PHYLOGEOGRAPHY OF *CAREX EBURNEA* (CYPERACEAE) AND THE SYSTEMATICS OF THE *CAREX EBURNEA* COMPLEX

A Thesis

by

EMILY LAURA GILLESPIE

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APPROVED BY:

Zack E. Murrell
Chairperson, Thesis Committee

Mary U. Connell, Thesis Committee

R. Wayne Van Devender, Thesis Committee

Richard N. Henson, Thesis Committee

Steven W. Seagle
Chairperson, Department of Biology

Edelma D. Huntley
Interim Dean, Graduate Studies and Research

ABSTRACT

PHYLOGEOGRAPHY OF *CAREX EBURNEA* (CYPERACEAE) AND THE SYSTEMATICS OF THE *CAREX EBURNEA* COMPLEX (May 2005)

Emily Laura Gillespie, B.A., University of North Carolina at Asheville

M.S., Appalachian State University

Thesis Chairperson: Zack Murrell

The *Carex eburnea* complex (*Carex* Section *Albae*) is comprised of two named species, *C. eburnea* Boott and *C. mckittrickensis* Ball. *Carex eburnea* is widely distributed on limestone in North America and *C. mckittrickensis* exists at a single station in the Guadalupe Mountains of Texas. The purposes of the current study were 1) to generate a distribution map of the *C. eburnea* complex, 2) to test the validity of the segregate *C. mckittrickensis*, 3) to determine if past migratory routes can be inferred for the complex, and 4) to describe the genetic and morphometric structure of the *C. eburnea* complex within the context of competing species concepts. A distribution map was generated for the complex using 938 specimens from 13 herbaria and compared with known limestone outcrops in North and Central America. Morphometric analysis was performed on 124 specimens; twelve ratios were generated from 14 measurements to produce shape-related measurements to compare morphological variation within Section *Albae* and within the *C. eburnea* complex. Populations were sampled for DNA analysis throughout the range of the complex. Sixty-eight ISSR primers

were screened, of which seven were variable and reproducible; 52 bands were included in the data set. Molecular data were used to generate distance, parsimony, maximum likelihood and Bayesian trees. Principal Components Analysis and Discriminant Function Analysis demonstrated that *C. mckittrickensis* could be easily differentiated from *C. eburnea* with the untransformed morphometric data, but clustered with *C. eburnea* using the ratios, suggesting that the differences between these two taxa are primarily a function of size. *Carex mckittrickensis* was nested within *C. eburnea* in a Neighbor Joining distance analysis, as well as in parsimony and Bayesian analysis. The parsimony and Bayesian analyses indicated that the northwestern-most populations of *C. eburnea* are basal, and that populations in the south and east are derived. Molecular and morphological analyses suggest that the *C. mckittrickensis* population is no more divergent than any other population of *C. eburnea* and does not merit recognition as a species. These data also suggest that the ancestor to the *C. eburnea* migrated from Asia into North America via the Bering Land Bridge. The lack of structure among lineages of *C. eburnea* suggests that the species radiated recently from a northwest North American origin and over time has been restricted to different habitats, creating an unresolved polytomy of lineages that may be best described as a metaspecies sensu Brandon and Mishler.

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Thanks to Dr. Mary Connell (ASU) for radically changing how I felt about molecular biology and for serving on my thesis committee; Drs. Wayne Van Devender and Richard Henson (ASU) for their input as additional members of my thesis committee.

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allowed me to collect at Watauga Lake and Windowcliffs, both in Tennessee, as well as Ketona Dolomite Glade in Alabama. Ms. Rhea Rylee (Ozark -St. Francis National Forests) gave me permission to collect in the Ozarks. Dr. Carolyn Parker (U. Alaska-Fairbanks) provided specimens from Alaska for DNA extraction. Mr. Fred Armstrong (Guadalupe Mtns National Park) gave me permission to collect *Carex mckittrickensis* in Texas and Mr. Bill Bigelow (volunteer, GMNP) hiked through remote parts of McKittrick Canyon with Zack Murrell and I, looking for "some obscure plant." Dr. Peter Ball (U. Toronto) offered technical advice and shared aspects of his data collection on *Carex mckittrickensis* early in my work.

The following herbaria provided specimens on loan for either morphometric or molecular analyses, and for distribution construction: U. Tennessee, Knoxville (TENN), U. Michigan (MICH), Harvard University (GH), Botanical Research Institute of Texas (BRIT), Washington State U. (WS), U. Alaska-Fairbanks (ALA), The Field Museum (F), U. Texas at Austin (TEX and LL), New York Botanical Garden (NY) and Rancho Santa Ana Botanic Garden (RSA). The Smithsonian Herbarium (US) and The University of South Carolina (USCH) allowed me to examine specimens on site. The value of these collections in a systematic study cannot be overstated.

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DEDICATION

For my aunt, Ms. Carol Jackson, for always showing up when I needed her, and for being strangely difficult to shock.

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INTRODUCTION

Issues of species boundaries and species delimitation are central to all aspects of biology. Arguments can be made that correctly identifying species in nature may affect our ability to defend crops against species-specific herbivores or parasites, to manage exotic pests that attack forests, or to properly control organisms that may serve as vectors of human disease. Understanding boundaries between species also impacts our basic understanding of biodiversity and our attempts at conservation by influencing what units of biodiversity are recognized and protected (Cracraft 2000).

The Biological Species Concept (BSC) has arguably been the prevailing species concept for much of the past 60 years. The BSC, first formally proposed by Mayr (1942; 1963), recognizes a species as a group of actually or potentially reproducing populations, which are reproductively isolated from other such populations. The BSC applies poorly to plants in general, because plants often produce viable hybrids and plants tolerate chromosomal mutations such as triploidy with greater success than animals. In many plant groups, reproductive isolation is impossible to determine, and therefore these species are difficult to delimit using the BSC. Consequently, delimitation of species using the BSC may result in paraphyletic or polyphyletic groups of populations, which promotes a loss of lineage-based information.

The Evolutionary Species Concept (ESC) proposed by Simpson (1962) and modified by Wiley (1978) defines a species as a population (or populations) with a separate evolutionary trajectory, niche and historical fate. This species concept may highlight important shifts in ecology, but may recognize either monophyletic or paraphyletic groups of populations as a species.

The Phylogenetic Species Concept (PSC), generally attributed to Cracraft (1983), defines a species as the smallest diagnosable phylogenetic unit that is united by a synapomorphy. Adherents to the PSC require both grouping and ranking to follow monophyletic groups. It is well known that many plant species recognized using the BSC or ESC are paraphyletic or polyphyletic when the PSC and monophyly are applied.

The Phylogenetic Species Concept as interpreted by Mishler and Brandon (1987) recognizes a species as the smallest phylogenetic unit possible, but allows for the absence of a synapomorphy, which might be evident due to a recent radiation. This is accomplished by invoking a "metaspecies" designation. Mishler and Brandon define a metaspecies as some number of unresolved populations, none of which are recognized by a synapomorphy. A species defined in this way would be neither monophyletic nor paraphyletic, and a representative phylogram would appear as an unresolved polytomy or a pectinate phylogram.

Carex Section *Albae* is comprised of four members, all occurring in the northern hemisphere. Based on analysis of herbarium records in the current study, *Carex alba* Scopoli is distributed throughout central and western Europe,

and is typically found on calcareous rock. *Carex ussuriensis* Komarov is distributed in eastern Europe and throughout China and the Korean Peninsula, but its habitat is poorly documented on herbarium records.

The *Carex eburnea* complex is comprised of two named species, *Carex eburnea* Boott and *Carex mckittrickensis* Ball. *Carex eburnea* is found in North America, from Alaska to Newfoundland and southward into the Ozark Mountains, the Cumberland Plateau and the Southern Appalachian Mountains. Southern disjunct populations occur in central Alabama and in the Sierra Madre Mountains in the Mexican states of Queretaro and San Luis Potosi. *Carex eburnea* is reportedly found nearly exclusively on limestone, and exists on cliff faces and rock outcrops, in cedar glades and bogs and in treeless habitats such as alvar and tundra. Co-occurring tree species include spruce (*Picea* sp.) in the American northwest and northern white cedar (*Thuja occidentalis*) in the upper midwest and in the northeast. Northern white cedar is also found along the Southern Appalachians as disjunct populations, where it sometimes co-occurs with *C. eburnea*. In the southeastern United States and in Mexico, *C. eburnea* co-occurs with junipers (*Juniperus* sp.) and oaks (*Quercus* sp.), respectively. Despite fairly frequent collections deposited in herbaria, *C. eburnea* has never been the subject of ecological or phylogenetic studies.

Carex mckittrickensis occurs at a single station in South McKittrick Canyon in the Guadalupe Mountains of Texas, where it is found on limestone seeps near juniper, Texas madrone and Douglas fir. Specimens from this locality were segregated from *C. eburnea* by Ball (1998) based on morphological

characters, particularly perigynium beak length and pistillate and staminate scale lengths. Ball noted that aside from the McKittrick Canyon individuals, *C. eburnea* exhibits very little morphological variation across its range, and that little ecological difference is evident between *C. mckittrickensis* and *C. eburnea*.

Microsatellites are noncoding DNA regions common in eukaryotic systems that are comprised of variable numbers of 2-3 base pair repeats. Because of the structure of these DNA regions, they undergo insertions and deletions with relative ease. The result is hypervariability at the species level. The use of microsatellite DNA regions has been developed into a PCR-based technique called Inter-Simple Sequence Repeats (ISSR) (Zietkiewicz et al. 1994). This technique has recently emerged as a tool for use among and within species. Compared to older microsatellite techniques such as RAPDs (Randomly Amplified Polymorphic DNA), ISSRs use longer primers, a primer anchor, and higher annealing temperatures during PCR amplification. Together, these properties result in higher reproducibility of bands than RAPDs (Wolfe 1998). Matos et al. (2001) compared the utility of ISSRs to traditionally used techniques such as RAPDs and isozymes. This study showed that ISSR data are more reproducible and less prone to artifacts than RAPD data, and often lack much of the interpretation ambiguity and safety hazard of isozyme methods.

ISSR data have been used to detect genetic diversity at the cultivar level in barley (Fernandez et al. 2002), grapes (Herrera et al. 2002) and millet (Salimath et al. 1995). This technique has also been used to determine genetic structure in wildflowers such as Queen Anne's Lace (Bradeen et al. 2002), in

commercially important tea (Mondal 2002) and in the common research subject *Arabidopsis thaliana* (Barth et al. 2002). Phylogenetic studies have been conducted using ISSRs in rice (Joshi et al. 2000). Therefore, ISSRs are an appropriate molecular tool for determining relationships at the inter- and intraspecific level in plants.

Morphological data have been used extensively in answering questions about relationships among all taxonomic ranks in plants, and is a well-established technique. In particular, character evolution in *Carex* has been studied extensively using morphological data (reviewed by Reznicek 1990). Because morphological characters are often overlapping in closely related taxa, multivariate analysis has been used to evaluate species boundaries and explore geographical variation within several different plant groups, including *Cornus* (Murrell 1994, 1996), *Spiraea* (Anders and Murrell 2001), *Ixeris* (Whang et al. 2001), *Hedera* (Ackerfield and Wen 2002) and others.

The distribution of *Carex* Section *Albae* in Asia and North America suggests that the ancestor of the Section was distributed on one or both continents. Ball (1990) speculated that *Carex* species with an eastern North American/southeast Asian distribution could have migrated across the Bering Land Bridge during the late Tertiary or Quarternary. The distribution of species in Section *Albae* similarly supports the idea that migration via the Bering Land Bridge may have led to speciation in North America and/or Eurasia. This land bridge has been available in warmer periods throughout the Pliocene and Pleistocene (Graham 1999). Many studies of North American species have

focused on Pleistocene events to explain distributions and divergence. However, Klicka and Zink (1997) found that divergence times in migratory bird sister species were more likely correlated with Pliocene events. Regardless of the precise timing, it is expected that the warming and cooling periods of the past seven million years have impacted the distribution of species in the Section.

Based upon the work of Mayewski et al. (1981) it is possible to reconstruct past climates. This information can then be compared with phylogenetic evidence from *Carex* Section *Albae* to test hypotheses concerning speciation and divergence in the group. Given that two species of Section *Albae* (*C. alba* and *C. ussuriensis*) are present in Europe and Asia, and the third taxon (*C. eburnea* complex) is present in North America, three possible scenarios for the possible origin and migration of the Section exists. First, a circumboreal ancestor may have given rise to all three species. Second, a North American ancestor may have given rise to the Eurasian taxa, and third, a Eurasian ancestor may have given rise to the North American taxon.

The purposes of the current study were 1) to generate an accurate distribution map of the *C. eburnea* complex using herbarium records, 2) to describe the genetic and morphometric structure of the *C. eburnea* complex within the context of competing species concepts, 3) to use morphometric and molecular data to test the validity of the segregate *C. mckittrickensis*, and 4) to determine if past migratory routes can be suggested for this species complex.

MATERIALS AND METHODS

Distribution Map

A distribution map was generated for the *Carex eburnea* complex using historical records from 13 herbaria. Herbaria were chosen because they were either large herbaria with widespread collections or regional herbaria with collections of special interest. These herbaria included The University of Michigan (MICH), The University of Texas Austin (TEX and LL), Washington State University (WS), Harvard University (GH), Field Museum (F), Smithsonian Institution (US), New York Botanical Garden (NY), Appalachian State University (BOON), The University of Alaska- Fairbanks (ALA), The University of South Carolina (USCH), The University of Tennessee, Knoxville (TENN) and The University of Alabama (UNA).

A total of 938 specimens were examined. Locality, habitat and collection data were recorded for each specimen. Canadian and Mexican records were recorded at a scale similar to the United States counties, since equivalent units are not commonly used in these two countries. Duplicate county records were disregarded, and a distribution map was generated using ArcMap 3.2 (ESRI Inc., Redlands CA, USA).

Limestone Map

A distribution map of North American limestone outcrops was constructed using a stratigraphic atlas (Cook and Bally 1975). For each period from the Cambrian to the Tertiary (10 maps total), the distribution of limestone was hand-traced. All 10 maps were condensed into a composite map. Known rock outcrops from each period were overlaid with the composite limestone distribution, resulting in a map showing only present-day limestone outcrops. It should be noted that this map was intended to be only an estimate of the distribution of limestone in North America.

Specimen Collection

Live *C. eburnea* specimens were collected by permit or permission at Watauga Lake (TN), Windowcliffs (TN), Blanchard Springs Recreation Area (AR), Fort Hill (MD), Natural Bridge (VA), Clifton Gorge (OH), Bailey's Harbor (WI) and Bruce Peninsula (Ontario). Live *C. mckittrickensis* was collected at McKittrick Canyon (TX). At these locations (Table 1), either leaf clippings or whole plants were collected and refrigerated until DNA extraction was performed. In all cases, plants were collected greater than 3 m apart to avoid collection of ramets, following McClintock and Waterway (1993). Vouchers from each locality were collected as whole plants and were deposited in the herbarium of Appalachian State University (BOON).

Dried *C. eburnea* herbarium specimens were borrowed to generate locality representatives for Denali National Park (AK), Fort Greely Military

Table 1. Sources of Specimens Used for Molecular Analysis.

Abbrev.	Site Name	Location
ALK *	Denali National Park	Central Alaska
ALK *	Fort Greely Military Reserve	Central Alaska
CLF	Clifton Gorge	Central Ohio
FOR	Fort Hill	Western Maryland
KET	Ketona Dolomite Glade	Central Alabama
MAN *	Spruce Woods Provincial Park	Southern Manitoba
MCK	McKittrick Canyon	Guadalupe Mtns. Nat. Park, Texas
MIN *	Stearns County, MN	Central Minnesota
MIN *	Lake County, MN	Northern Minnesota
NAT	Natural Bridge	Western Virginia
ONT	Pendell Point	Bruce Peninsula, Ontario
OZK	Blanchard Springs Rec. Area	Ozark Mountains, Arkansas
MEX *	Queretaro, Mexico	Eastern Sierra Madres
MEX *	San Luis Potosi, Mexico	Eastern Sierra Madres
WAT	Watauga Lake	Ridge and Valley, Tennessee
WIN	Windowcliffs	Cumberland Plateau, Tennessee
WIS	Bailey's Harbor	Door Peninsula, Wisconsin

* Dried Specimens

Base (AK), Spruce Woods Provincial Park (Manitoba), Lake County (MN), Stearns County (MN), Queretaro (Mexico) and San Luis Potosi (Mexico) (Table 1).

Outgroup Selection

Outgroups were selected based on intra- and intersectional relationships in the genus *Carex*. *Carex alba* was chosen as an outgroup based on its position within *Carex* Section *Albae*. *Carex brunnea* was also chosen as an outgroup, based on its position in a presumed related Section (*Carex* Section *Graciles*) (Roalson et al. 2001).

DNA Extraction

DNA extraction of *C. eburnea* and *C. mckittrickensis* tissue was carried out using a DNEasy Plant Mini Kit (Qiagen, Valencia CA, USA). For live material, extraction was performed without modification to the DNEasy kit instructions. DNA extraction of dried specimens was carried out following modifications of Drábková et al. (2002). These modifications included an increased volume of Lysis Buffer (450 µL), a longer 65°C incubation during cell disruption (30 min) and a longer elution (10 min). Following the recommendations of the DNEasy kit instructions for dried specimens, the procedure was carried out on 30 mg of dry tissue and eluted using half the usual volume of Elution Buffer (50 µL). DNA isolations were verified by gel electrophoresis on a 1% agarose gel at 100 v for one hour. The gel was stained

in ethidium bromide and visualized using the Alpha Innotech Digital Imaging and Analysis System (Alpha Innotech Corp., San Leandro CA, USA).

ISSR Analysis

Initial screening of 11 *C. eburnea* individuals from the first three localities collected (WAT, WIN and KET) was carried out using 68 ISSR primers (obtained from the University of British Columbia Biotechnology Laboratory (Table 2). Seven primers were determined to be variable and reproducible. Variable primers were used in PCR amplification of 86 individuals from 17 localities plus four *C. alba* and one *C. brunnea.* Fifteen individuals were excluded later because of inconsistent or poor amplification, leaving 76 individuals for analysis. Each 25 µL reaction consisted of 2.9 µL dH_20, 2.5 µL buffer, 3.0 µL $MgCl_2$, 0.5 µL dNTPs, 0.2 µL *Taq* polymerase, 3.4 µL primer (1.5 µM) and 12.5 µL target DNA (1/100). DNA amplification was performed using a GeneAmp® PCR System 9700 Thermocycler. An initial denaturation of 90 sec at 94°C was followed by 35 cycles of (40 sec at 94°C, 45 sec at 45°C and 90 sec at 72°C) and a final cycle of (45 sec at 94°C, 45 sec at 45°C and 5 min at 72°C). Amplified products were analyzed by gel electrophoresis on a 1.5% agarose gel (50% Seakem ® LE Agarose, 50% NuSieve ® 3:1 Agarose) at 30 v for 5 hrs. Resulting fragments were visualized with ethidium bromide and the Alpha Innotech System. Bands were manually scored as present/absent. The data were analyzed using PAUP* 4.0b10 (Swofford 2002) to produce trees from UPGMA, Neighbor Joining (using Nei-Li option), Parsimony (heuristic

Table 2. ISSR Primers Screened. Primers used in analysis are underlined.

801 ATA TAT ATA TAT ATA TT	848 CAC ACA CAC ACA CAC ARG
802 ATA TAT ATA TAT ATA TG	850 GTG TGT GTG TGT GTG TYC
803 ATA TAT ATA TAT ATA TC	851 GTG TGT GTG TGT GTG TYG
805 TAT ATA TAT ATA TAT AC	852 TCT CTC TCT CTC TCT CRA
807 AGA GAG AGA GAG AGA GT	854 TCT CTC TCT CTC TCT CRG
809 AGA GAG AGA GAG AGA GG	856 ACA CAC ACA CAC ACA CYA
810 GAG AGA GAG AGA GAG AT	858 TGT GTG TGT GTG TGT GRT
811 GAG AGA GAG AGA GAG AC	860 TGT GTG TGT GTG TGT GRA
812 GAG AGA GAG AGA GAG AA	863 AGT AGT AGT AGT AGT AGT
813 CTC TCT CTC TCT CTC TT	864 ATG ATG ATG ATG ATG ATG
814 CTC TCT CTC TCT CTC TA	866 CTC CTC CTC CTC CTC CTC
815 CTC TCT CTC TCT CTC TG	867 GGC GGC GGC GGC GGC GGC
817 CAC ACA CAC ACA CAC AA	868 GAA GAA GAA GAA GAA GAA
818 CAC ACA CAC ACA CAC AG	869 GTT GTT GTT GTT GTT GTT
820 GTG TGT GTG TGT GTG TC	870 TGC TGC TGC TGC TGC TGC
821 GTG TGT GTG TGT GTG TT	871 TAT TAT TAT TAT TAT TAT
823 TCT CTC TCT CTC TCT CC	873 GAC AGA CAG ACA GAC A
824 TCT CTC TCT CTC TCT CG	874 CCC TCC CTC CCT CCC T
825 ACA CAC ACA CAC ACA CT	878 GGA TGG ATG GAT GGA T
828 TGT GTG TGT GTG TGT GA	879 CTT CAC TTC ACT TCA
829 TGT GTG TGT GTG TGT GC	880 GGA GAG GAG AGG AGA
830 TGT GTG TGT GTG TGT GG	882 VBV ATA TAT ATA TAT AT
831 ATA TAT ATA TAT ATA TYA	884 HBH AGA GAG AGA GAG AG
832 ATA TAT ATA TAT ATA TYC	886 VDV CTC TCT CTC TCT CT
833 ATA TAT ATA TAT ATA TYG	888 BDB CAC ACA CAC ACA CA
834 AGA GAG AGA GAG AGA GYT	889 DBD ACA CAC ACA CAC AC
836 AGA GAG AGA GAG AGA GYA	890 VHV GTG TGT GTG TGT GT
837 TAT ATA TAT ATA TAT ART	891 HVH TGT GTG TGT GTG TG
839 TAT ATA TAT ATA TAT ARG	893 NNN NNN NNN NNN NNN
841 GAG AGA GAG AGA GAG AYC	894 TGG TAG CTC TTG ATC ANN NNN
842 GAG AGA GAG AGA GAG AYG	896 AGG TCG CGG CCG CNN NNN NAT G
843 CTC TCT CTC TCT CTC TRA	897 CCG ACT CGA GNN NNN NAT GTG G
845 CTC TCT CTC TCT CTC TRG	899 CAT GGT GTT GGT CAT TGT TCC A
847 CAC ACA CAC ACA CAC ARC	900 ACT TCC CCA CAG GTT AAC ACA

search) and Maximum Likelihood (following Farris et al. 1996). Bootstrap values were not obtained for the Parsimony analysis, because of computer RAM limitations. In an effort to remediate for this deficiency, three independent Bayesian analysis were carried out using MrBayes (Huelsenbeck and Ronquist 2001), following Lewis (2001). Duplicate PCR amplifications of all seven primers were run for all individuals, and ambiguous data were excluded.

Morphometric Analysis

Herbarium specimens were selected for morphometric analysis on the basis of maturity and quality (Appendix A). Ninety-one *C. eburnea* specimens were chosen to represent the entire distribution. Due to rarity of specimens, seven *C. mckittrickensis* specimens were selected (two were excluded due to immaturity of the specimens). Nine *C. alba*, six *C. ussuriensis* and eleven *C. brunnea* were used for outgroup comparison. Measurements chosen were based upon the eleven used by Ball (1998) and then expanded to include 29 measurements representing a variety of reproductive and vegetative characters. Abbreviations used are presented along with measurement definitions in Table 3.

Perigynium length (PL), width (PW), and the position of the widest point in millimeters from the base (PWdPt), as well as the pistillate scale length (PSL) and width (PSW) were used to estimate the overall size or robustness of the reproductive structures. The distinctness and ornamentation of the perigynium were considered categorically in order to discern significant differences among or within species in *Carex* Section *Albae*. Anther length (AL) and style length (SL)

Table 3. Characters used in morphometric analysis

Perigynium Beak Length	PBL	Distance from narrowing of perigynium to apex of beak
Perigynium Length	PL	Distance from base of perigynium to narrowing at base of beak
Perigynium Width	PW	At widest point
Perigynium Widest Point	PWdPt	From base to widest point (mm from base)
Pistillate Scale Length	PSL	From scale base to scale apex
Pistillate Scale Width	PSW	At widest point
Perigynium Nerves Distinct?	NDIST	Nerves are visible or not (qualitative)
Nerves Ornamented?	NORN	Nerves with projections or hairs or none (qualitative)
Anther Length	AL	Entire length
Style Length	SL	Distance from base to stigma
Inflorescence Internode Length	PINT	Distance between perigynia scales (for PINTL, PINT2, PINT3 and PINT4)
Leaf Width	LW	At widest point of a randomly chosen typical leaf
Marginal Teeth per mm	#TH	# of teeth per cm, near base of leaf
Marginal Tooth Length	THL	Entire length of randomly chosen typical tooth
Culm Height to First Branch	CHtoB1	Distance from rhizome to lowest branch on a randomly chosen typical culm
Typical Leaf Length	LL	Distance from rhizome to apex of leaf on a randomly chosen typical leaf
Culm Height	CH	Distance from rhizome to culm apex
Bract Length	B	Distance from node to bract apex, for each bract on a culm (for B1-B4)
Culm Internode Length	INT	Entire internode, from bract base to next bract base (for INT1, INT2 and INT3)
Rachis Length	R	Distance from base of bract to first perigynium (for R1, R2 and R3)
Bract #1 to Male Bract	B1toMB	Distance rom base of bract to base of lowest bract on male inflorescence

were considered only when present and mature. Internode length (INT or PINT) and rachis length (R) were measured in order to describe the compaction of branches. Number (TTH) and size (TTHmm) of marginal teeth on leaves were measured in order to discern intra- or interspecific differences. Leaf length (LL) and width (LW), as well as culm height (CH) and culm height to first branch (CHtoB1) were measured to estimate overall robustness of the plant. Bract length (B) was chosen to estimate inflorescence branch compaction as a function of plant robustness.

Measurements were obtained using an ocular micrometer and/or millimeter ruler and a Meiji EMZ dissecting scope. Morphometric data were entered into an MS Excel spreadsheet (Microsoft Corporation, Redmond WA, USA) and converted from micrometer units or centimeters to millimeters. Fifteen of the 29 measurements were omitted from further analyses because architectural differences among the species generated large amounts of missing data for some measurements. Therefore, descriptive statistics for 14 measurements were calculated, including means and standard deviations for each species. Millimeter values for all specimens were imported into SAS Enterprise Guide (SAS Institute, Cary NC, USA) for analysis. Twelve ratios (Table 4) were generated from the untransformed data in an attempt to diminish the effects of individual size differences on multivariate analyses. Tests performed included Principle Components Analysis (PCA) and Discriminant Function Analysis (DFA) using both untransformed and ratio (transformed) data.

Table 4. Ratios Used in Multivariate Analyses.

Ratio	Abbreviation
Perigynium Width : Perigynium Length	PW:PL
Perigynium Widest Point : Perigynium Length	PWdPt:PL
Perigynium Beak Length : Perigynium Length	PBL:PL
Pistillate Scale Length : Perigynium Length	PSL:PL
Pistillate Scale Width : Perigynium Length	PSW:PL
Perigynium Internode 1 : Perigynium Internode 1 + Perigynium Internode 2	PINT1:PINT1+PINT2
Perigynium Internode 2 : Perigynium Internode 1 + Perigynium Internode 2	PINT2:PINT1+PINT2
Culm Internode 1 : Culm Height to first branch	INT1:CHtoB1
Culm Internode 1 : Culm Height	INT1:CH
Bract 1 : Bract 2	B1:B2
Rachis 1 : Culm Internode 1	R1:INT1
Leaf Length : Culm Height	LL:CH

Comparisons included four *Carex* Section *Albae* taxa plus *C. brunnea* and *C. eburnea* versus *C. mckittrickensis*. In an attempt to determine if morphological structure is evident in different habitats, specimens were clustered on the basis of the dominant canopy tree with which they co-occur. Categories included 'Northwest' for the northwestern U.S. (typically dominated by spruce), 'White Cedar' for the northeast, 'Red Cedar' for the southeast, 'Tex/Mex' for the southwest (where oak or juniper are dominant) and 'White Disjunct' for the white cedar disjuncts in the Southern Appalachians.

RESULTS

Distribution

The distribution generated in this study represents the first specimen-based analysis of the broad range of the *Carex eburnea* complex. The distribution map (Figure 1) demonstrates that the complex is found nearly contiguously across much of North America, from Alaska to New Brunswick in the north, to Arkansas, Tennessee and North Carolina in the south. Disjuncts occur at one locality in central Alabama, two localities in the Eastern Sierra Madres of Mexico and a single site in McKittrick Canyon in the Guadalupe Mountains of western Texas. The Mexican disjunct sites were unknown until recently; specimens were first collected in 2003 by A. A. Reznicek (U. Michigan) (Pers. comm./unpublished data). The McKittrick Canyon site has been collected several times in the past 50 years, and was recently described as a new species by Ball (1998). The Texas and Mexico sites represent the southwestern-most stations for the complex. The species complex is apparently absent from most of the western United States, the southeastern coastal plain, and central Indiana, Illinois and Ohio.

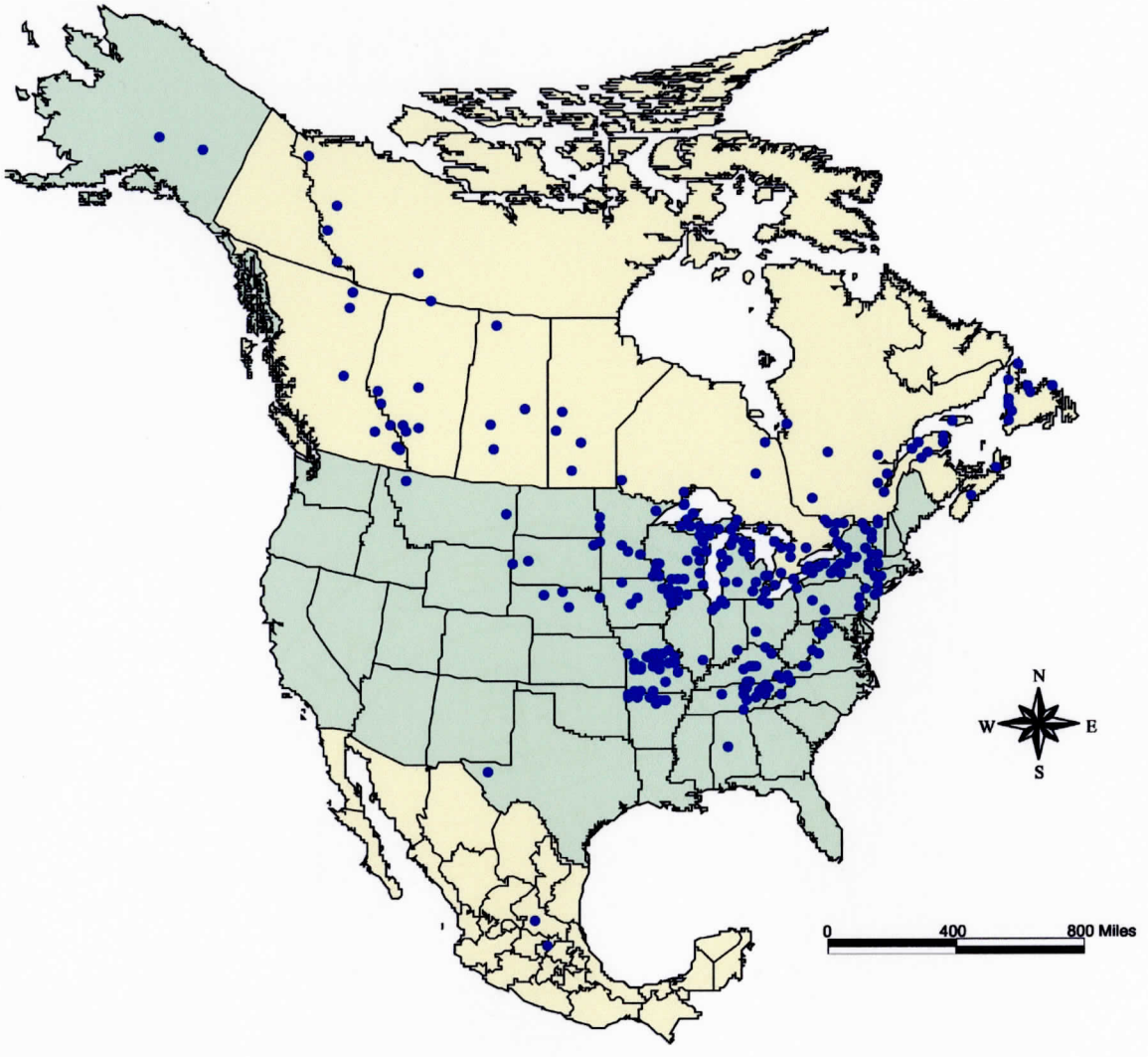


Figure 1. Distribution Map of the *Carex eburnea* Complex. Dots Represent County-Level Records (n=938) from 13 United States Herbaria.

Limestone Distribution

Limestone outcrops (Figure 2) are distributed across the North American continent, but they are rare or absent on the Pacific coast and the Great Plains. Localized areas of limestone are found along the Rocky Mountains, around the Great Lakes and in the Ridge and Valley physiographic province. Limestone is absent in central Indiana, Illinois and Ohio. *C. eburnea* is also absent in these locations. In several locations, limestone is present, but no records of *C. eburnea* exist. These areas include central Texas, northern Ontario and the Rocky Mountains. In other areas, *C. eburnea* records exist, but limestone outcrops do not. These areas include South Dakota, Nebraska, New Brunswick, Nova Scotia and southern Alberta. In some of these cases, herbarium records indicate that limestone exists, but the resolution of the limestone map has not shown the outcrop. In other cases, this could not be determined. In most locations, presence of limestone coincides with the distribution of the *Carex eburnea* complex, suggesting that historical records have accurately captured the species' limitation to limestone.

ISSR Analysis

Fifty-two variable bands were generated from seven primers for an average of 7.2 bands per primer (Appendix B). PAUP analysis was used to generate distance, parsimony and maximum likelihood trees for 76 individuals from 17 localities (Figure 3) plus outgroup representatives. The basal position of

Figure 2. Distribution of Limestone Outcrops in North America. Shaded areas represent a composite of outcrops from the Cambrian through the Tertiary.

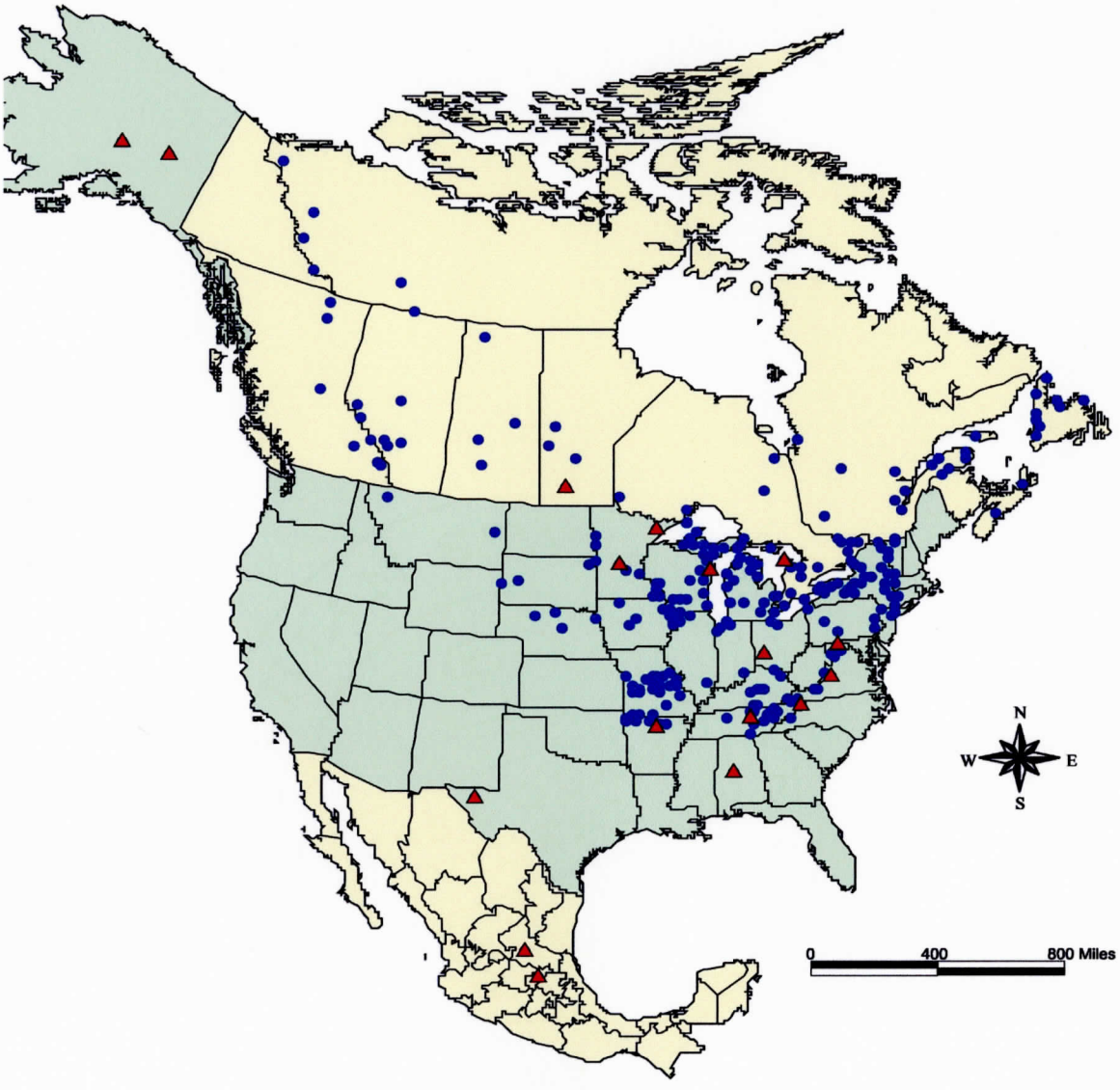


Figure 3. Collection Sites Used for Molecular Analysis. Sites appear as red triangles and are shown in the context of the distribution (blue dots).

MCK within the ingroup on the UPGMA tree (Figure 4) demonstrates that this site differs from others in terms of both bands present and bands absent. MEX clustered with WIN and FOR in a derived clade, and ALK, MAN and MIN formed a large clade. However, the Nei Li Neighbor Joining tree (Figure 5), which clusters individuals on the basis of shared present bands, shows MCK as a derived clade within a larger clade including ALK, MIN and MAN. WAT appears as unresolved individuals basal to a clade of KET individuals along with a single NAT individual. OZK and ONT together form a clade.

Three trees were generated from independent Bayesian analyses. In the first Bayesian tree (Figure 6), MAN, MIN and ALK are basal to a large unresolved inner clade. This tree also places MEX most closely related to WIN. In the second Bayesian tree (Figure 7), MIN and ALK are basal to the inner clade, but MAN is part of the inner clade. This tree also has some intraspecific paraphyly, with an individual from WAT clustering with OZK and an individual from NAT clustering with KET. The third Bayesian tree (Figure 8) has MIN and ALK basal, and also shows MEX clustering in a clade with WIN.

A consensus produced from three million MCMC generations (Figure 9) shows the northwest populations basal to the rest of the complex. In this tree, MCK and MEX appear in a clade along with WIN. CLF and WIS form a clade together within the 'inner-most' large clade. The rest of the large inner clade is comprised of OZK, WAT, KET, NAT and ONT individuals.

A 50% majority rule maximum likelihood analysis was carried out using a bootstrapping technique following Farris et al. (1996) (Figure 10). This tree

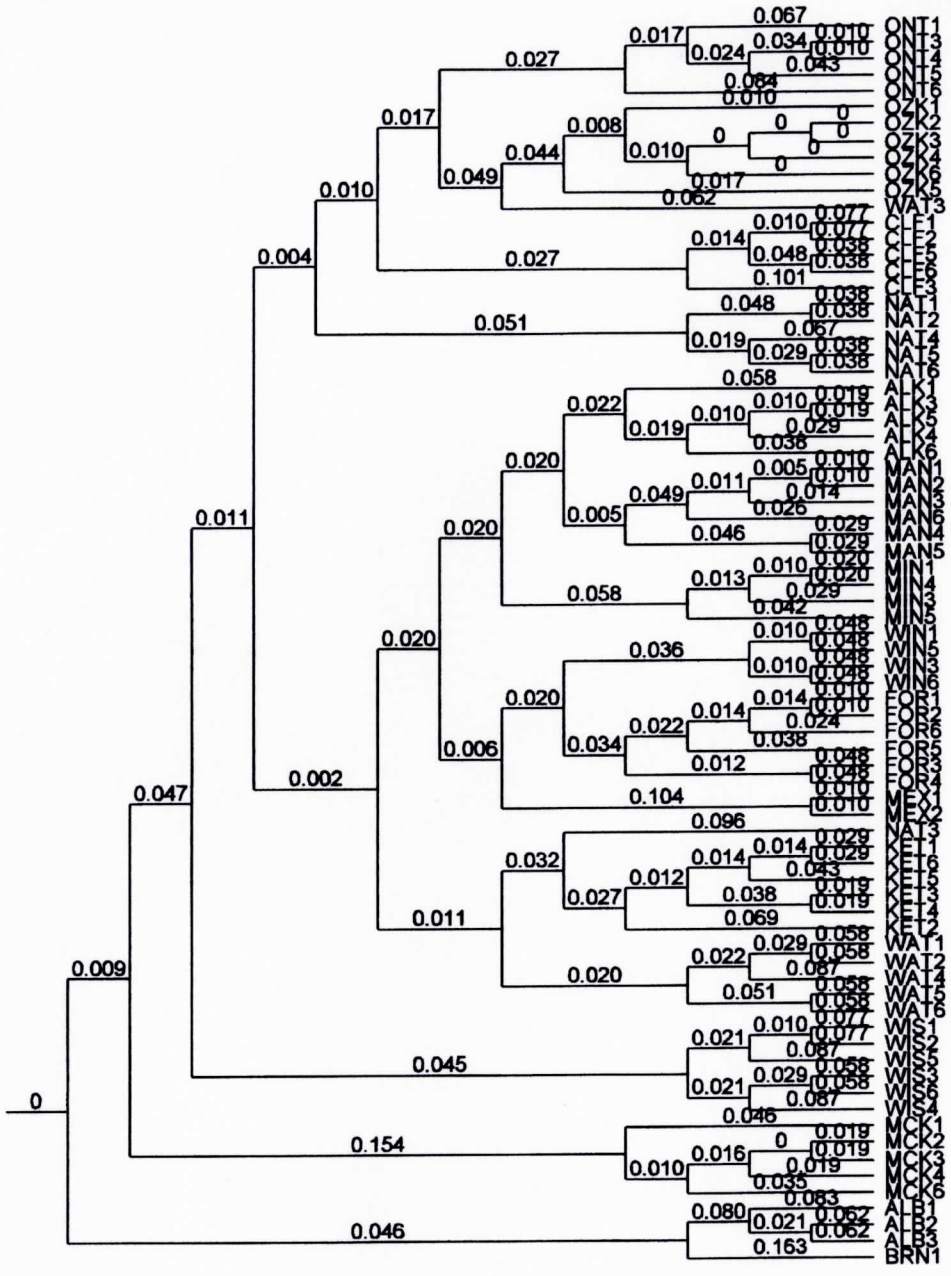


Figure 4. UPGMA Tree Generated from Analysis of 76 Individuals. Branch lengths are shown.

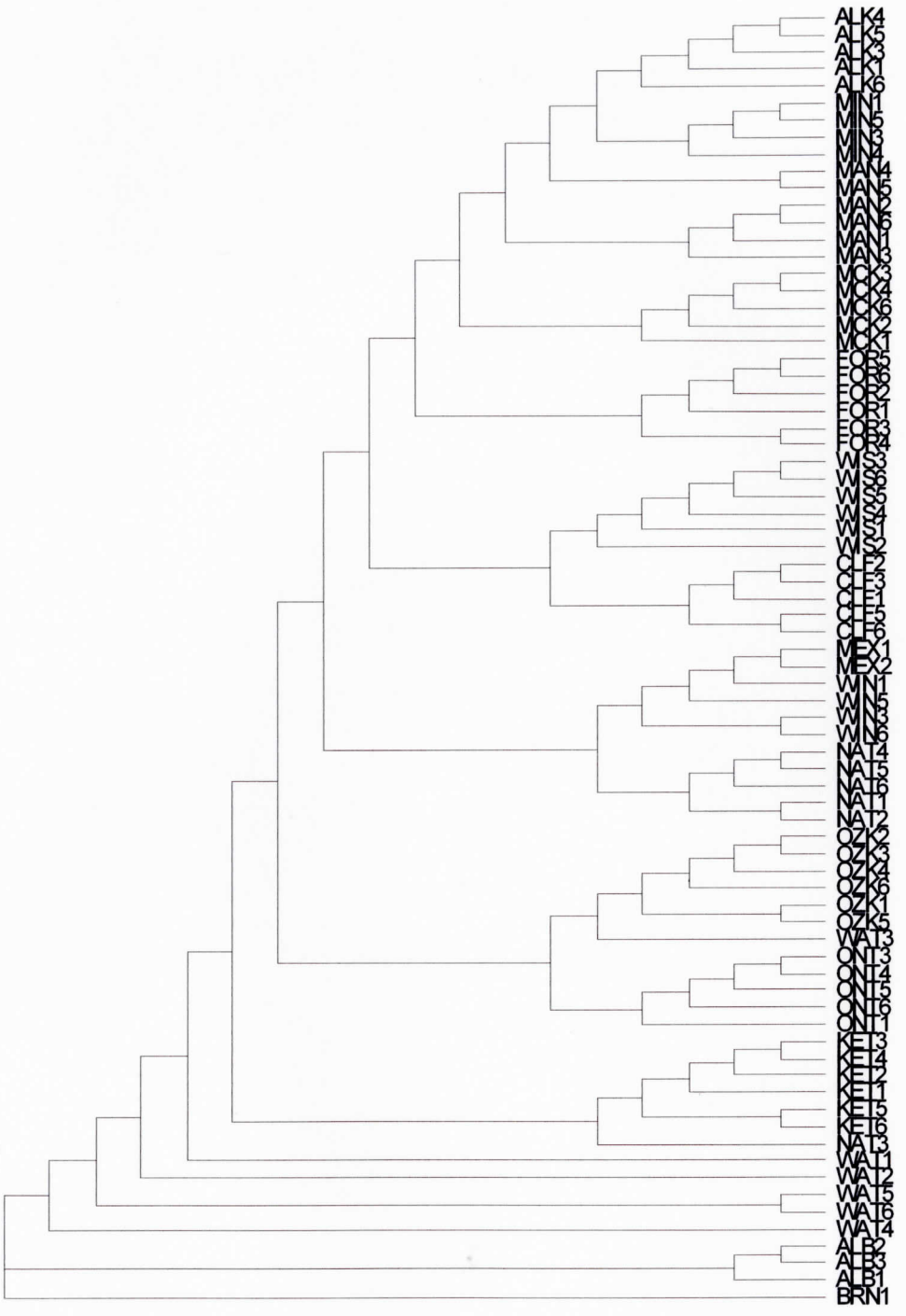


Figure 5. Neighbor Joining (Nei Li) Tree Generated from Analysis of 76 Individuals.

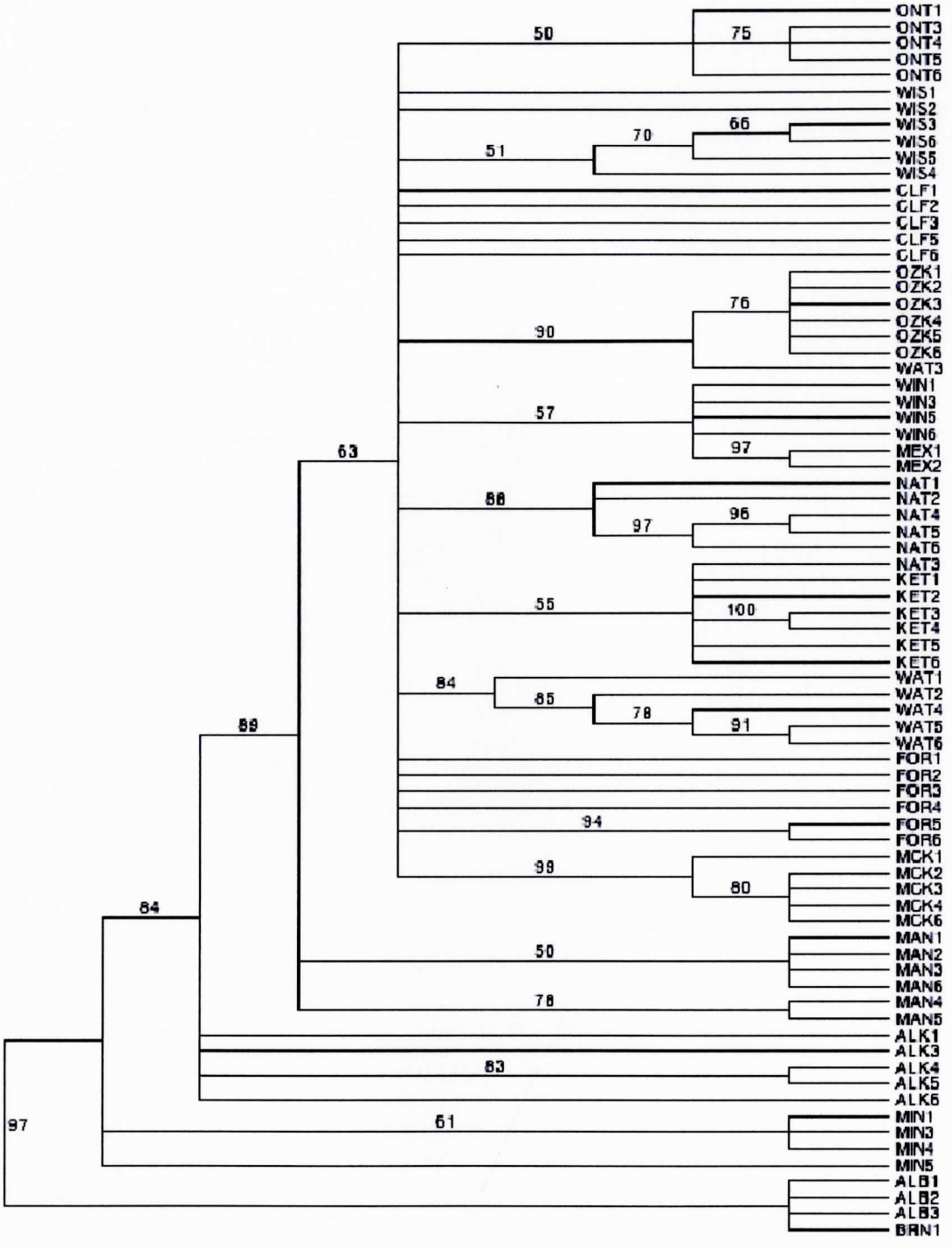


Figure 6. 50% Majority Rule Consensus Bayesian Tree (1 of 3) Generated from Analysis of 76 Individuals. Bootstrap values are shown.

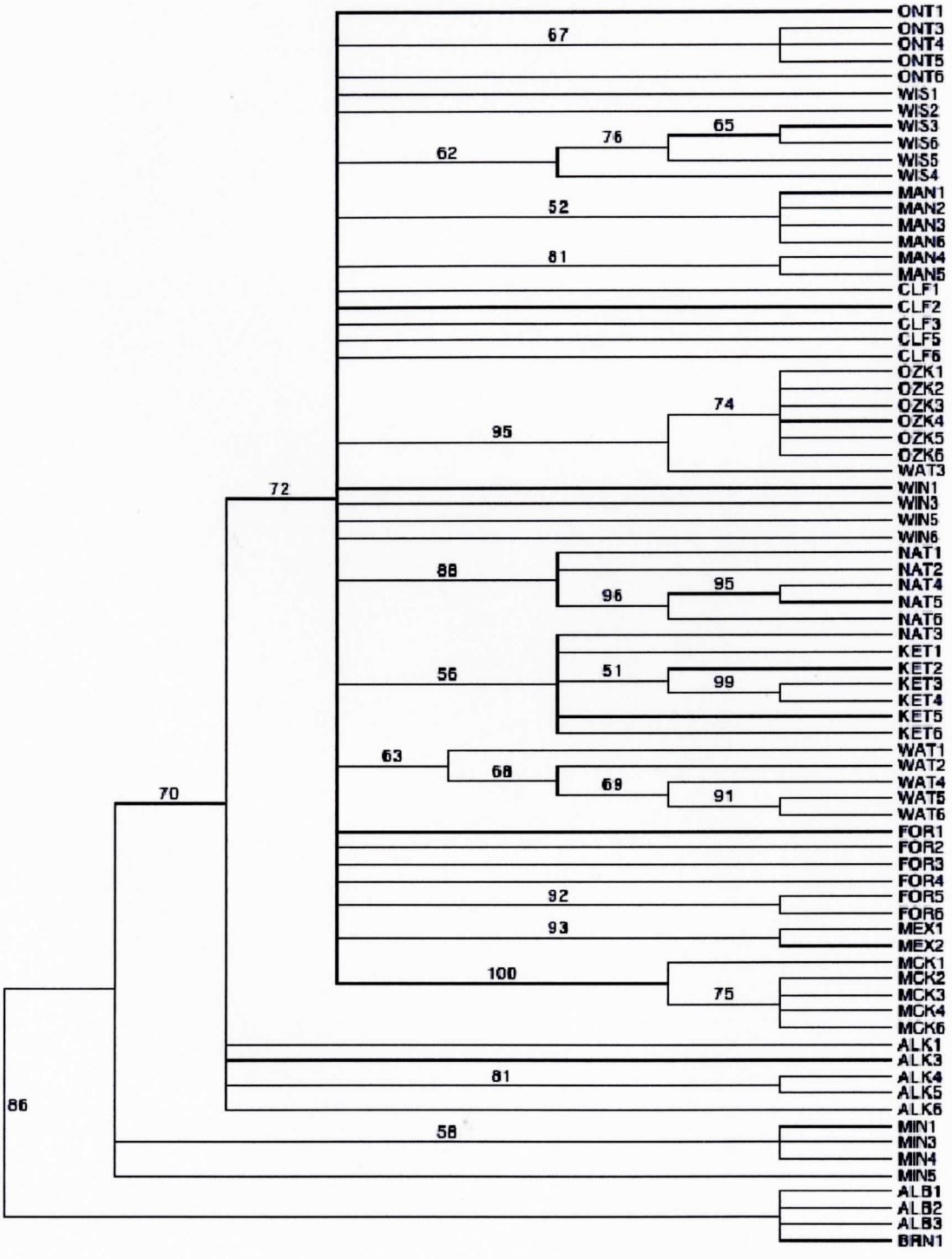


Figure 7. 50% Majority Rule Consensus Bayesian Tree (2 of 3) Generated from Analysis of 76 Individuals. Bootstrap values are shown.

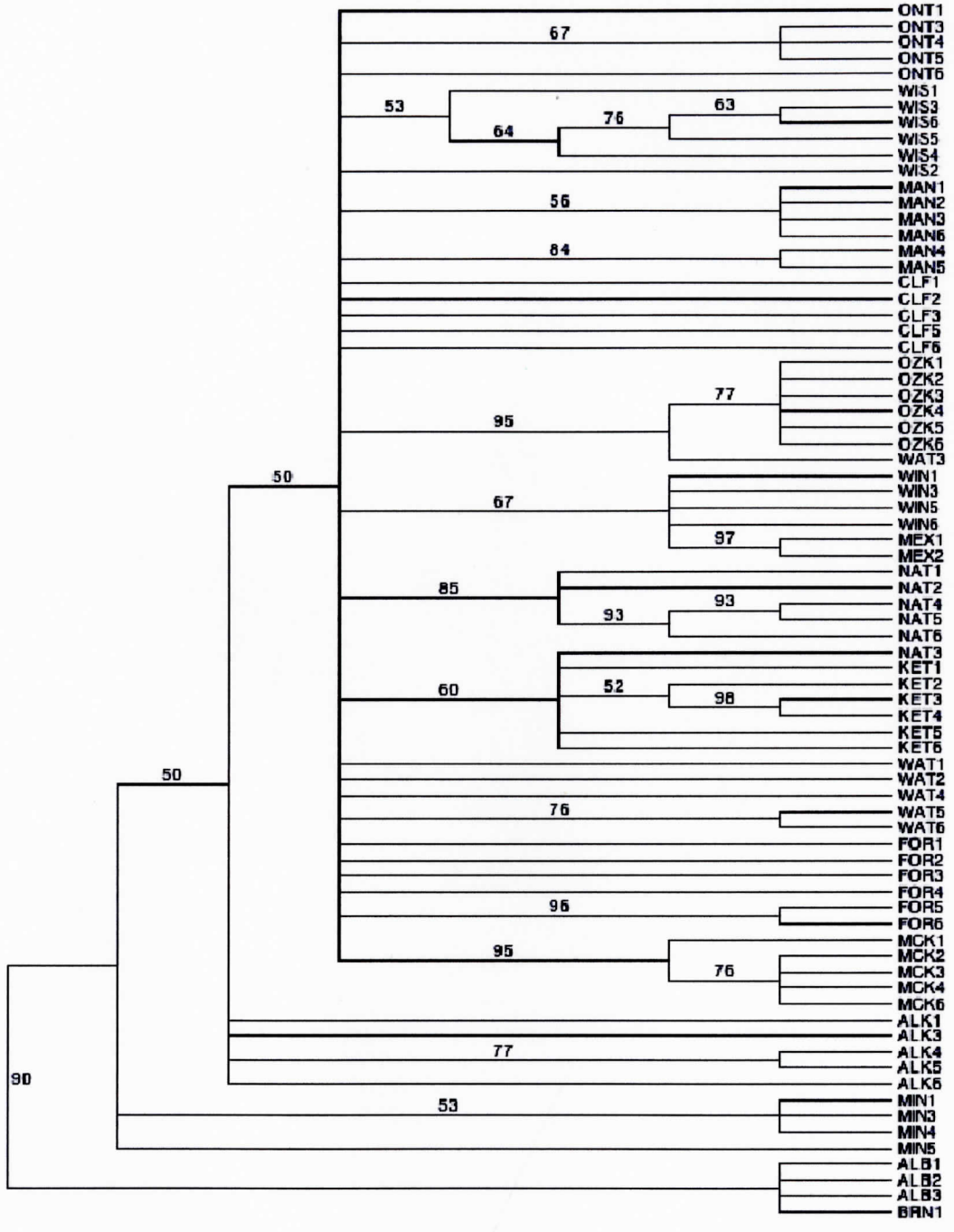


Figure 8. 50% Majority Rule Consensus Bayesian Tree (3 of 3) Generated from Analysis of 76 individuals. Bootstrap values are shown.

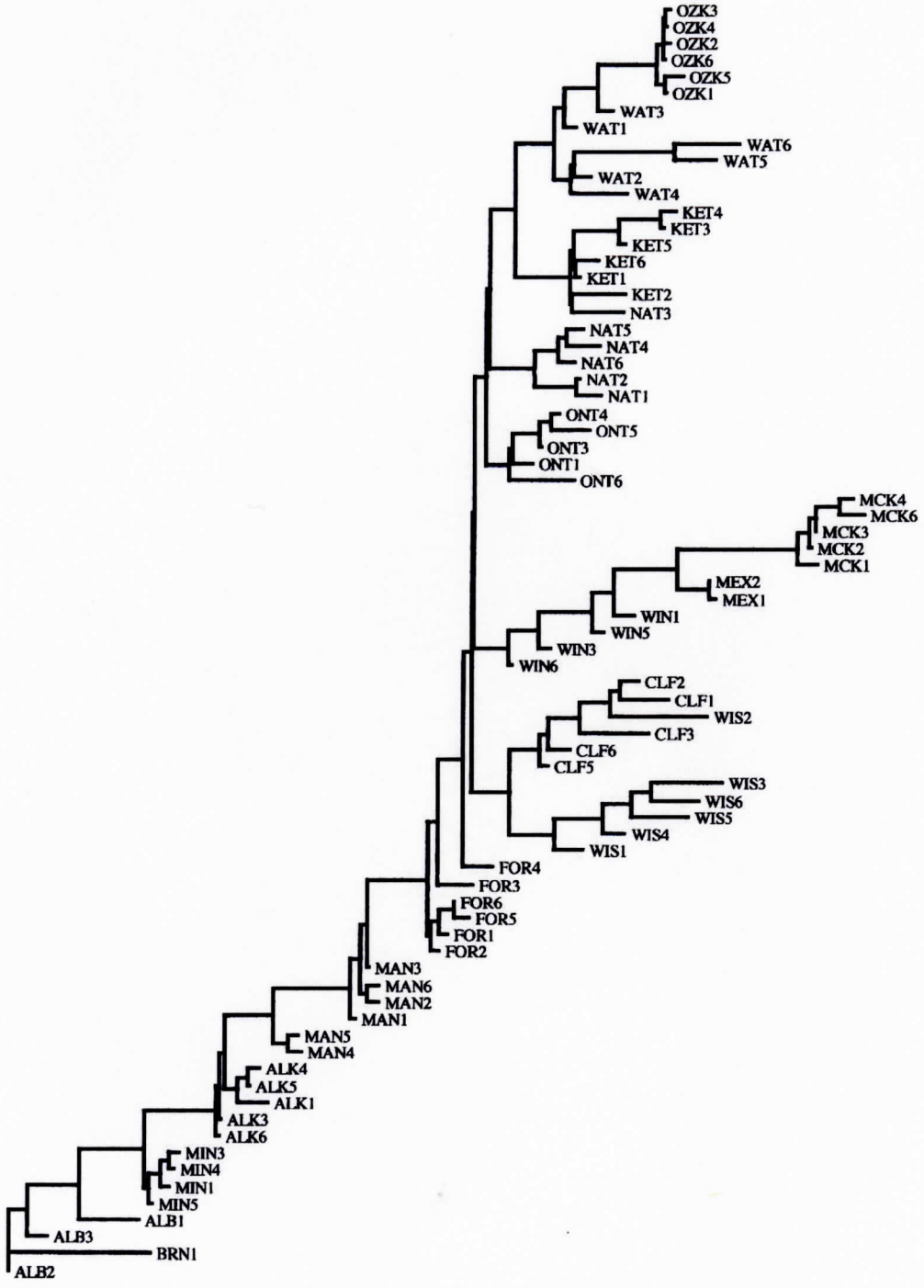


Figure 9. Consensus Tree from Bayesian Analysis of 76 Individuals. Branch lengths are estimated using parsimony and indicate relative number of stepwise changes among taxa. Tree length=316, Consistency Index=0.1646, Homoplasy Index=0.8354, Retention Index=0.6471.

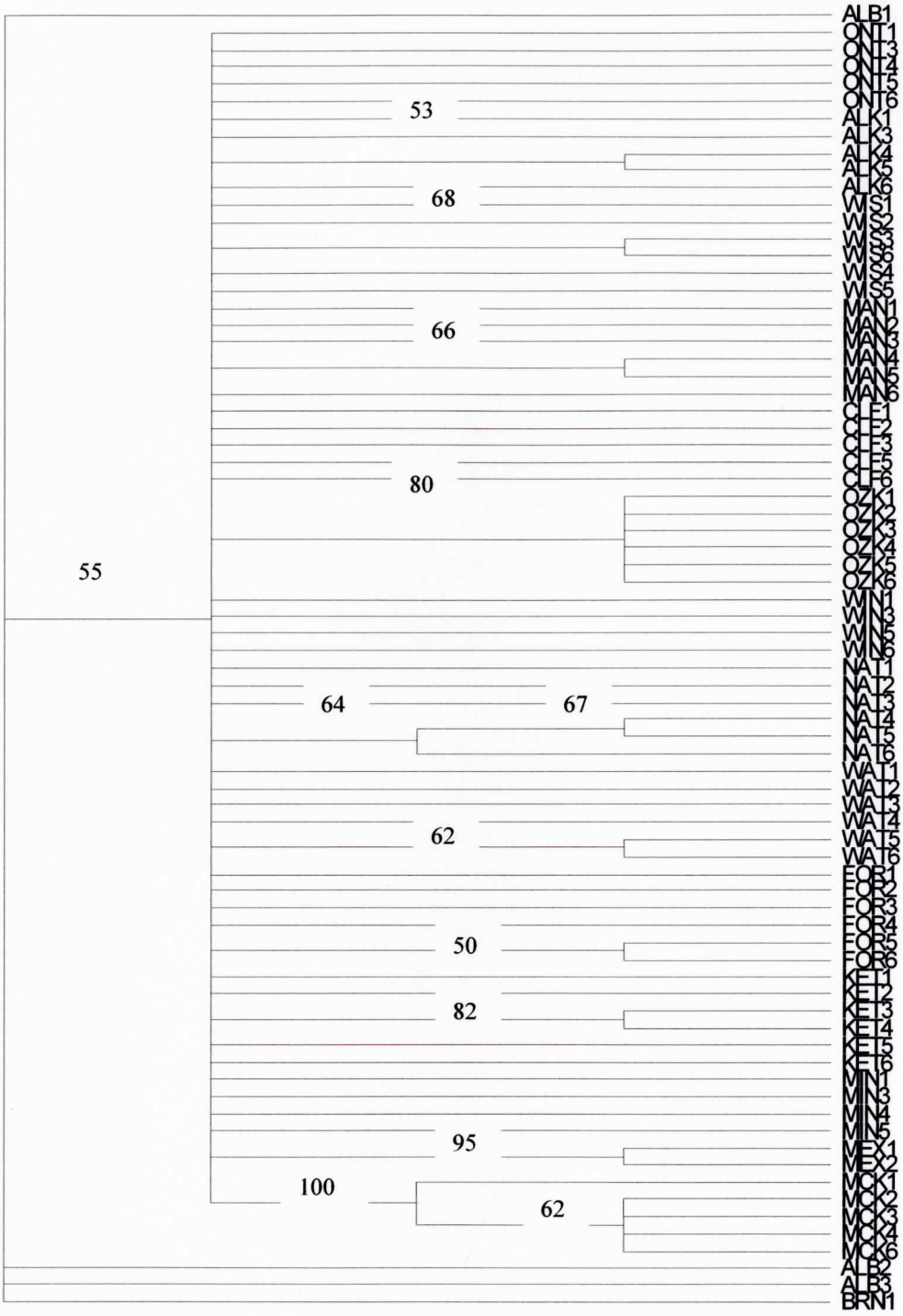


Figure 10. Maximum Likelihood Analysis of 76 Individuals. 50% majority rule consensus generated by resampling trees generated from 10,000 heuristic searches. Bootstrap values are shown.

shows total collapse of the *Carex eburnea* complex into an unresolved polytomy, whose bootstrap value is 55. Within the polytomy, the MCK clade is supported with a value of 100. A heuristic search by PAUP generated 40 best trees (tree length=296) and the consensus tree (Figure 11) placed MIN individuals basal and the ONT/WAT/OZK clade was most derived.

Morphometrics

Morphometric analysis was used to compare 124 specimens from five species. Ninety-one individuals from the *Carex eburnea* complex were used to compare morphologies across the range (Figure 12) of the complex, along with 6-11 individuals from each of the other taxa (*C. alba*, *C. brunnea* and *C. ussuriensis*).

Principle Components Analysis of the four *Carex* Section *Albae* taxa and the putative outgroup taxon *C. brunnea* (*Carex* Section *Graciles*) showed some separation of the groups in both untransformed (size) and transformed (shape) data. *Carex eburnea* formed a very tight cluster in the analysis of untransformed data (Figure 13A). *Carex brunnea* and *C. alba* showed some overlap, with *C. ussuriensis* somewhat separate. *Carex mckittrickensis* clustered between *C. eburnea* and the other three taxa. PCA analysis of the transformed data (Figure 13B) showed similar spatial positions of the taxa, but less separation, suggesting that some separation in the untransformed data analysis was due to size variation. Univariate analysis of all five taxa showed that *C. eburnea* means are

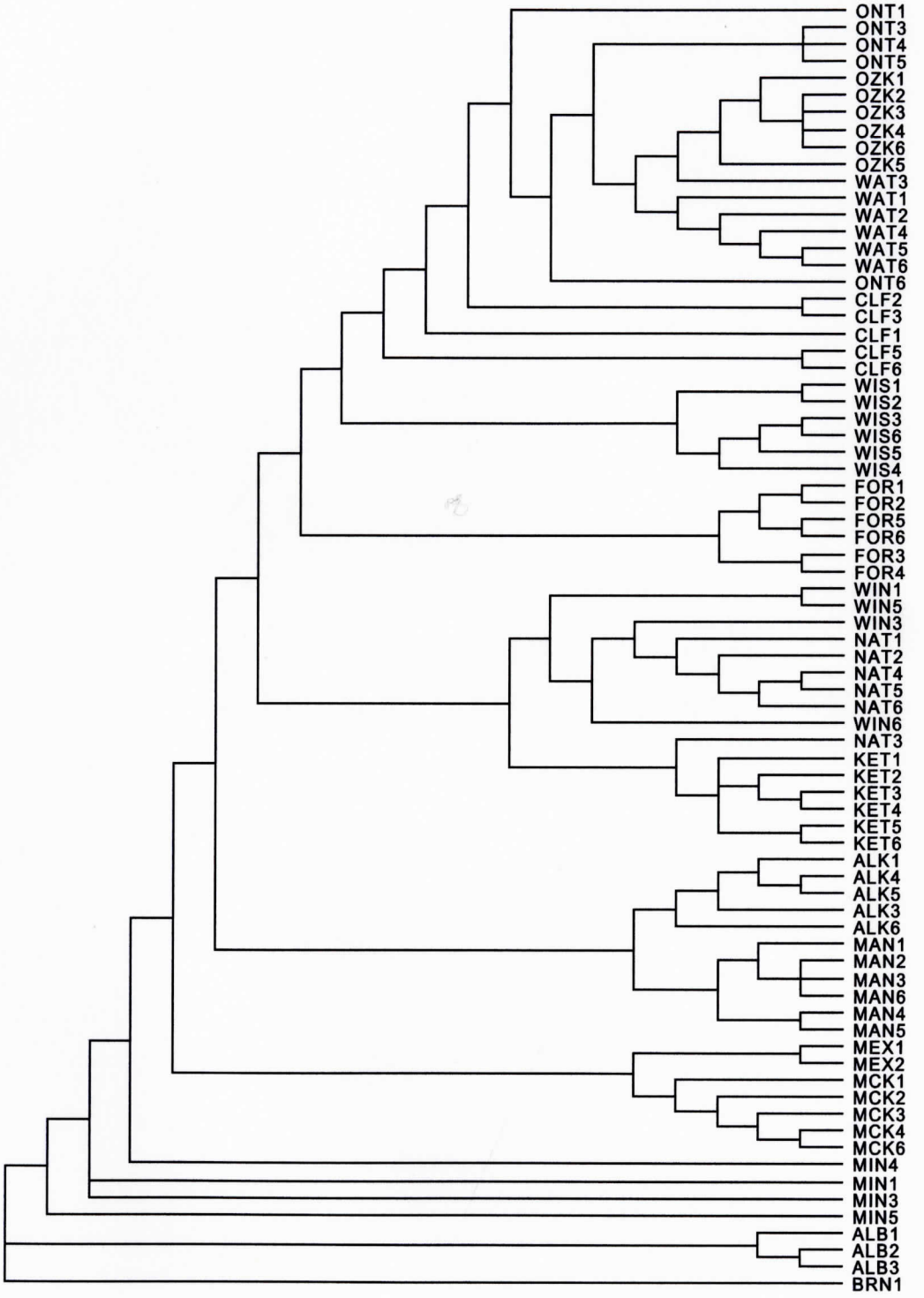


Figure 11. Heuristic Strict Consensus of Parsimony Analysis of 76 Individuals. Consensus of 40 best trees (tree length=296).

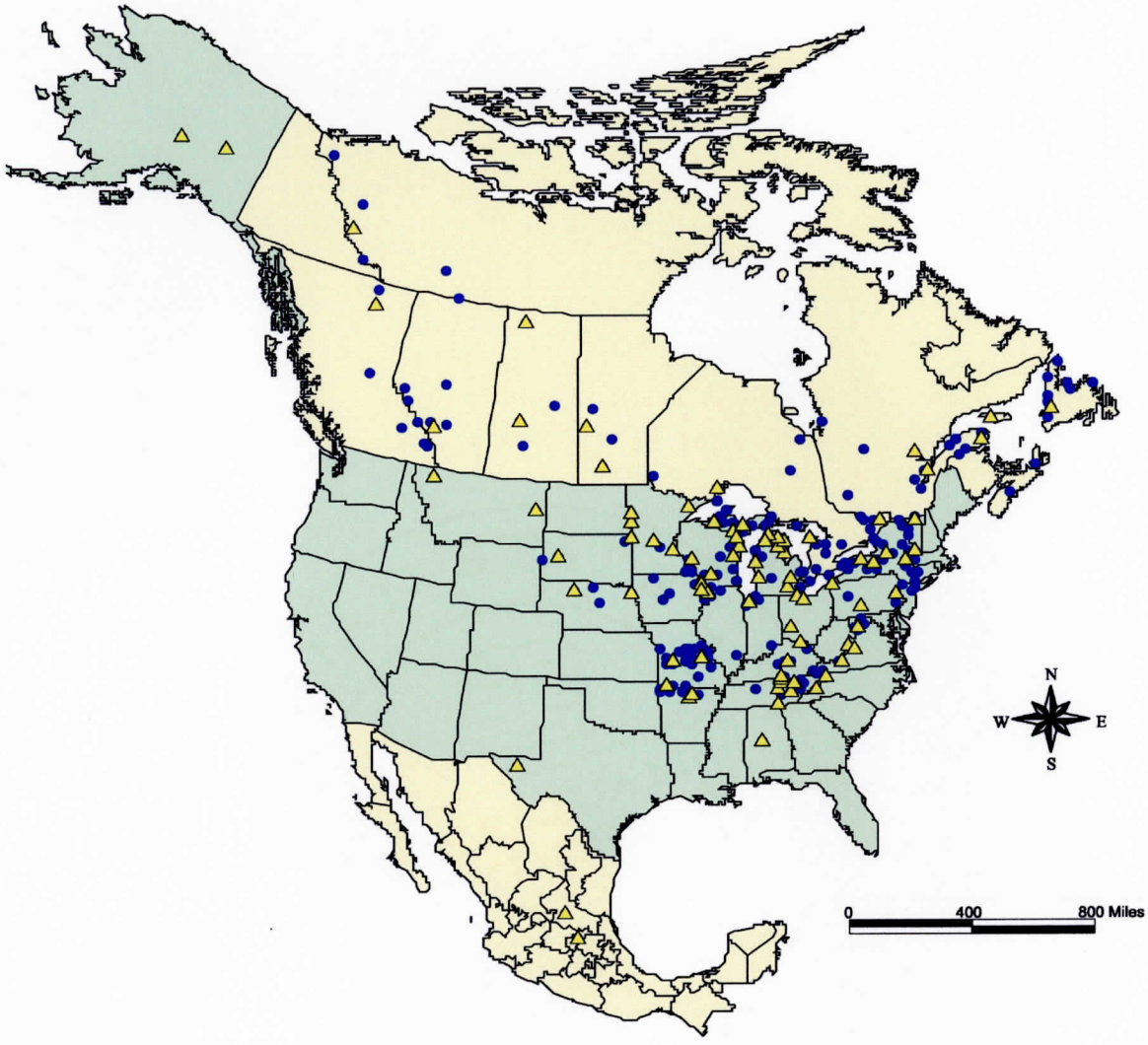


Figure 12. Specimens Used in Morphometric Analysis. Specimens used are represented by yellow triangles and are shown in the context of the distribution (blue dots).

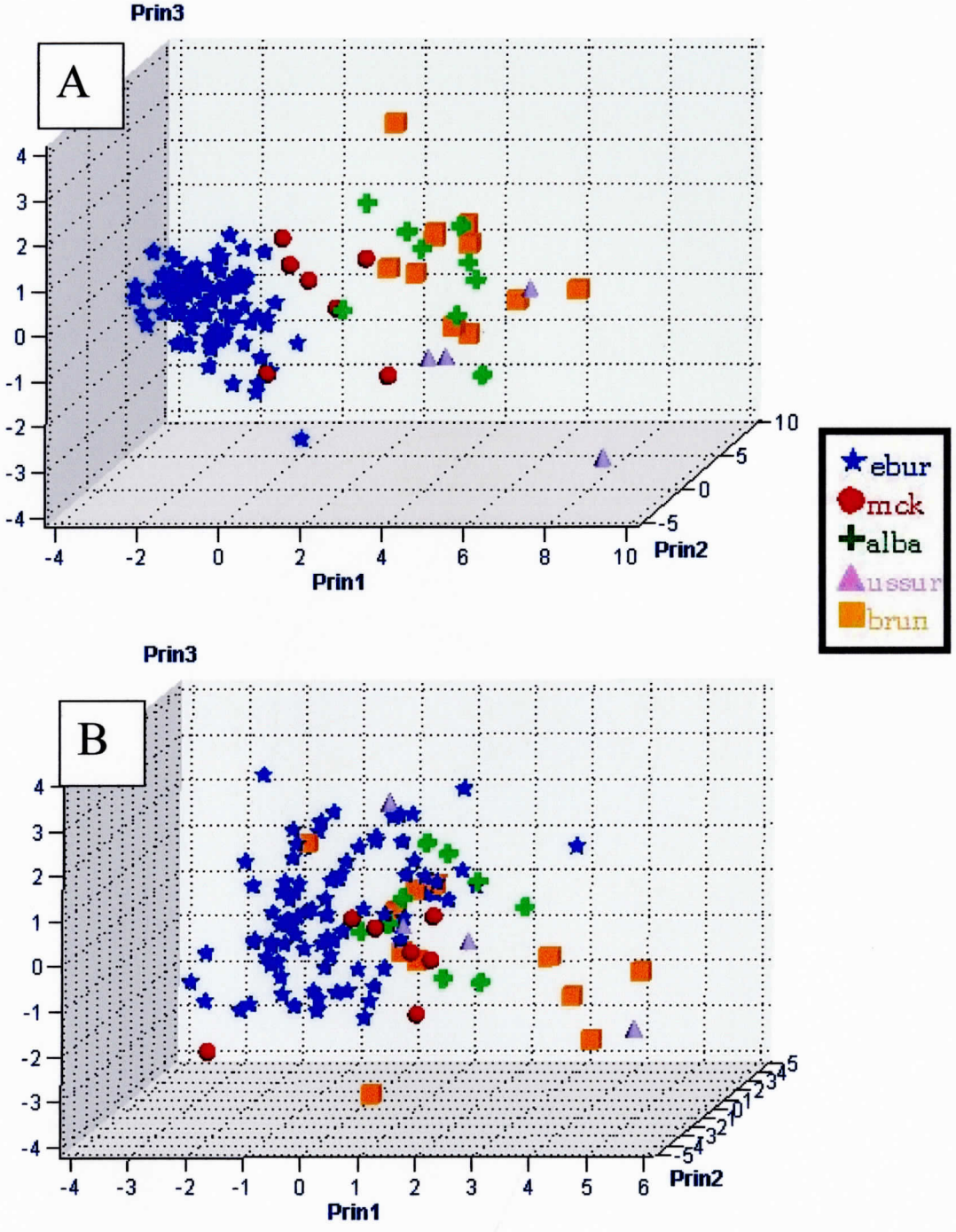


Figure 13. Principle Components Analysis of Morphometric Data for four *Carex* Section *Albae* taxa plus *C. brunnea*. A) Untransformed B) Transformed.

smaller than the other four taxa in all 14 measurements (Figures 14-27). Principle Components Analysis of untransformed variables between *C. eburnea* and *C. mckittrickensis* (Figure 28A) shows relatively clear separation of the two taxa. Discriminant Function Analysis of untransformed data (Figure 28B) indicates that both taxa are correctly identified 100% of the time on the basis on untransformed data. PCA of untransformed data (Figure 29A) between the two taxa, however, showed little discrete clustering. Discriminant Function Analysis (Figure 29B) shows that 10.2% of the time, *C. eburnea* is mistakenly identified as *C. mckittrickensis,* and *C. mckittrickensis* is misidentified as *C. eburnea* 14.3% of the time using these data.

When specimens were clustered into groups based on the dominant canopy tree, PCA showed some clustering occurred when untransformed data (Figure 30A) were examined and similar clustering occurred using transformed data (Figure 30B), although individuals clustered more tightly overall.

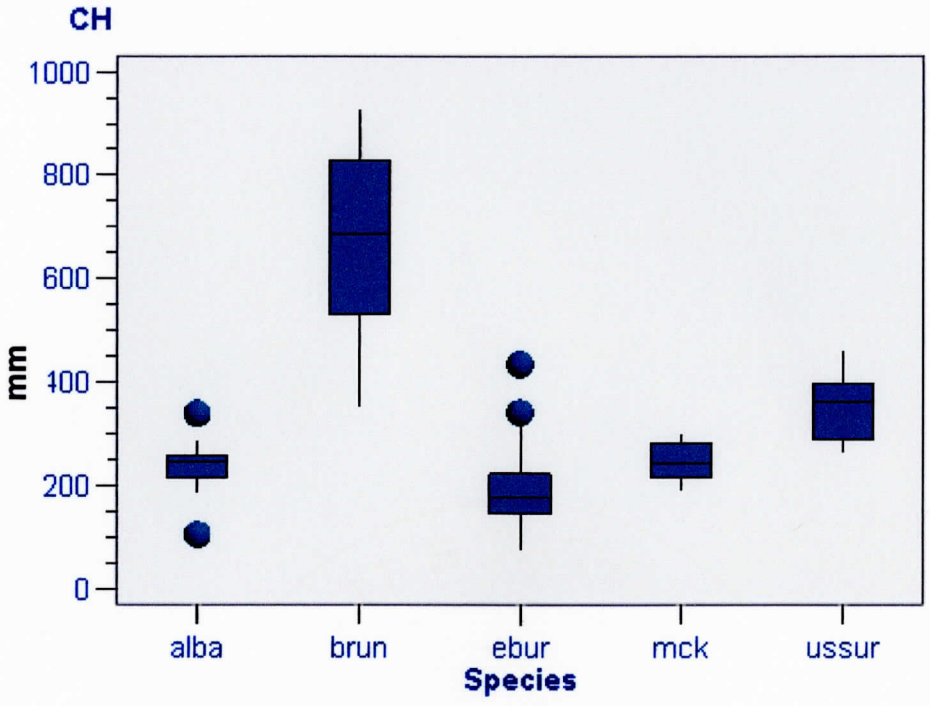


Figure 14. Univariate Analysis of Culm Height (CH). Means, standard deviations and outliers are shown.

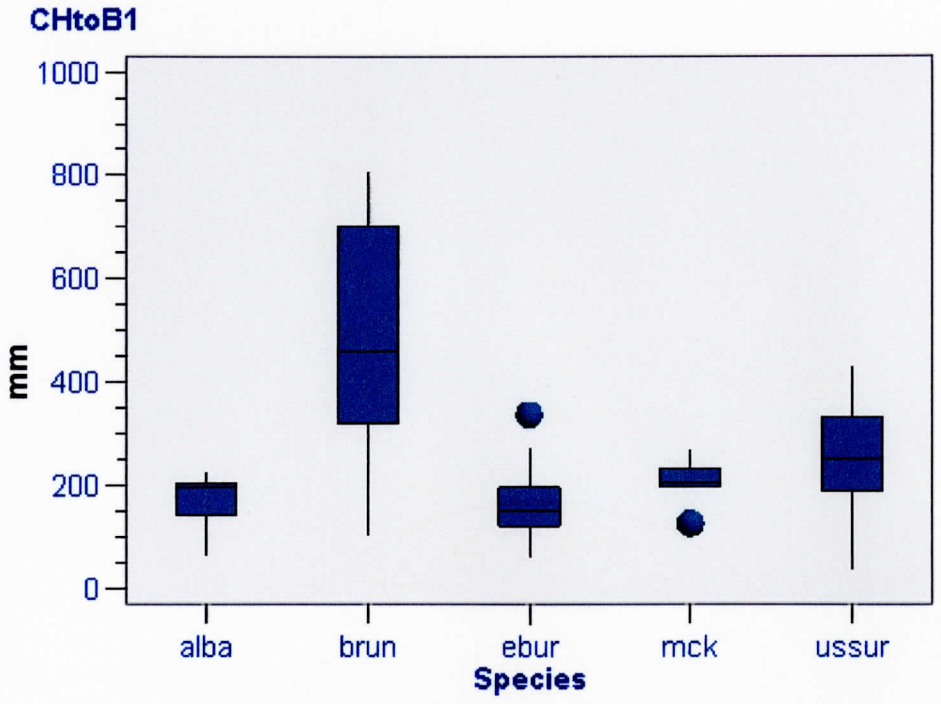


Figure 15. Univariate Analysis of Culm Height to First Bract (CHtoB1). Means, standard deviations and outliers are shown.

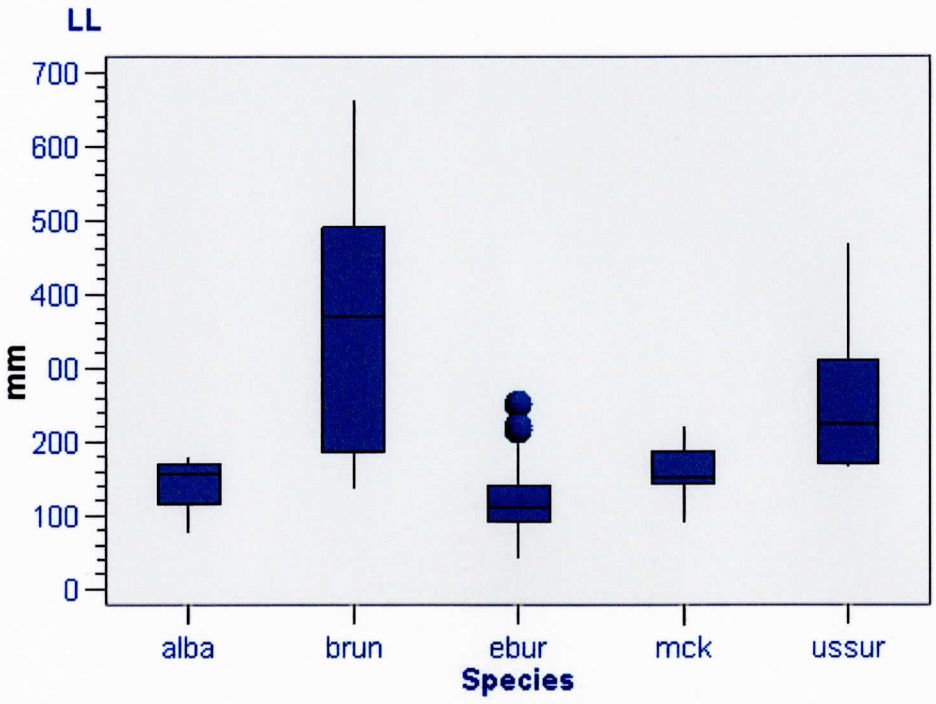


Figure 16. Univariate Analysis of Leaf Length (LL). Means, standard deviations and outliers are shown.

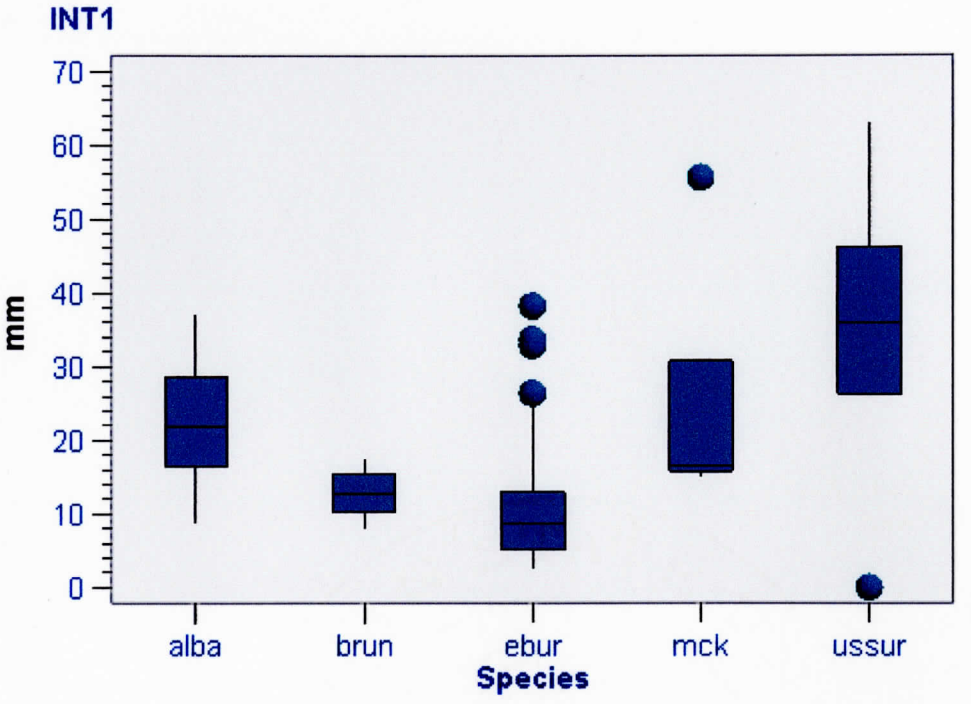


Figure 17. Univariate Analysis of Culm Internode 1 Length (INT1). Means, standard deviations and outliers are shown.

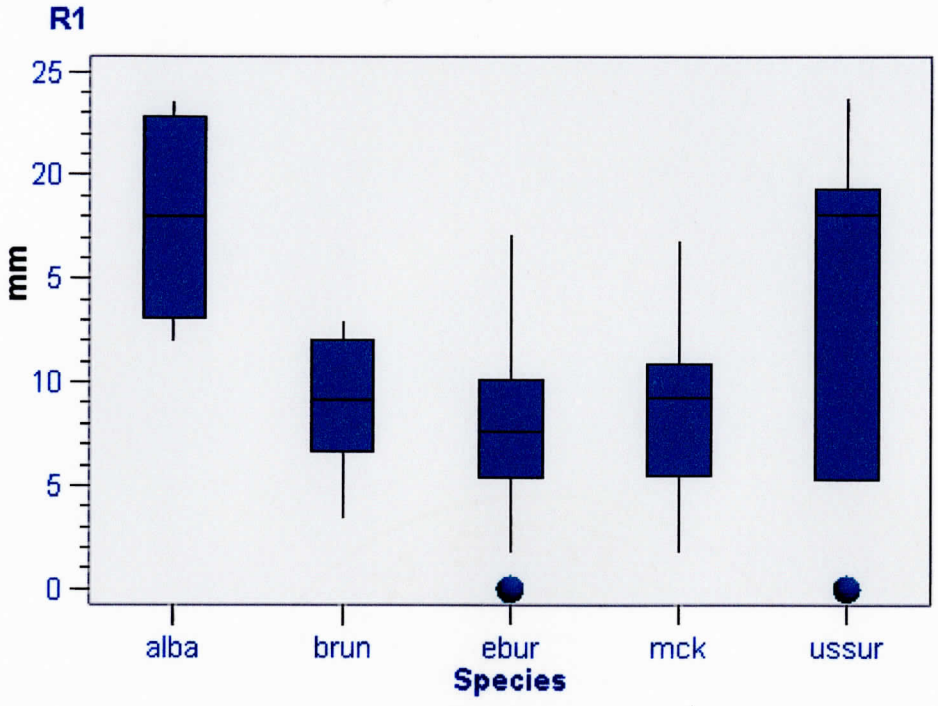


Figure 18. Univariate analysis of Rachis 1 Length (R1). Means, standard deviations and outliers are shown.

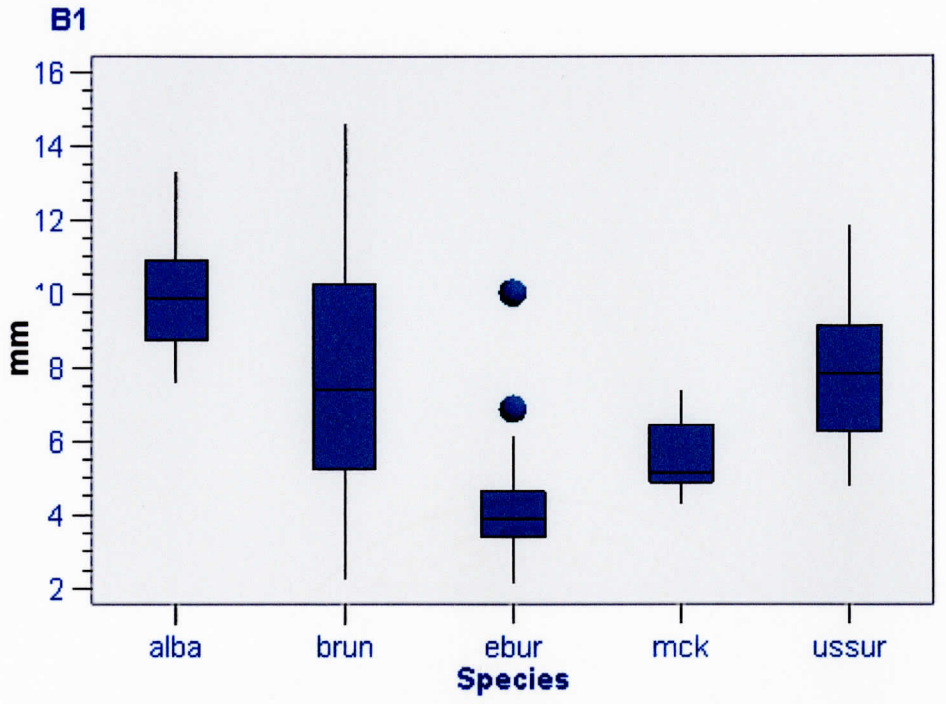


Figure 19. Univariate analysis of Bract 1 Length (B1). Means, standard deviations and outliers are shown.

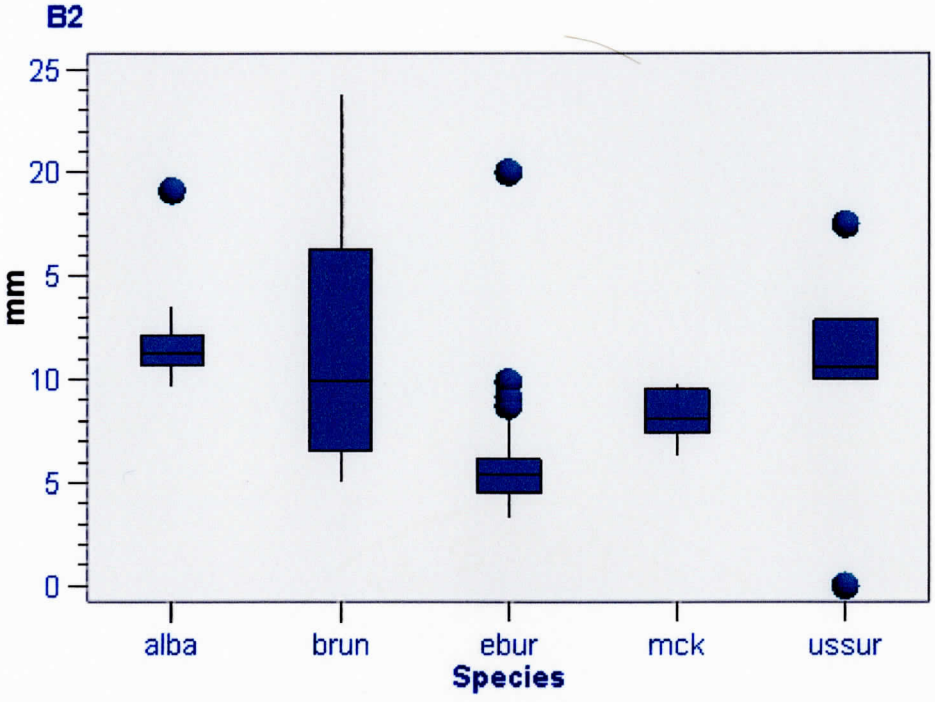


Figure 20. Univariate analysis of Bract 2 Length (B2). Means, standard deviations and outliers are shown.

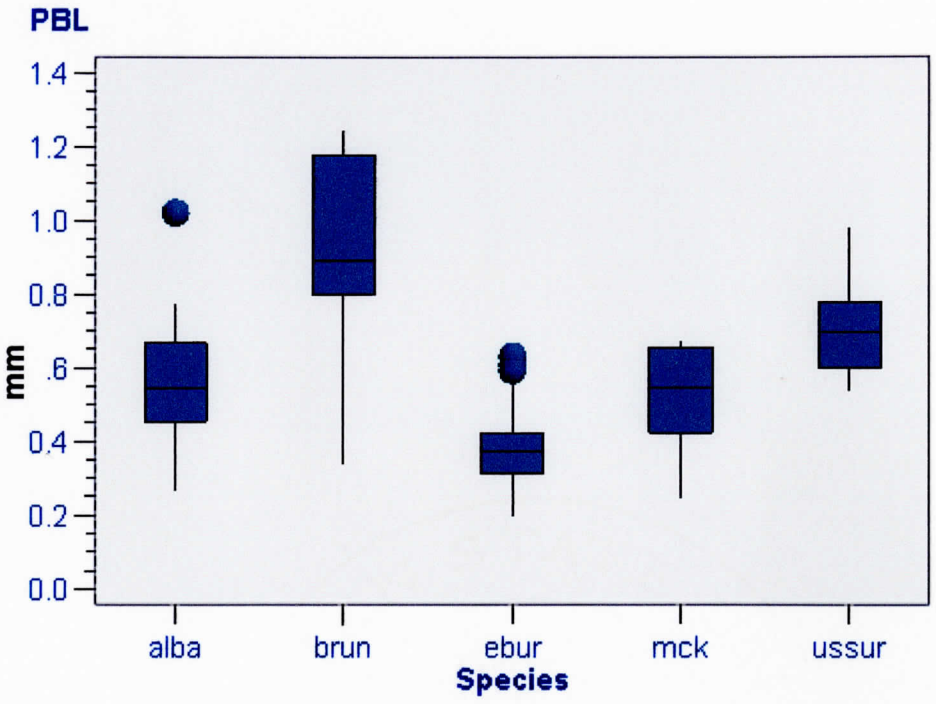


Figure 21. Univariate analysis of Perigynium Beak Length (PBL). Means, standard deviations and outliers are shown.

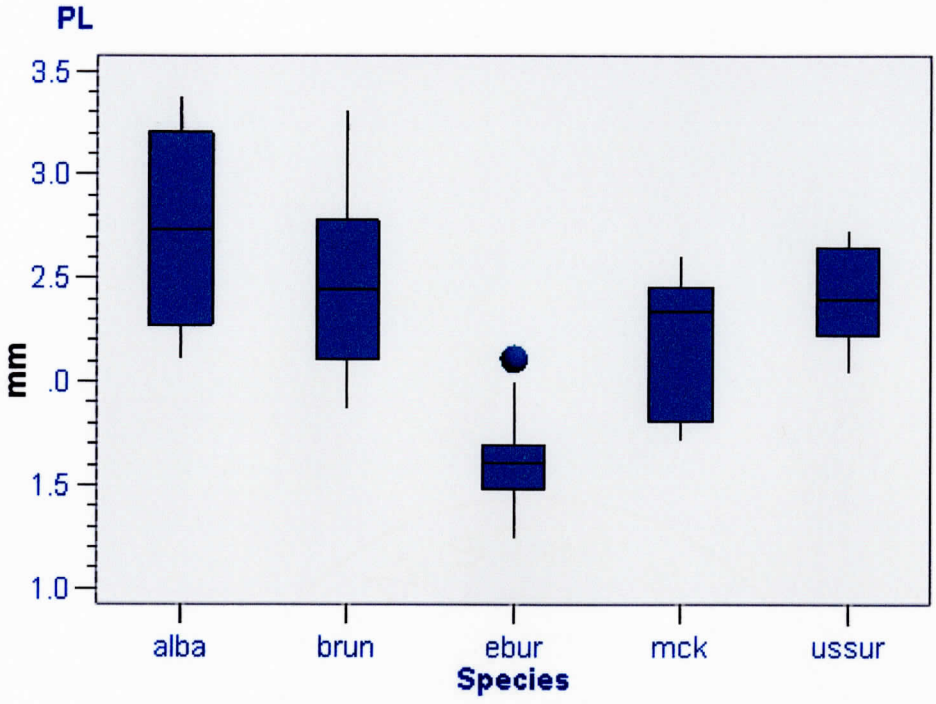


Figure 22. Univariate analysis of Perigynium Length (PL). Means, standard deviations and outliers are shown.

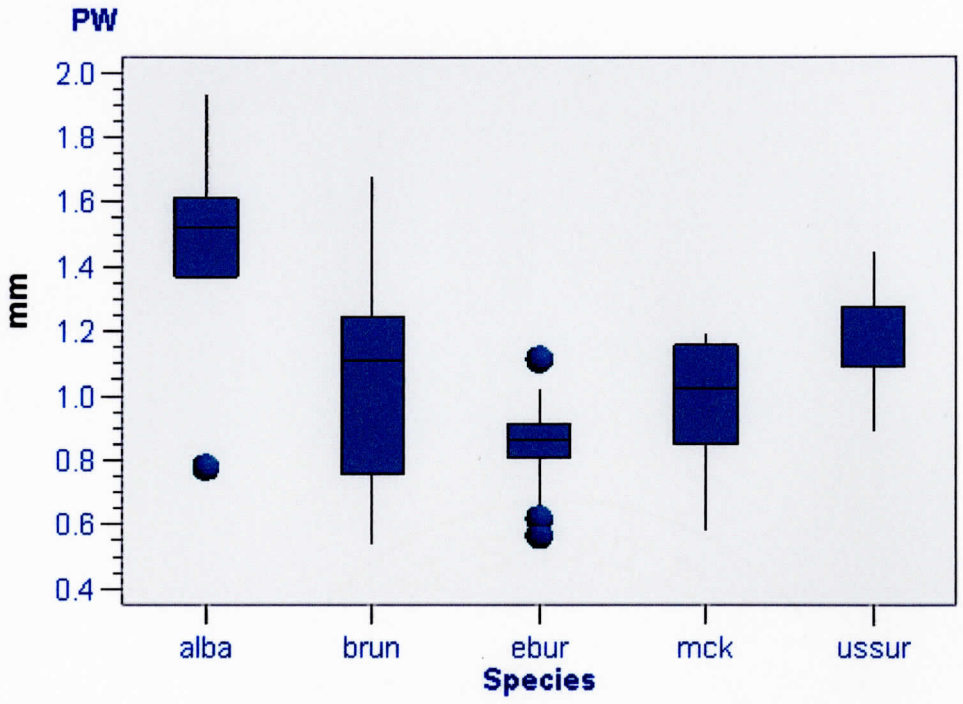


Figure 23. Univariate analysis of Perigynium Width (PW). Means, standard deviations and outliers are shown.

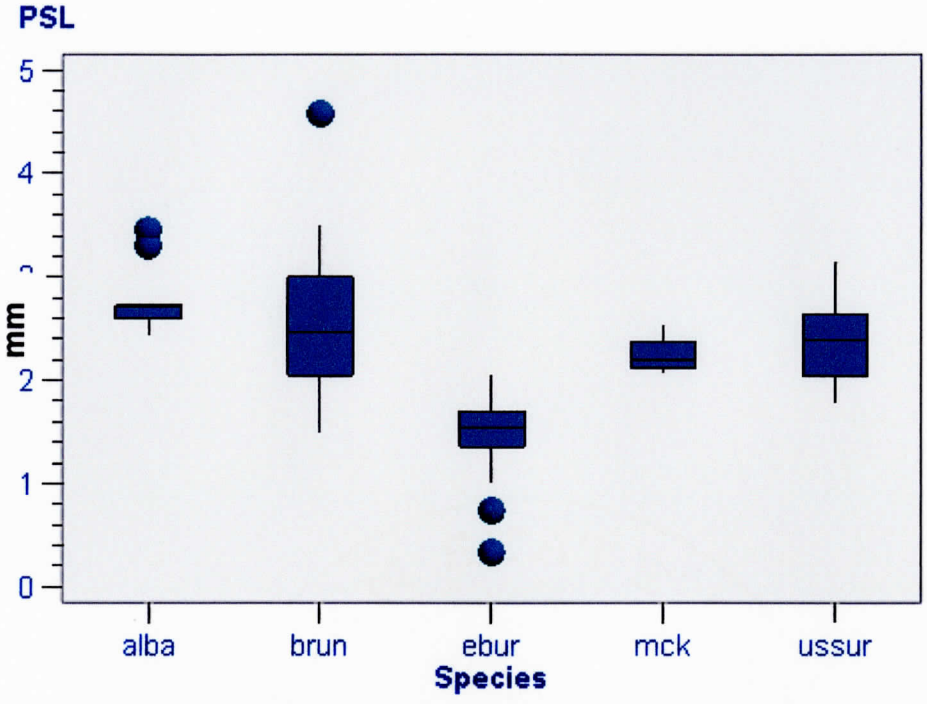


Figure 24. Univariate analysis of Pistillate Scale Length (PSL). Means, standard deviations and outliers are shown.

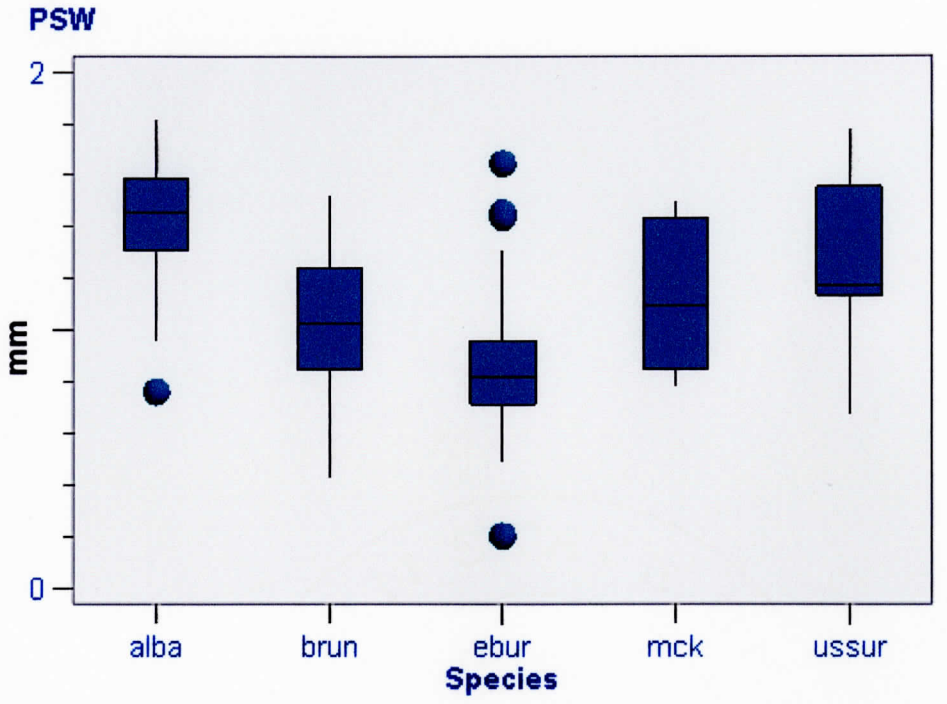


Figure 25. Univariate analysis of Pistillate Scale Width (PSW). Means, standard deviations and outliers are shown.

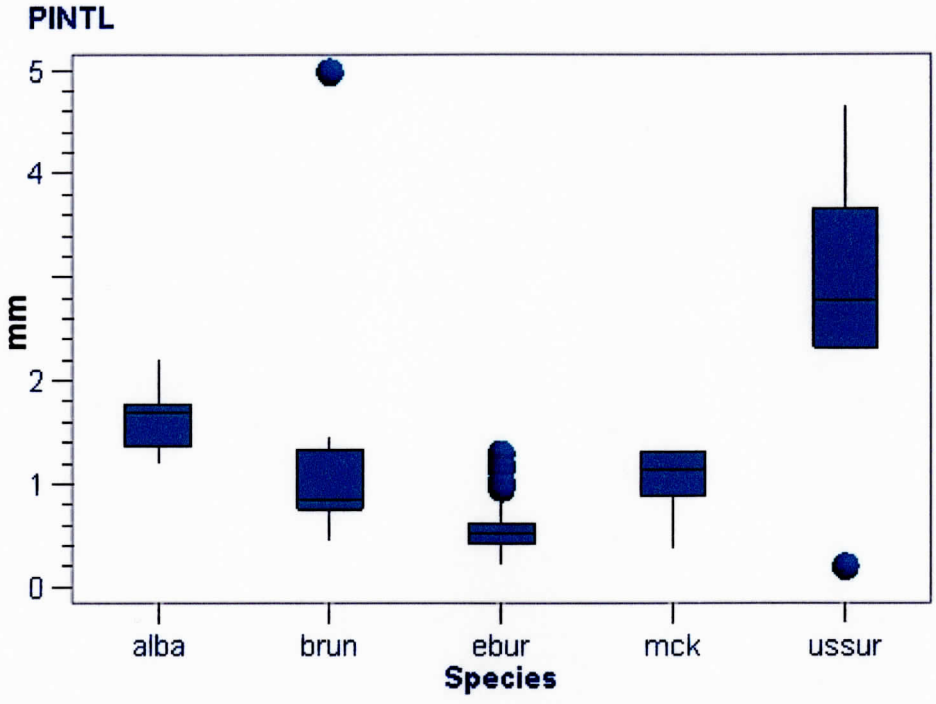


Figure 26. Univariate analysis of Lowest Inflorescence Internode Length (PINTL). Means, standard deviations and outliers are shown.

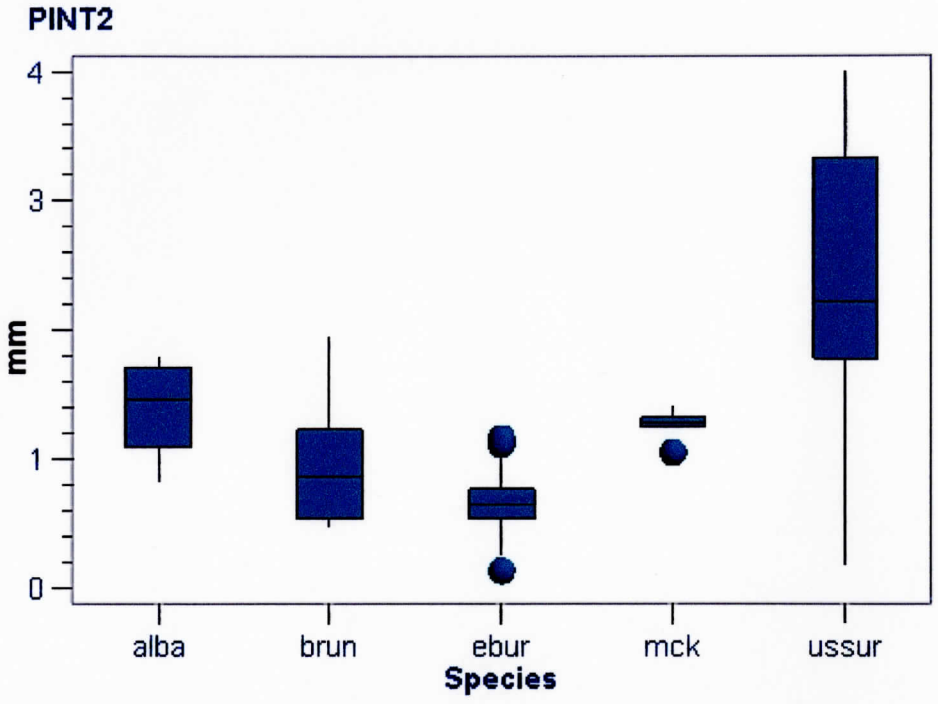
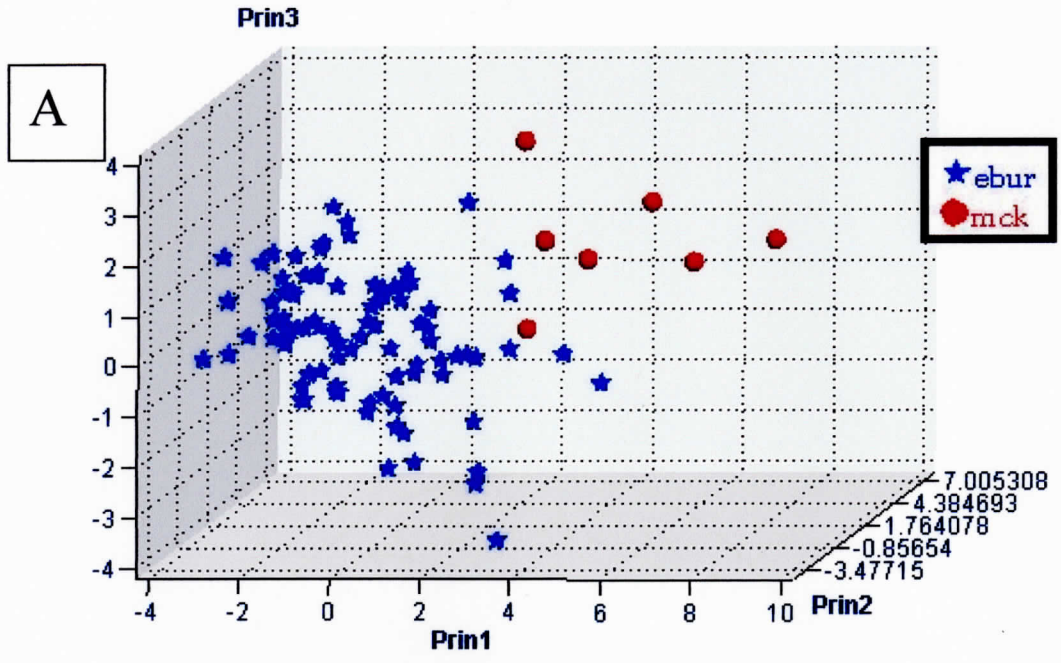


Figure 27. Univariate analysis of Second Inflorescence Internode Length (PINT2). Means, standard deviations and outliers are shown.

A

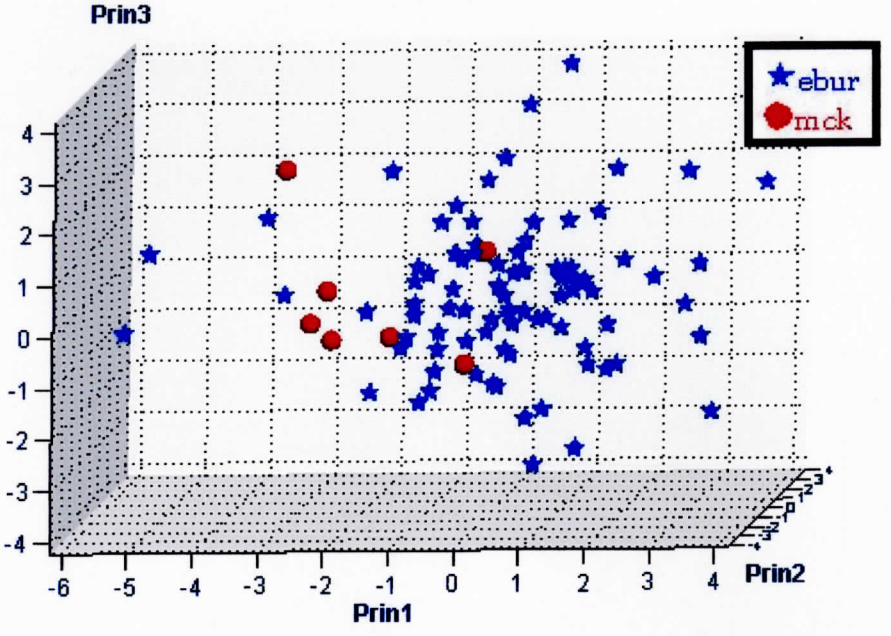


B

From Species	ebur	mck	Total
ebur	n=90 100.0%	n=0 0.0%	n=90 100.0%
mck	n=0 0.0%	n=7 100.0%	n=7 100.0%

Figure 28. Comparison of *C. eburnea* and *C. mckittrickensis* using untransformed data. A) Principle Components Analysis B) Discriminant Function Analysis.

A



B

From Species	ebur	mck	Total
ebur	n=79 89.8%	n=9 10.2%	n=88 100.0
mck	n=1 14.3%	n=6 85.7%	n=7 100.0%

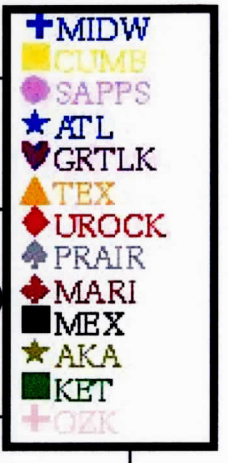


Figure 29. Comparison of *C. eburnea* and *C. mckittrickensis* using transformed data. A) Principle Components Analysis B)Discriminant Function Analysis.

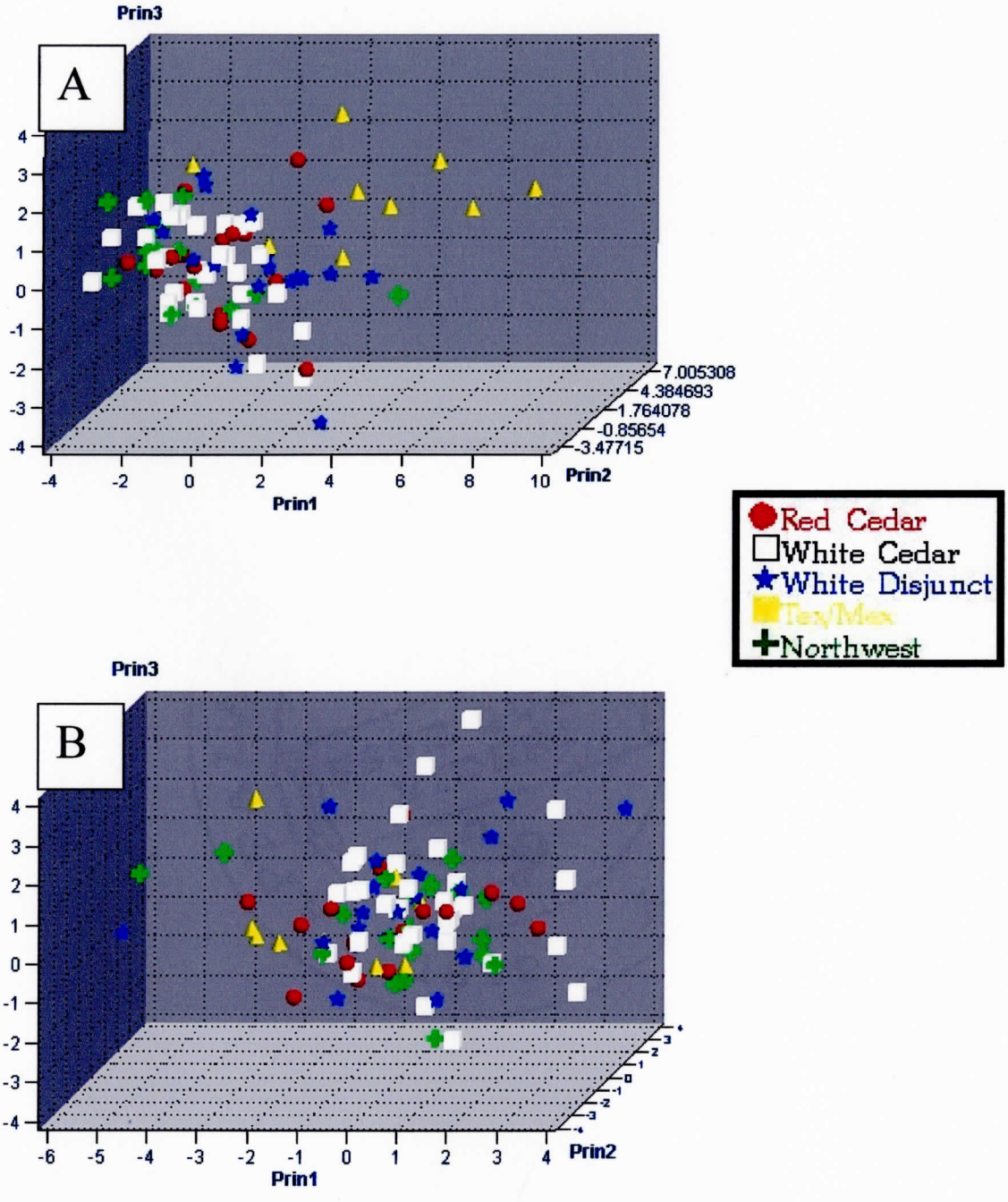


Figure 30. Principle Components Analysis of Morphometric Data Assigned into Categories on the Basis of Dominant Canopy Type A) Untransformed B) Transformed.

DISCUSSION

The *Carex eburnea* distribution in North America, coupled with its restriction to limestone habitats and consequent occurrence in isolated populations makes it an interesting group to study. Little work has been done on the complex and no molecular studies have been carried out prior to this research. By using both molecular and morphometric approaches, a snapshot of genetic and morphologic structure can be used to assess the segregation of *C. mckittrickinsis* from *C. eburnea*, to suggest past migratory routes for *C. eburnea* and to evaluate the complex in terms of alternative species concepts.

The major finding of the study was that the morphometric and molecular analyses do not support the segregation of *C. mckittrickensis* from *C. eburnea*. Ball (1998) differentiated *C. mckittrickensis* from *C. eburnea* based on size differences. He noted that pistillate scale length and perigynium beak length were larger in *C. mckittrickensis* and more similar to the larger Eurasian species. Principle Components Analysis of untransformed data in this study mirrors Ball's analysis in separating *C. mckittrickensis* from *C. eburnea*. However, the ISSR results, the transformed morphometric data, the distributional data and the ecological data all showed that segregation of the Texas population is not merited, provides no biologically meaningful information, and promotes a chaotic classification for the complex.

This study uses molecular, morphological and ecological data to attempt to address three questions: 1) What is the genetic and morphometric structure of the *C. eburnea* complex? 2) Is *C. mckittrickensis* a valid segregate species? and 3) Can past migratory routes be inferred for this species complex? For each of these questions, the choice of outgroups is critical to the analysis. Prior to these analyses, it was cautioned that *C. brunnea* may be too distantly related to *Carex* Section *Albae* to serve as an outgroup in the study, and that Section *Albae* may in fact have no close relatives (A.A. Reznicek, pers. comm.). However, because none of the molecular analyses clustered *C. brunnea* within the ingroup, and because sufficient homologous characters and character states were available for morphometric analysis, *C. brunnea* was considered a suitable outgroup for character polarization in this analysis. It is also evident that *C. alba* was a suitable outgroup taxon in this study. As with *C. brunnea*, *C. alba* did not cluster in the ingroup based on molecular data, making it another suitable outgroup taxon in this analysis. Therefore, the use of these two outgroups in the analyses is appropriate.

The various phylogenetic analyses (Parsimony, Maximum Likelihood and Bayesian analyses) of ISSR data all demonstrate that *C. mckittrickensis* is embedded within *C. eburnea*. Based upon these results, recognition of *C. mckittrickensis* would render *C. eburnea* paraphyletic. Neighbor Joining distance analysis, which shows only shared band presence, shows Watauga Lake (WAT) specimens as being most different at the individual level, and Ketona Glade (KET) as being most different at the population level. The UPGMA analysis was

the only molecular analysis that supported Ball's segregation of *C. mckittrickensis*, and the UPGMA analysis is problematic in that by clustering taxa on the basis of absence of bands, there is an overemphasis on characters that are likely to be homoplasious. Therefore, in summary of the ISSR data, the phylogenetic analyses do not support segregation of *C. mckittrickensis*, since this lineage shares more synapomorphies with other derived lineages than it does with several lineages that apparently diverged earlier. In this case, recognition of *C. mckittrickensis* renders *C. eburnea* paraphyletic. If species segregation were based upon the Neighbor Joining analysis, then the Ketona population is a more likely candidate to be recognized as a segregate species (this result will be explored more fully in the discussion of ecological variation). Finally, because any mutation in the primer regions will lead to a loss of a band at a site, the clustering of individuals based upon shared absence, in addition to the shared presence used in NJ analysis, leads to clustering of individuals that do not share homologous character states. This fact renders the results from the UPGMA analysis suspect, and should not be considered significant in the recognition of species boundaries.

Although the findings from the morphometric analysis of the untransformed data were in agreement with Ball's study, showing *C. mckittrickensis* clustering separately from the rest of *C. eburnea*, the analysis based upon size was not supported when shape differences are considered. The analysis of untransformed data clusters the two species, and several of the *C. mckittrickensis* specimens are deeply embedded within the *C. eburnea*

cluster. Examination of the raw measurements reveals that the Arkansas specimens are intermediate in size between *C. mckittrickensis* and other *C. eburnea* specimens, and fill the gap in Ball's morphological differentiation of the two taxa. Because there is continuous size variation and overlap of character states between *C. mckittrickensis* and *C. eburnea*, *C. mckittrickensis* does not merit segregation. The perceived morphological gap that Ball identified in his analysis was obscured when more specimens were included in the data set. The analysis using transformed data unequivocally obscures any morphological gaps within the complex.

Data gathered on the ecology of the various localities from herbarium specimens and field site visits show that the *C. eburnea* complex is highly variable in terms of canopy and associated species, with the only common thread in the ecology at the various sites being that they are all limestone outcrops. *Carex eburnea* is found associated with white cedar, red redar, spruce, pine, oak, alvar pavement and tundra. The Texas site, on a steep, north facing cliff face, associated with red cedar and oaks, is no more ecologically unique than the Ketona Dolomite community (Allison and Stevens 2001), the Arkansas locality, which is dominated by both pine and red cedar, or Windowcliffs, where red and white cedars co-occur.

Carex eburnea sensu lato co-occurs with four tree species over much of its range (red cedar, northern white cedar, spruce and oak). There are many disjunct populations of white cedar within the red cedar range in the Ridge and Valley Province and the Cumberland Plateau of the Southern Appalachian

Mountains. In the multivariate analysis of *C. eburnea* across these four ranges plus the disjunct northern white cedar range, specimens from the disjunct areas were recognized as a group. A northwest group and a southwest group were also apparent. These analyses demonstrate that there is some separation between those plants co-occurring with white cedar and red cedar. This is more evident in the ratio data, which suggests that there may be some ecological differentiation occurring within the species. Based upon these ecological data, we can see some potential for insipient speciation among the ecological variants, but there is clearly no discernable unit that can be recognized as a segregate species.

The distributional data provide no support for segregation of the Texas population from the rest of the complex. Ball had no knowledge of sites recently discovered in eastern Mexico, and he therefore recognized McKittrick Canyon as an extreme disjunct population. The compilation of the known distribution presented in this study indicates that the McKittrick Canyon locality is not unique in the distribution of *C. eburnea*, but is one of several localities that could be considered disjunct, including eastern Mexico, Arkansas, and Alabama. When we consider each of the disjuncts in turn we can begin to understand the ramifications of recognizing *C. mckittrickensis* as a segregate species. The Arkansas material could also be considered disjunct. However, the intermediate morphology of the Arkansas specimens suggests a relatedness of the Ozarks to the Guadalupe Mountains, and the argument could be made to segregate the Arkansas material with the Texas material. Similarly, the disjunct Mexican

material is found in multiple populations and is recognizable on the basis of molecular data. Again, the argument could be made to segregate the Mexican material as a separate species. The Alabama disjunct material is in a unique ecological setting and could again be segregated as a distinct species. Recognition of any of these disjuncts as a species is in concordance with an Evolutionary Species Concept and would have the negative impact of rendering the rest of the lineage paraphyletic, which would in turn result in a loss of lineage-based information.

Ball segregated the Texas material to a new species, based upon perceived size differences and a perceived disjunct locality. The data presented here show that the *C. eburnea* complex is comprised of many divergent populations, when examined using molecular data, morphometric data, distributional data and/or ecological data; however, none of these differences co-occur in a single locality or a group of geographically related localities that would merit recognition of a segregate species. If an evolutionary species concept were applied to the complex, segregation of the Texas and Arkansas material, based upon size, or segregation of Ketona, Mexico or Texas populations separately, based upon distribution, or the separation of regions based upon co-occurrence of canopy species, would render the entire species complex paraphyletic, and no group would have diagnosable features.

The pattern of genetic and morphological structure within *C. eburnea* is one of lineages that may currently be unresolveable using molecular or morphological analyses. The 50% majority rule Maximum Likelihood tree

demonstrates that while the *Carex eburnea* complex retains its distinction from *C. alba*, little structure remains within the complex upon consensus, suggesting that the individuals used in this analysis are poorly differentiated using molecular data. These data suggest that *C. eburnea* may be undergoing rapid, recent differentiation and may best be currently described as a metaspecies.

Parsimony analysis suggests that lineages from the northwest part of the range of the *Carex eburnea* complex are basal. The pattern of maximum glacial ice indicates an unglaciated area in the northwest United States along the U.S.-Canada border (Graham 1999). These data suggest that the northwest may have been a glacial refugium in the past. Additionally, limestone exists in the Rocky Mountains of the United States and across the upper midwest, as well as across Texas. *Carex eburnea* is unknown from most of these limestone outcrops. These outcrops could have served as migratory pathways in the past, however. Interpretation of molecular data, glacial ice maxima and North American limestone distribution suggest that the range of *C. eburnea* may have been relatively undisturbed by glaciation in the western part of North America but considerably constricted in eastern North America. During glacial retreat, *C. eburnea* may have migrated across limestone in the upper Midwest, down through the Ozark Mountains and through Texas into eastern Mexico. During past (and current) interglacials, the range of *C. eburnea* may have become more restricted, leaving disjuncts in the southern part of the range, such as Alabama, Texas and Mexico.

The basal position of the northwestern-most *C. eburnea* populations suggests that the nearest relative is Eurasian. The three scenarios outlined in the introduction suggested that a circumboreal ancestor, a North American ancestor, or a Eurasian ancestor may have given rise to the Section. Although the data from this study are not overwhelming, the presence of two species in Eurasia and a single species in North America with its basal-most populations in the Northwest part of the continent suggest a Eurasian origin for the Section, and a more recent dispersal across the Bering Land Bridge, to give rise to the *C. eburnea* complex. This pattern also fits with the supposed metaspecies model for the *C. eburnea* complex in that the northwestern origin then gave rise to divergent lineages scattered across the North American continent.

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APPENDIX A
Herbarium Specimens Used in Morphometric Analyses

Species: *C. alba*=alba, *C. eburnea*=ebur, *C. mckittrickensis*=mck, *C. ussuriensis*=ussur and *C. brunnea*=brun

Sp.	Collector(s)	ID #	Herb	Country	State/Province	County/Area	Canopy
alba	T Vestergren	12Aug1922	MICH	Austria			
alba	M Racikorrski	25Jun1911	GH	Denmark			
alba	Olunier	May1832	GH	France			
alba	A Kneucker	?Jun1896	GH	Germany	Baden-Wurttemburg		
alba	Ervuer	242	GH	Germany	Bavaria		
alba	El Nyarady	527	GH	Romania			
alba	M Deyl	?Jun1938	GH	Slovakia			
alba	P Hainard & G Tcheremissinoff	18Aug73	GH	Switzerland			
alba	A.S.P.	13,181	GH	Tyrol			
brun	MS Clemens	Jan1945	MICH	Australia	Queensland		
brun	KS Chow & Wan	79059	MICH	China	Guangxi		
brun	M Bartholomew et al.	1709	GH	China	Hubei Province		
brun	SK Lau	4289	GH	China	Kiangsi Province		
brun	Hu & But	21372	GH	Hong Kong			
brun	T Koyama	839	MICH	Japan	Hondo		
brun	DH Nicolson	2245	MICH	Nepal			
brun	MS Clemens	12May1941	MICH	New Guinea			
brun	RR Stewart	11Sept1959	BRIT	Pakistan			
brun	ADE Elmer Herbarium	11495	F	Philippines	Mindanao		
brun	RL Wilbur & GL Webster	947	MICH	USA	Hawaii	Maui	
ebur	WC McCalla	2349	NY	Canada	Alberta	Banff	NWEST
ebur	HR DeSelm	91-902	TENN	Canada	British Columbia		NWEST
ebur	BA Ford	0113	MICH	Canada	Manitoba		NWEST
ebur	BA Ford & RFC Naczi	9930	BOON	Canada	Manitoba		NWEST

Sp.	Collector(s)	ID #	Herb	Country	State/Province	County/Area	Canopy
ebur	VE McNeilus	91-635	TENN	Canada	Manitoba		NWEST
ebur	E Rouleau	494	NY	Canada	Newfoundland		WHITE
ebur	A Bouchard, S Hay & L Brouillet	95250	GH	Canada	Newfoundland	St. Barbe	WHITE
ebur	WJ Cody & RL Gutteridge	7535	F	Canada	Northwest Territory		NWEST
ebur	P Richardson	16659	BOON	Canada	Ontario		WHITE
ebur	JA Calder, WJ Cody & D Erskine	7083	NY	Canada	Ontario	Carleton	WHITE
ebur	EC Abbe & DW Bierhorst	5215	TENN	Canada	Ontario	Thunder Bay	WHITE
ebur	M Victorin & Rolland-Germain	20,159	F	Canada	Quebec		WHITE
ebur	R Gauthier, M Garneau & C Roy	88-112	NY	Canada	Quebec	Choucotimi	WHITE
ebur	M Victorin	15082	NY	Canada	Quebec		WHITE
ebur	M Victorin & PL Rolland-Germain	20,159	NY	Canada	Quebec		WHITE
ebur	GF Ledingham	TENN4157	TENN	Canada	Saskatchewan		NWEST
ebur	HM Raup	6520	F	Canada	Saskatchewan		NWEST
ebur	AA Reznicek, S Zamudio & G Ocampo	11066	BOON	Mexico	Queretaro		TEX
ebur	AA Reznicek, S Zamudio & G Ocampo	11082	BOON	Mexico	San Luis Potosi		TEX
ebur	C Roland, M Duffy & A Blakesley	4145	ALA	USA	AK	Denali	NWEST
ebur	CL Parker and D DiFolco	6364	ALA	USA	AK	Yukon V	NWEST
ebur	M Duffy	98-377	ALA	USA	AK	Big Delta Quad	NWEST
ebur	C Roland	5002	ALA	USA	AK	Denali	NWEST
ebur	EL Gillespie, ZE Murrell & GL Walker	16606	BOON	USA	AL	Bibb	RED
ebur	P Hyatt	5219	BRIT	USA	AR	Izard	RED
ebur	ZE Murrell & EL Gillespie	16614	BOON	USA	AR	Stone	RED
ebur	RF Thorne	10033	F	USA	IA	Dubuque	RED
ebur	VH Chase	12352	MICH	USA	IL	JoDaviess	RED
ebur	CC Deam	49,801	MICH	USA	IN	Porter	DISJ
ebur	LH Jordal	3439	MICH	USA	KY	Clinton	DISJ
ebur	LE McKinney	6150	MICH	USA	KY	Garrard	DISJ
ebur	RH Read	250	MICH	USA	MI	Alger	WHITE

Sp.	Collector(s)	ID #	Herb	Country	State/Province	County/Area	Canopy
bur	JH Wiersema	1114	MICH	USA	MI	Charlevoix	WHITE
ebur	D Henson	2058	MICH	USA	MI	Dickinson	WHITE
ebur	EG Voss	1714	MICH	USA	MI	Emmet	WHITE
ebur	BT Hazlett	4337	MICH	USA	MI	Leelanau	WHITE
ebur	SR Hill	29519	MICH	USA	MI	Mackinac	WHITE
ebur	EG Voss	12795	MICH	USA	MI	Mackinac	WHITE
ebur	BT Hazlett	581	MICH	USA	MI	Mason	WHITE
ebur	VE McNeilus	96-580	TENN	USA	MI	Menominee	WHITE
ebur	C Billington	4Jul1917	MICH	USA	MI	Oakland	DISJ
ebur	D Henson	2053A	MICH	USA	MI	Ontonagon	WHITE
ebur	CW Bazuin	6160	F	USA	MI	Ottawa	WHITE
ebur	JP Hubbard	193	MICH	USA	MI	Presque Isle	WHITE
ebur	FJ Hermann	8526	F	USA	MI	Washtenaw	WHITE
ebur	GA Wheeler	10783	MICH	USA	MN	Clay	NWEST
ebur	GA Wheeler	10776	MICH	USA	MN	Norman	NWEST
ebur	CO Rosendahl & FK Butler	2851	NY	USA	MN	Ramsey	RED
ebur	GA Wheeler	11069	MICH	USA	MN	Traverse	NWEST
ebur	MD Lee	MDL2773	TENN	USA	MN	Stearns	RED
ebur	LB Gerdes	LBG4073	TENN	USA	MN	Lake	WHITE
ebur	LB Gerdes	LBG4173	TENN	USA	MN	Lake	WHITE
ebur	CA Morse & EF Smith	1202	MICH	USA	MO	Barry	RED
ebur	EJ Palmer	35967	NY	USA	MO	Benton	RED
ebur	H Eggert	20Apr1897	NY	USA	MO	Franklin	RED
ebur	P Lesica	4290	NY	USA	MT	Dawson	NWEST
ebur	P Lesica	5090	MICH	USA	MT	Flathead	NWEST
ebur	CC Freeman & SP Churchill	1216	NY	USA	NB	Cherry	NWEST
ebur	S Rolfsmeier	5175	MICH	USA	NB	Dakota	RED
ebur	JR Bozeman, JF Logue and AE Radford	45353	MICH	USA	NC	Madison	DISJ

Sp.	Collector(s)	ID #	Herb	Country	State/Province	County/Area	Canopy
ebur	HD House	13170	NY	USA	NY	Albany	WHITE
ebur	KM Wiegand	6051	MICH	USA	NY	Cayuga	WHITE
ebur	FW Hunnewell Herbarium	4666	NY	USA	NY	Clinton	WHITE
ebur	RE Shanks	729	TENN	USA	NY	Monroe	WHITE
ebur	CH Peek	198258	NY	USA	NY	Oneida	WHITE
ebur	RFC Naczi	3876	MICH	USA	OH	Adams	DISJ
ebur	JS McCormac	2597	MICH	USA	OH	Erie	DISJ
ebur	EL Gillespie, ZE Murrell & GL Walker	16613	BOON	USA	OH	Greene	DISJ
ebur	SA Reznicek & AA Reznicek	8177	MICH	USA	OH	Ottawa	DISJ
ebur	S Grund & LL Smith	1617	MICH	USA	PA	Blair	RED
ebur	JK Bissell	JKB:1985:172	MICH	USA	PA	Erie	RED
ebur	TC Porter	22May1890	MICH	USA	PA	Northhampton	RED
ebur	HE Hayward	1098	F	USA	SD	Meade	NWEST
ebur	PC Durr	93	TENN	USA	TN	Campbell	DISJ
ebur	EL Gillespie, GL Walker & MC Estep	16604	BOON	USA	TN	Johnson	DISJ
ebur	DH Webb	5116	TENN	USA	TN	Marion	DISJ
ebur	R Kral	42315	NY	USA	TN	Overton	DISJ
ebur	BE Wofford	90-14	TENN	USA	TN	Pickett	DISJ
ebur	EL Gillespie, ZE Murrell & GL Walker	16605	BOON	USA	TN	Putnam	DISJ
ebur	BE Wofford & KD McFarland	84-23	TENN	USA	TN	Roane	DISJ
ebur	TF Wieboldt	6032	MICH	USA	VA	Bath	DISJ
ebur	TF Wieboldt	8482	MICH	USA	VA	Montgomery	DISJ
ebur	ZE Murrell & EL Gillespie	16607	BOON	USA	VA	Rockbridge	DISJ
ebur	CH Knowlton	TENN4165	TENN	USA	VT	Bennington	WHITE
ebur	William Boott Herbarium	3Jul1854	NY	USA	VT	Orleans	WHITE
ebur	LH Schicetto	154.19.119	F	USA	WI	Brown	WHITE
ebur	NA Harriman & TL Eddy	21737	BOON	USA	WI	Door	WHITE
ebur	EL Greene	19May1914	NY	USA	WI	Grant	RED

Sp.	Collector(s)	ID #	Herb	Country	State/Province	County/Area	Canopy
ebur	LJ Mehrhoff	14607	MICH	USA	WI	Sauk	RED
ebur	TG Hartley, RF Thorne et al.	518	NY	USA	WI	Trempealeau	RED
ebur	AW Cusick	23,548	MICH	USA	WV	Hardy	DISJ
mck	BH Warnock	18250	TEX/LL	USA	TX	Culberson	TEX/MEX
mck	DS Correll & IM Johnston	19187	LL	USA	TX	Culberson	TEX/MEX
mck	BH Warnock & MC Johnston	16468	TEX/LL	USA	TX	Culberson	TEX/MEX
mck	DS Correll & IM Johnston	19187	BRIT	USA	TX	Culberson	TEX/MEX
mck	JA Moore & JA Steyermark	3375	GH	USA	TX	Culberson	TEX/MEX
mck	JA Moore & JA Steyermark	3575	MICH	USA	TX	Culberson	TEX/MEX
mck	DS Correll & IM Johnston	19187	NY	USA	TX	Culberson	TEX/MEX
ussur	V Komarov	288	GH	China	Manchuria		
ussur	BV Skvortzov	15Aug1938	GH	China	Manchuria		
ussur	BV Skvortzov	May201958	GH	China	Manchuria		
ussur	V Komarov	18Jun1897	GH	Korea			
ussur	V Verholat	5568	GH	USSR			
ussur	V Verholat	5568	MICH	USSR			
ussur	V Komarov		MICH	USSR			

APPENDIX B
Data Matrix Generated From ISSR Analyses

Data matrix generated from ISSR analyses. Matrix represents 52 variable bands.

ONT1	0	0	1	1	0	1	1	0	0	0	1	1	1	1	0	0	1	1	1	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	0
ONT3	0	1	0	1	0	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	1	1	1	1	1	0	1	1	1	0	1	0	0
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WIS1	1	0	0	1	0	1	0	0	0	1	1	1	1	1	0	1	0	1	1	1	0	0	0	1	1	0	0	0	0	0	1	0	0	0	1	1	1	0	1	1	1	1	1	0	0	0	1	1	0	0	0	0
WIS2	1	0	0	1	0	1	1	0	1	1	1	1	1	1	0	1	1	1	0	1	0	1	0	1	1	0	0	1	0	0	1	1	0	0	1	1	1	0	1	1	1	1	1	0	0	0	1	1	1	0	0	0
WIS3	1	0	0	1	0	1	0	0	0	0	1	1	0	1	0	1	0	1	1	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	0	0
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MAN1	1	0	1	1	0	1	1	0	0	0	1	1	1	1	0	0	1	1	1	1	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	1	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0
MAN2	1	0	1	1	0	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	1	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0
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CLF1	1	0	0	1	0	1	1	0	1	0	1	1	1	1	0	1	0	1	1	1	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0	1	1	0	1	1	1	1	1	1	0	1	0	1	0	1	0	0
CLF2	1	0	0	1	0	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1	0	1	0	1	0	1	0	0	0	1	1	1	0	0	0	1	0	1	0	1	1	1	1	1	0	0	1	1	1	1	0	0
CLF3	1	0	1	1	0	1	1	0	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	0	0	1	0	0	0	0	1	1	0	1	1	1	0	1	1	1	1	1	0	0	0	1	1	1	1	1	0	0
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OZK1	0	0	0	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1	0	1	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	1	1	0	1	0	0
OZK2	0	0	0	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1	0	1	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	1	0	0	1	1	0	1	0	0
OZK3	0	0	0	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1	0	1	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	1	0	0	1	1	0	1	0	0
OZK4	0	0	0	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1	0	1	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	1	0	0	1	1	0	1	0	0
OZK5	0	0	0	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1	0	1	0	0	0	0	1	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	1	1	0	1	0	0
OZK6	0	0	0	1	0	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1	0	1	0	0	0	0	1	1	1	0	0	0	1	1	1	1	1	1	0	1	0	0	1	1	0	1	0	0
WIN1	0	0	0	0	1	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0	1	1	1	1	1	1	1	0	0	1	0	0	0	0
WIN3	0	0	0	0	1	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	1	0	1	1	1	0	0	0	0	1	1	0	0	0	1	0	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0
WIN5	0	0	0	0	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	1	1	0	0	0	1	1	0	1	1	1	1	1	1	1	0	1	1	0	0	0	0
WIN6	0	0	0	0	1	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1	1	1	1	1	0	0	0	1	1	0	1	0	0
NAT1	0	0	0	0	0	1	1	0	0	0	1	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	0	0	1	0	0	0	1	1	1	1	0	0	0	1	1	0	0	0	0
NAT2	0	0	0	1	0	1	1	0	0	1	1	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	1	1	1	1	0	0	0	1	1	0	1	0	0
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WAT1	0	0	0	1	0	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	0	1	0	0	1	0	0	0	0	0	1	1	0	1	0	1	0	1	1	0	1	1	1	1	0	0	1	1	0	0	0	0
WAT2	0	0	0	1	0	1	1	0	0	0	1	1	1	1	1	1	1	0	1	1	0	1	0	1	1	1	0	0	0	0	1	1	0	1	0	1	0	1	1	0	1	1	1	1	0	0	1	1	0	1	0	0
WAT3	0	0	1	1	0	1	1	0	0	1	1	1	1	1	1	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0	1	?	?	0	0	0	0	1	1	1	1	1	1	1	0	0	1	1	0	1	0	0
WAT4	0	0	1	1	0	1	1	0	0	0	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	1	0	1	0	1	0	1	1	0	1	1	1	0	0	0	1	1	0	0	0	0
WAT5	0	0	1	1	0	1	1	0	0	1	1	1	1	1	0	1	1	0	0	1	0	1	1	1	1	1	0	0	0	0	1	1	0	1	0	1	0	1	1	0	1	1	0	0	0	0	1	0	1	1	0	0
WAT6	0	0	1	1	0	1	1	0	0	0	1	1	1	1	1	1	1	0	0	1	0	1	0	1	0	1	0	0	0	0	1	1	0	1	0	1	1	0	1	0	1	1	0	0	0	0	1	0	1	1	0	0
FOR1	1	0	0	1	0	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	1	1	0	1	0	1	1	0	1	1	1	1	1	1	0	0	0	1	0	1	0	0
FOR2	1	0	0	1	0	1	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	1	1	0	1	0	1	1	0	1	1	1	1	1	1	0	0	0	1	1	1	0	0
FOR3	1	0	0	0	0	0	1	0	0	1	1	1	1	1	0	0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	1	0	1	0	0	1	1	0	1	1	1	1	1	1	0	0	0	1	0	1	0	0
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FOR5	1	0	0	1	0	1	0	0	0	1	0	1	1	1	0	0	1	1	1	1	0	0	0	1	1	1	0	0	0	1	1	1	0	1	0	1	1	0	1	1	1	1	1	1	0	0	0	1	1	0	0	0
FOR6	1	0	0	1	0	1	0	0	0	1	0	1	1	1	0	0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	1	1	0	1	0	1	1	0	1	1	1	1	1	1	0	0	0	1	0	1	0	0
KET1	0	0	0	1	0	1	1	0	0	0	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	1	1	0	1	0	1	1	1	1	1	0	1	1	0	0	0	1	1	0	1	0	0
KET2	0	0	0	1	0	1	0	0	0	1	0	1	0	1	1	0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	1	1	0	1	0	1	1	1	1	1	0	1	1	0	0	0	1	0	0	1	0	0
KET3	0	0	1	1	0	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	0	0	0	1	1	1	0	0	0	0	1	1	0	1	0	1	1	1	1	1	0	1	1	0	0	0	1	1	0	1	0	0

KET4	0	0	0	1	0	1	1	1	0	0	0	0	1	1	1	0	1	1	1	1	1	0	0	1	1	1	0	0	0	0	1	1	0	1	0	1	1	1	1	1	0	1	1	0	0	0	1	1	0	1	0	0
KET5	0	0	1	1	0	1	1	0	0	0	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	1	1	1	1	1	0	1	1	0	1	0	0	1	0	1	0	0
KET6	0	0	0	1	0	1	1	0	0	0	1	1	1	1	1	0	1	1	1	1	0	0	0	1	1	0	0	1	0	0	1	1	0	1	0	1	1	1	1	1	0	1	1	0	0	0	1	1	1	1	0	0
MIN1	0	0	1	1	0	0	1	0	0	1	1	?	0	1	0	0	1	0	1	1	0	0	0	1	0	1	0	0	0	0	1	1	0	1	0	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0
MIN3	0	0	1	1	0	0	1	0	0	0	1	?	0	1	0	0	1	0	1	1	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0
MIN4	0	0	1	1	0	0	1	0	0	0	1	1	1	1	0	0	1	0	1	1	0	0	0	1	0	1	0	0	0	0	1	1	0	1	0	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0
MIN5	0	0	1	1	0	0	1	0	0	0	1	1	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	1	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0
MEX1	0	1	0	0	1	0	1	0	0	0	1	1	1	1	1	0	1	1	1	1	0	0	0	1	0	1	0	0	0	0	1	1	0	1	0	1	0	0	1	1	1	0	1	1	0	0	0	0	0	0	0	0
MEX2	0	1	0	0	1	0	1	0	0	0	1	1	1	1	1	0	1	1	1	1	0	0	0	1	0	1	0	0	0	0	1	1	0	1	0	1	0	0	1	1	1	0	1	1	0	0	0	1	0	0	0	0
MCK1	0	1	1	0	0	0	1	0	0	0	1	1	1	0	1	0	0	1	1	1	0	0	1	0	0	1	0	0	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0	0	1	1	1	1	0
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MCK3	0	1	1	0	0	0	1	0	0	0	1	1	1	0	1	0	1	1	1	1	0	0	1	0	0	1	1	0	0	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0	1	1	1	1	1	1
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ALB2	0	0	1	0	0	0	1	0	0	0	1	0	0	1	1	0	1	0	0	0	0	?	?	?	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	?	0	1	1	0	0	0	0	0	1	0	0	0
ALB3	0	1	1	0	0	0	1	0	0	0	1	1	0	1	1	0	1	0	0	0	0	?	?	?	1	0	0	1	0	0	0	1	0	1	0	1	0	0	1	?	1	1	1	0	1	0	1	0	1	0	0	0
BRN1	0	0	?	?	?	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	1	1	1	1	0	0	0	0	?	0	0	1	0	1	0	0	0	0	0	0	0

VITA

Emily Laura Gillespie was born in Sylva, North Carolina on March 9, 1973 to James and Judith Gillespie. She lived on the Cherokee Indian Reservation until moving to Asheville, North Carolina, where she graduated from Erwin High School in 1991. After completing a B.A. in Biology at the University of North Carolina at Asheville in 2000, she attended Appalachian State University in Boone, North Carolina. She completed her M.S. in Biology in May, 2005 and will enter the Biology doctoral program at Wake Forest University in Winston-Salem, North Carolina in August 2005.