the same population. The Hertford Co., North Carolina population, indicated in bold, represents a syntopic occurrence of var. *macropoda* and var. *floridana.* Numbers along branches represent bootstrap support.

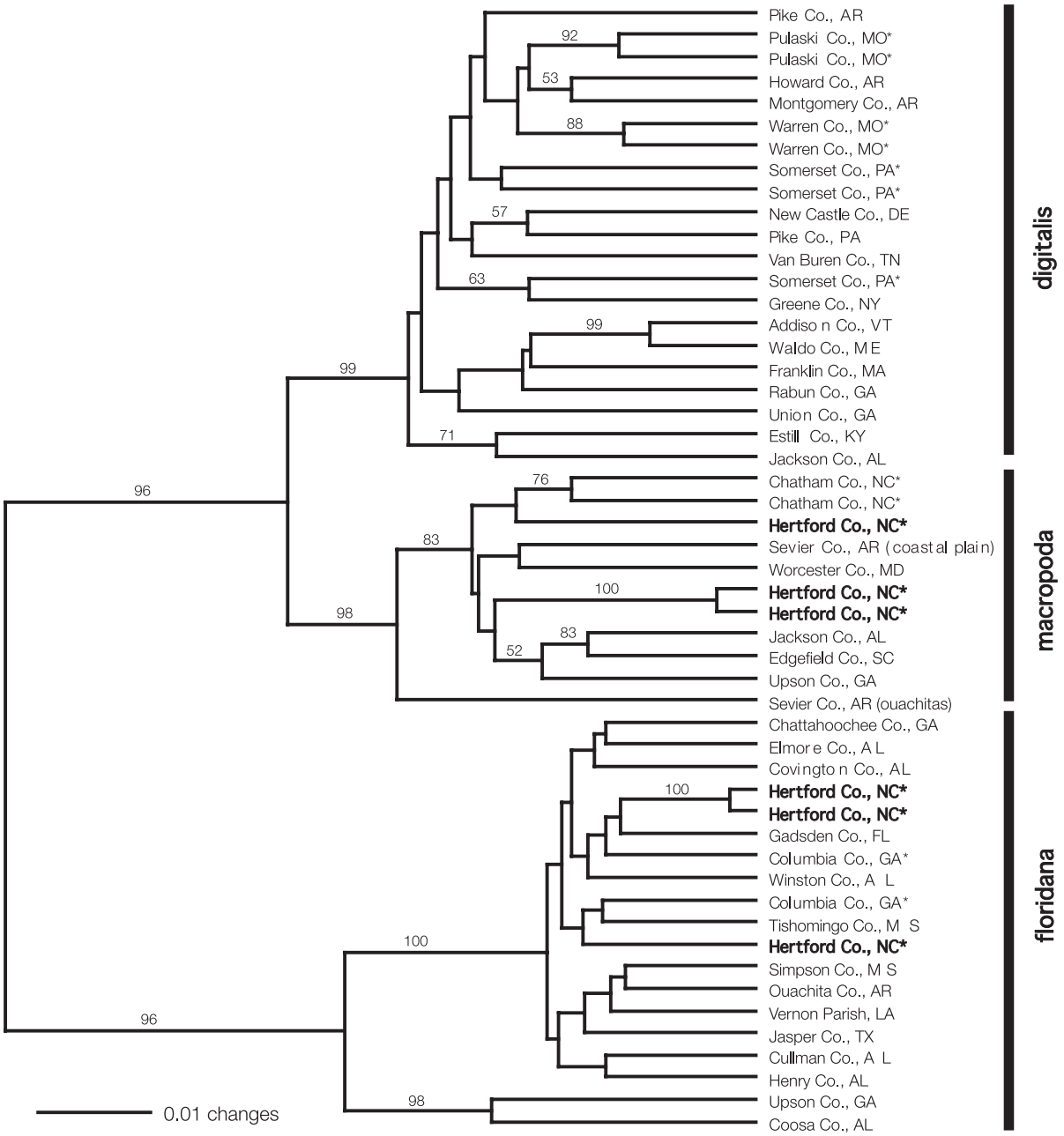
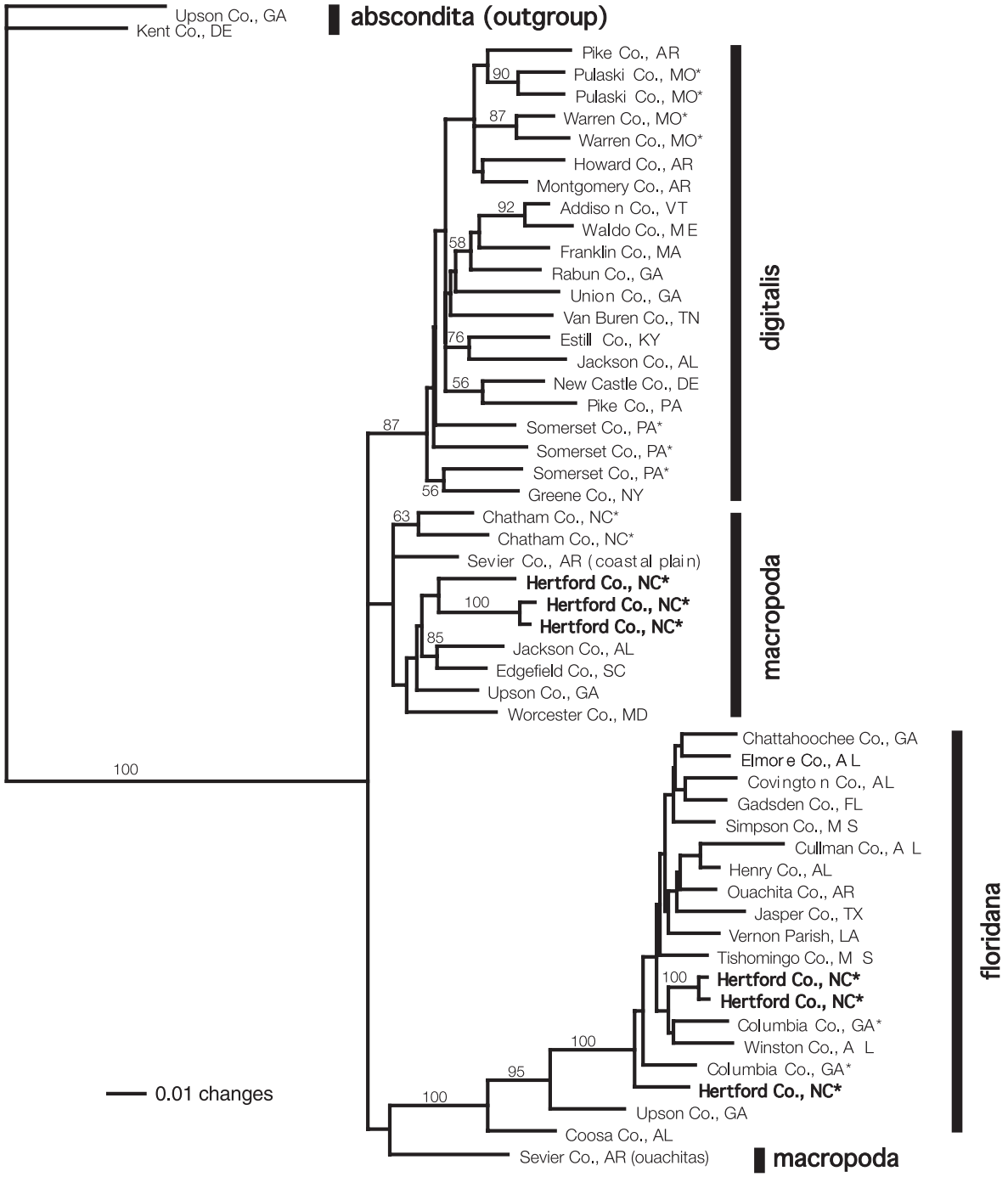


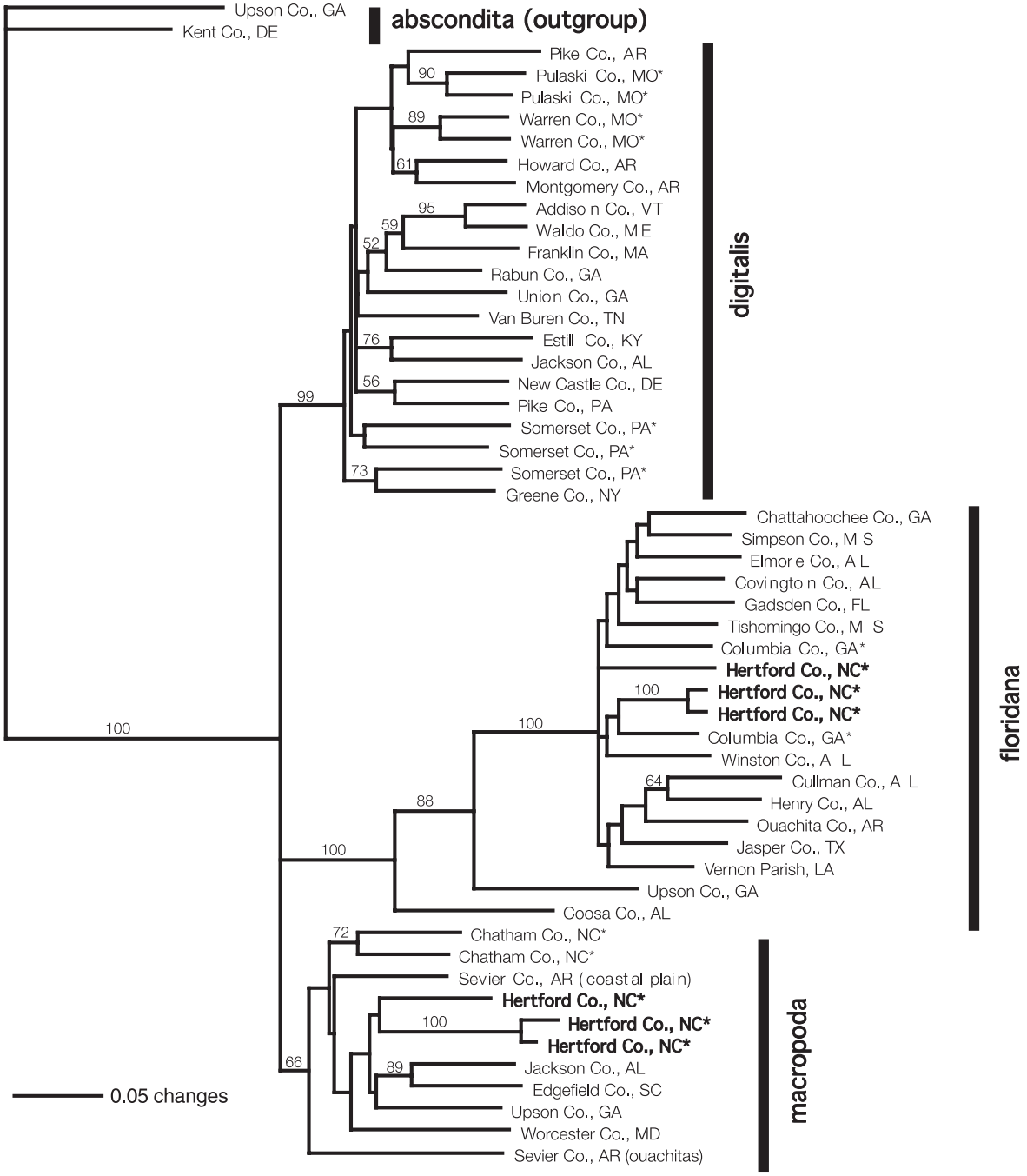
Fig. 2. Neighbor-joining analysis (NJ) of three varieties of *Carex digitalis* using amplified fragment length polymorphism (AFLP) data and a Nei Li (NEILI) association matrix. An asterisk (*) marks individuals collected from the same population. The Hertford Co., North Carolina population, indicated in bold, represents a syntopic occurrence of var. *macropoda* and var. *floridana.* Numbers along branches represent bootstrap support.



study. Of the polymorphic loci scored, 152 were taxonomically informative (i.e., bands were present in one or two taxa with a frequency of >50%; Table 2). Variety *floridana* was the most distinctive taxon, being distinguished by 65 high-frequency bands. In contrast, var. *digitalis* and var. *macropoda* possessed 20 and 24 high-frequency bands, respectively. The presence of shared high-frequency bands also helped to define taxon pairs. Variety *macropoda* and var. *digitalis* shared 28 bands, while a comparison of var. *macropoda* and var. *floridana* revealed 11 shared bands. Variety *digitalis* and var. *floridana* shared only 4 bands.

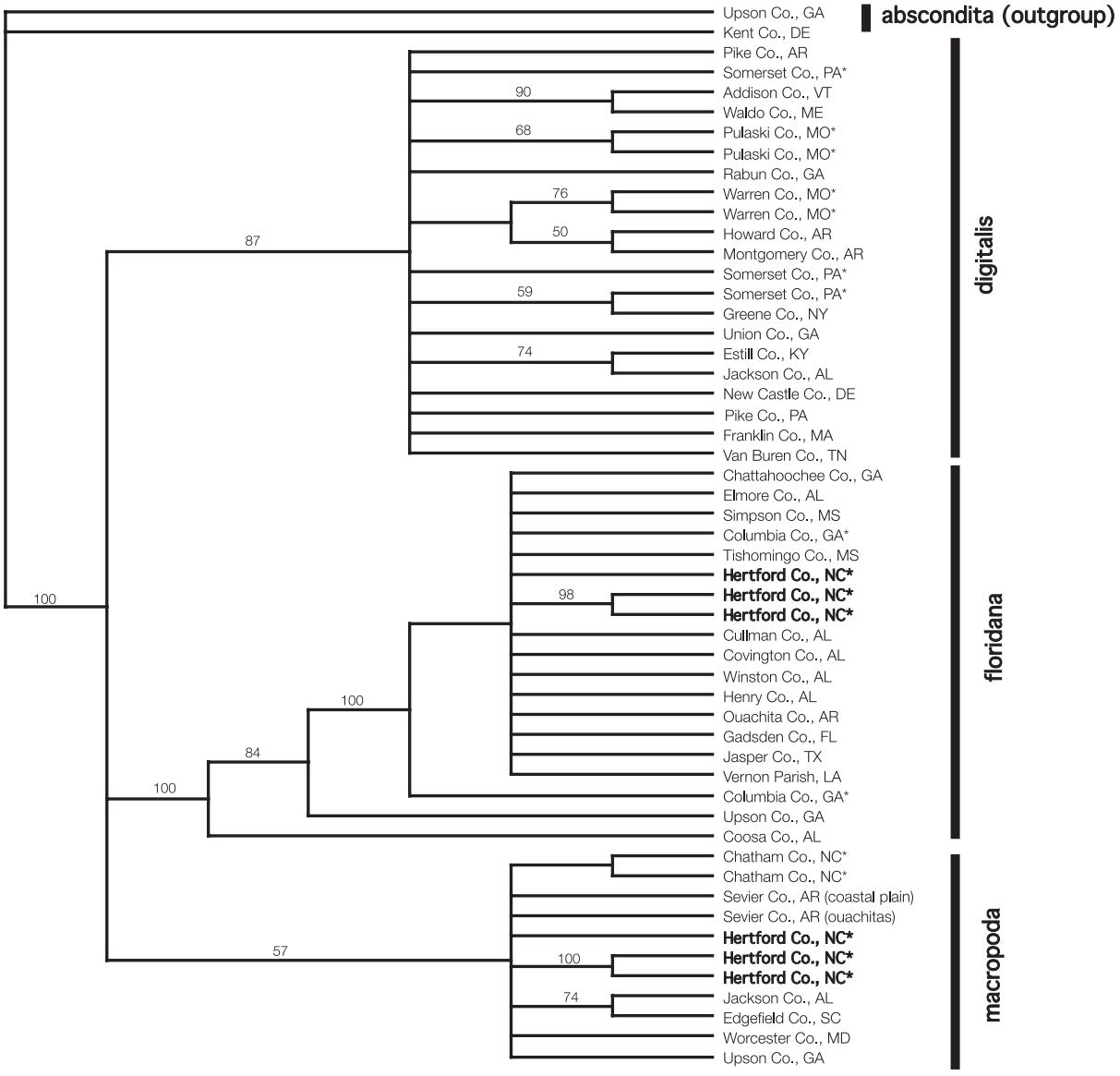
The UPGMA analysis using a NEILI association matrix showed the presence of two strongly supported taxon clusters (BS = 96%), one corresponding with var. *floridana,* the other to vars. *digitalis/macropoda* (Fig. 1). Within the second cluster, var. *digitalis* and var. *macropoda* form strongly supported taxon assemblages (BS = 99% and 98%, respectively) with no individuals misclassified. Syntopically occur-

Fig. 3. Neighbor-joining analysis (NJ) of three varieties of *Carex digitalis* using amplified fragment length polymorphism (AFLP) data and a mean number of pairwise character differences (MEAN) association matrix. An asterisk (*) marks individuals collected from the same population. The Hertford Co., North Carolina population, indicated in bold, represents a syntopic occurrence of var. *macropoda* and var. *floridana.* Numbers along branches represent bootstrap support.



ring taxa (var. *macropoda* and var. *floridana* populations from Hertford Co., North Carolina) were distinct with no evidence of intergradation. Within each taxon cluster, individuals from the same population often grouped closely together (e.g., Pulaski Co. and Warren Co., Missouri populations of var. *digitalis*; Chatham Co., North Carolina populations of var. *macropoda*) although this did not always occur (e.g., Somerset Co., Pennsylvania populations of var. *digitalis*; Hertford Co., North Carolina populations of vars. *macropoda* and *floridana*). Population structuring at larger geographic scales was not evident, with populations from disparate geographic regions found within the same cluster (Fig. 1). However, substantial variation was detected in samples of var. *floridana* from Upson Co., Georgia, and Coosa Co., Alabama and the Sevier Co., Arkansas (oauchita) population of var. *macropoda*. A re-extraction and AFLP analysis of the DNA from these individuals confirmed the divergent nature of these accessions (B.A. Ford, unpublished data). A

Fig. 4. Strict consensus of 121 most parsimonious trees resulting from heuristic searches of an amplified fragment length polymorphism (AFLP) data set representing three varieties of *Carex digitalis*. An asterisk (*) marks individuals collected from the same population. The Hertford Co., North Carolina population, indicated in bold, represents a syntopic occurrence of var. *macropoda* and var. *floridana.* Numbers along branches represent bootstrap support.



UPGMA analysis of a MEAN character association matrix revealed the same overall pattern as that using NEILI (results not shown).

The NJ analysis using a NEILI matrix revealed a polytomy of three groups corresponding with the three taxa. However, the Sevier Co., Arkansas (oauchita) population of var. *macropoda* was placed as sister to the var. *floridana* clade. The geographically proximate Sevier Co, Arkansas (coastal plain) population of var. *macropoda* was located within the core var. *macropoda* clade. Geographic structuring and variation within each of the three major clades was similar to that found in the cluster analysis. Bootstrap values for the var. *digitalis* and var. *floridana* clades were strong (BS = 87% and 100%, respectively). However, support for the var. *macropoda* clade was poor (<50%) (Fig. 2). A heuristic analysis of the NEILI matrix produced a single tree that was similar to the NJ tree (results not shown).

A NJ analysis using a MEAN character association matrix also revealed a polytomy of three clades each corresponding with the three taxa. However, all individuals of var. *macropoda* were assigned to that clade (Fig. 3). Clade support was strong for the var. *digitalis* and var. *floridana* clades (BS = 99% and 100%, respectively) but was only moderate for var. *macropoda* (BS = 66%). Relationships within each of the clades were similar to those found in previous analyses.

Heuristic parsimony searches found 121 most parsimonious trees of 1562 steps in length (CI = 0.209; RI = 0.623, 38 characters potentially parsimony informative). The strict

consensus of these trees shows a polytomy of three clades, each corresponding with the three varietal taxa (Fig. 4). Branch support for the var. *digitalis* and var. *floridana* clades was very good or strong (BS = 87% and 100%, respectively). However, support for the var. *macropoda* clade was weak (BS = 57%). There was little resolution of relationships within the three major clades, with the noteworthy exception being three individuals of var. *floridana* (Upson and Columbia Co., Georgia; Coosa Co., Alabama) (Fig. 4).

Discussion

The results of the AFLP analysis show the presence of three distinct groups, with each corresponding with the three varieties of *C. digitalis* recognized by Fernald (1950). Even when members of this complex occur syntopically, genetic distinctiveness is maintained (Hertford Co., North Carolina populations of var. *macropoda* and var. *floridana*). Our findings parallel earlier allozyme studies of the *C. willdenowii* Willd. and *C. jamesii* Schwein. complexes (Ford et al. 1998*a*; Ford and Naczi 2001), in which genetic data supported the presence of previously undetected species diversity.

The relatively few high-frequency bands that are shared between var. *digitalis* and var. *floridana* (only four) coupled with the relatively distant relationship suggested by UPGMA analyses was unexpected given the morphological similarity between these taxa. This same pattern was also found in our earlier study of the *C. jamesii* complex, in which a low genetic identity was found between the morphologically similar *C. jamesii* and *C. timida* Naczi & Ford relative to that found between *C. timida* and *C. juniperorum* Catling, Reznicek & Crins (Ford and Naczi 2001). This study corroborates our earlier observation that, at least in *Carex*, genetic divergence may not be correlated with striking morphological differences: genetically similar taxa are not always morphologically alike (Ford and Naczi 2001). Overall morphological similarity conflicting with genetic similarity could be due to possession of symplesiomorphies by morphologically similar taxa, as we hypothesized with the *C. jamesii* complex (Naczi and Ford 2001). A more distant relationship between var. *digitalis* and var. *floridana* is not supported in NJ and parsimony analyses, since these trees root along a polytomy.

Another parallel between this and our earlier study of the *C. jamesii* complex is the discovery of genetically divergent individuals from glacial refugia and (or) areas of narrow endemism. These areas include the Ouachita Mountains of Arkansas and eastern Oklahoma (var. *macropoda*, Sevier Co., Arkansas) as well as the Piedmont of the southeastern USA (var. *floridana*, Upson Co., Georgia; Coosa Co., Alabama). This study provides further evidence of the importance of these areas as not only regions of high species diversity, but as reservoirs of unique genetic variability in wide-ranging taxa.

Topological support for the var. *macropoda* clade, as well as many clades within the three taxon clusters, was poor. Studies by Koopman (2005) indicate that phylogenetic signal is not evenly dispersed across reconstructed trees. His results suggest that in plants, well-supported clades in AFLP analyses correspond with internal transcribed spacer (ITS)-1 sequence divergences of 10–30 nucleotides. Poor support was found for clades that had ITS-1 divergences of <10 or >35 nucleotides. A lack of resolution in divergent taxa is likely due to highly variable AFLP data with a low signal/noise ratio. In taxa with low levels of ITS divergence, the amount of AFLP data may be insufficient to resolve relationships (Koopman 2005). In this latter case, the addition of more AFLP markers was suggested as a way to construct more robust phylogenetic hypotheses (Koopman 2005). Our failure to recover a well-supported var. *macropoda* clade (and hence a fully resolved phylogeny of the *C. digitalis* complex), as well as our inability to gain a better understanding of intrataxon relationships, is unlikely due to high AFLP variability. For instance, the number of taxon-specific bands (24) for var. *macropoda* is lower than that found in the well-supported and highly variable var. *floridana* clade (65 taxon-specific bands) (Table 2). Furthermore, successive jackknifing of the data set (NJ + NEILI distances, 100 replicates, 25%, 50%, and 75% deletion, B.A. Ford, unpublished data) showed that clade support increased with the number of loci sampled. An increase in the number of primer combinations, along with an analysis of all the species in sect. *Careyanae*, should produce a more robust evolutionary hypothesis and determine the correct phylogenetic placement of all the taxa within the *C. digitalis* complex. Nevertheless, this study corroborates ongoing taxonomic investigations (R.F.C. Naczi and C T. Bryson, data not shown) of this group and provides yet another example of hidden species diversity within the North American *Carex* flora.

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