

***Calliscirpus* gen. nov. and its relationships within the hyperdiverse Cariceae + Dulichieae +
Scirpeae clade (Cyperaceae)**

by

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Thesis submitted to the
Faculty of Graduate and Postdoctoral Studies
University of Ottawa
In partial fulfilment of the requirements for the
Degree of Master of Science in the
Ottawa Carleton Institute of Biology

Thèse soumise à
La Faculté des études supérieures et postdoctorales
Université d'Ottawa
En vue de l'obtention de la maîtrise ès sciences à
L'Institut de Biologie d'Ottawa-Carleton

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Abstract

Eriophorum crinigerum (Scirpeae, Cyperaceae) has been placed in either the genus *Scirpus* (club-rushes) or *Eriophorum* (cottograsses), but a unique combination of bristle and inflorescence features suggests that it could represent a new generic lineage. In addition, prior field studies and initial analyses suggested that *E. crinigerum* could consist of two species. Using molecular, morphological, anatomical, embryological and geographical data, I examine relationships within the hyperdiverse Cariceae + Dulichieae + Scirpeae clade (ca. 2055 species) to which *E. crinigerum* belongs and I explore whether this species could contain undocumented species diversity. Results demonstrate not only that *E. crinigerum* represents a new genus within Cyperaceae, here called *Calliscirpus*, but it can be divided into two allopatric species, *C. criniger* and *C. brachythrix* sp. nov. *Calliscirpus brachythrix* is confined to the Sierra Nevada Mountains and *C. criniger* is confined to the Klamath-Siskiyou and North Coastal Mountains; all three mountain ranges are found within the California Floristic Province (USA) which is well known for its high generic and species endemism. My results suggest that *Calliscirpus* is distantly related to *Eriophorum*, but that it could be most closely related to the Cariceae, a cosmopolitan clade representing almost 40% of all Cyperaceae diversity.

Résumé

Jusqu'à maintenant, *Eriophorum crinigerum* (Scirpeae, Cyperaceae) a été placé soit dans le genre *Scirpus* (les scirpes), soit dans le genre *Eriophorum* (les linaigrettes), mais une combinaison unique de caractéristiques entourant ses soies et son inflorescence suggère qu'elle pourrait représenter un nouveau genre. De plus, des recherches sur le terrain et des analyses préliminaires suggèrent que *E. crinigerum* pourrait en fait consister de deux espèces. En utilisant des données moléculaires, morphologiques, anatomiques et géographiques, j'examine les relations phylogénétiques dans le clade hyperdiversifié des Cariceae + Dulicheae + Scirpeae (autour de 2055 espèces) dans lequel se trouve *E. crinigerum* et j'explore la possibilité que ce taxon puisse comprendre plus d'une espèce. Mes résultats démontrent non seulement que *E. crinigerum* constitue un nouveau genre pour la famille Cyperaceae, ici nommé *Calliscirpus*, mais que ce genre peut être divisé en deux espèces allopatriques, *C. crinigeri* et *C. brachythrix* sp. nov. *Calliscirpus brachythrix* est confinée aux montagnes Sierra Nevada alors que *C. criniger* est endémique aux montagnes Klamath-Siskiyou et North Coastal. Ces trois chaînes de montagnes sont situées à l'intérieur de la Province floristique de la Californie, une région bien connue pour son haut taux d'endémisme en genres et espèces. De plus, mes résultats suggèrent que *Calliscirpus* n'est pas proche parent d'*Eriophorum*, mais se positionne comme group sœur à la tribu des Cariceae, un clade cosmopolite qui représente presque 40% de toute la diversité de la famille des Cyperaceae.

Statement of Contributions

Comments from Julian Starr and Travis Schwantje were thoroughly appreciated for helping to improve drafts of this work.

Acknowledgements

This research, writing, and publication would not have been possible without the assistance, kindness, and patience of many people over the last two years. The following individuals have made this project a thoroughly enjoyable experience and are addressed in no particular order.

I would first like to thank my supervisor, Julian Starr, for all of his enthusiasm, help, advice, encouragement and time. It has been greatly appreciated. From his botanical guidance to his thorough editing of my manuscripts, he has been a wonderful supervisor. This research has been an opportunity of a lifetime and has been an incredibly enjoyable master's degree; I would like to thank him for the opportunity of not only giving me this project, but taking me on as a graduate student. I would also like to thank the members of my committee: Naomi Cappuccino, Jacques Cayouette, and Lynn Gillespie have all provided comments, advice, perspective and guidance as I undertook this project.

I would also like to thank Dr. Rob Naczi at the New York Botanical Garden for all of his help, encouragement, and edits during the early stages of writing Chapter 2 for publication.

A special thanks to Paul Sokoloff and Roger Bull, who taught me everything I needed to know about DNA, amplification and sequencing and who always had helpful advice or a listening ear during the laboratory work and the sequence editing process. Roger and Paul, you have made this such an enjoyable working environment, from the classical music (which I now play when I'm working hard) to the organized environment. Thanks.

Anna Ginter, Tamara Villaverde, Wayne Sawtell, Katya Boudko, Neda Amiri, Jocelyn Pender, Cassandra Robillard, Vashti King, Shan Leung, Michel Paradis, Frankie Janzen, Warren Cardinal-McTeague, Frederick Caron, Sabina Donadio, Alicia Alonso and the rest of my labmates who have kept my spirits high when I was near my breaking point. You have all made all the difference during this degree and I thank you for all of your support and friendship. In particular, I would like to thank Warren and Sabina for all of the tea, cocktails and bike rides.

Thank-you to Irene McKechnie for all of your advice and help with transitioning into graduate school, for the coffee breaks, and the squash games. Thanks for everything.

I would like to thank the curators of the following herbaria without whose specimens this study would not have been possible: A, CAN, CAS, CHSC, DAO, DOV, DS, JEPS, NY, OSC, PH, UC, US, WS. I would also like to thank Drs. Paul Hamilton and André Martel of the Canadian Museum of Nature (CMN) for help with ESEM and light microscopy work. The comments of two anonymous reviewers were greatly appreciated. This research was conducted as part of the requirements for a M.Sc. at the University of Ottawa (UofO) with support from an NSERC Alexander Graham Bell Canadian Graduate Scholarship, an Ontario Graduate Scholarship, an Excellence Scholarship from the UofO, and a Systematics Research Graduate Supplement (CMN) to CNG. This research was funded by an NSERC Discovery Grant to JRS. Thank-you to my family: Penny, Gary, Lisa, Neil, Daniel, Kim, Jeff, and Sandy for all of their support throughout out the last couple of years.

Lastly, I would like to thank Travis Schwantje for being there to celebrate when things were going well and for being my rock when things were going poorly. Your love, patience, humor and kindness made this thesis possible.

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Chapter 1: Introduction

The Cyperaceae Jussieu (sedge family) is the third largest monocot family with approximately 5500 species and 109 genera (Muasya *et al.* 2009). The family consists of 14 tribes, 5 of which are polyphyletic (Dhooge 2005). The following research focuses on the circumscription of the polyphyletic tribe Scirpeae Kunth ex Dumortier and its relationships to its sister tribes, Cariceae Pax and Dulicheae Rchb. ex J. Schultze-Motel (Dhooge 2005). Numerous studies have shown that these three tribes form a monophyletic group, hereafter known as the Cariceae + Dulicheae + Scirpeae clade although their relationships are poorly known (Dhooge 2005; Simpson *et al.* 2007; Muasya *et al.* 2009).

The Scirpeae tribe is composed of the genera *Scirpus* L., *Eriophorum* L., *Trichophorum* Pers., *Cypringlea* M.T.Strong, *Zameioscirpus* Dhooge & Goetgh., *Oreobolopsis* T.Koyama & Guagl. and *Phylloscirpus* C.B.Clarke (Dhooge *et al.* 2003; Strong 2003). These genera are grouped into this tribe because of their spirally arranged glumes that are usually all fertile, their presence of persistent perianth bristles, flowers with hypogynous scales, and the morphological and epidermal structure of the achene and the embryo types (Strong 2003; Goetghebeur 1998). The type specimen for Scirpeae belongs to the poorly understood genus *Scirpus*.

Scirpus s.l., originally described by Linnaeus (Linnaeus 1753, 1754), had only 24 species. The description was originally based upon very widespread and common morphological characters in the family. The use of these common characters resulted in as many as 200 to 300 species being included within *Scirpus* even though many of them were clearly unrelated to the true type for the genus, *Scirpus sylvaticus* L. (World Checklist of

Cyperaceae 2010). As defined by Wilson (1989), *Scirpus* s.s. is now circumscribed to include species with multispiculate, antheloid inflorescences with spikelets with imbricate scales where each scale subtends a bisexual flower that displays very short (shorter than the length of the scale) hypogenous bristles. This modern circumscription comprises only 64 Holarctic, Oceanic and South American species (Jung & Choi 2010; World Checklist of Cyperaceae 2010; Muasya *et al.* 2012), but the limits of the genus remain unclear (Dhooge 2005; Muasya *et al.* 2012) due to a series of intermediate taxa that cannot be easily placed in *Scirpus* or in any other genus in the Cariceae + Dulichieae + Scirpeae clade.

There are still many unclear relationships within *Scirpus* as well as between *Scirpus* and other closely related genera. This is particularly true in regards to its sister genus, *Eriophorum* (the cottongrasses) in which there are several species that blur the limits between *Scirpus* and *Eriophorum*. One such intermediate species is *Scirpus criniger* A. Gray (Gray 1868), a species that was transferred to *Eriophorum* by Beetle (1942) because the size, shape and colouration of its achenes (Gray 1868; Beetle 1942) and the length (much greater than achenes) and number (≥ 6) of its perianth bristles was more like an *Eriophorum*. However, *E. crinigerum* does not fit easily into *Eriophorum* either. Its bristles are barbed like *Scirpus* species and unlike typical *Eriophorum*, which have 10 or more bristles per flower, *E. crinigerum* normally has six and rarely has more than eight. Such an unusual combination of characters is not seen in any other genus, suggesting that this endemic to the California Floristic Province, a region known for high generic endemism (DellaSala *et al.* 1999; Myers *et al.* 1999; Thorne *et al.* 2009), could represent a completely separate and currently unrecognised generic lineage.

In addition to *Eriophorum crinigerum*, many other intermediate taxa have been identified as new lineages after their evolutionary relationships were analyzed, as well as new taxa that have been found to fill in gaps in the evolutionary tree of the Cariceae + Dulichieae + Scirpeae clade. *Zameioscirpus* Dhooge and Goetgh. was only described in 2003 and is composed of former *Scirpus* species. *Zameioscirpus* fell within a clade that was sister to the clade containing *Eriophorum* and *Scirpus* (Dhooge *et al.* 2003). *Khaosokia* D.A.Simpson, Chayam. & J.Parn, a monotypic genus described in 2005, has been situated as a basal element in the Cariceae + Dulicheae + Scirpeae clade in some studies (Dhooge 2005; Simpson *et al.* 2005), but its phylogenetic position and tribal affiliations are still unclear given the poor support and understanding of the evolutionary relationships throughout the entire clade (Simpson *et al.* 2005). There are also several other taxa that are currently blurring the limits between *Scirpus* and *Eriophorum*, such as *Scirpus maximowiczii* C.B.Clarke, *Eriophorum comosum* (Wall.) Nees, *Scirpus asper* J.Presl & C.Presl, *Cypringlea* M.T.Strong species and *Karinia* Reznicek & McVaugh species. In each case, these species have either been linked phylogenetically to species in different genera within the Cariceae + Dulichieae + Scirpeae clade or they represent segregates from the genus *Scirpus* s.l. Clarifying their relationships may significantly help to determine the limits not only of *Scirpus*, but of many other genera within Cariceae + Dulichieae + Scirpeae clade.

In order to clarify relationships within Scirpeae and the Cariceae + Dulichieae + Scirpeae clade in general, this thesis focuses on the circumscription of *Eriophorum crinigerum* using molecular, morphological, and embryological data. As this species represents one of the more difficult to place in either *Scirpus*, *Eriophorum* or in any other genus in the clade, a thorough understanding of its phylogenetic position could significantly

help with generic circumscription within the Cariceae + Dulichieae + Scirpeae clade. Moreover, evidence from the field and its unique character combinations, suggest not only that *Eriophorum crinigerum* could be a new Cyperaceae genus, but it may in fact harbour an undiscovered species. This has important conservation implications at multiple levels. This research has implications for the conservation of not just species, but of major evolutionary lineages as well.

Three gene regions were used for the molecular analysis of this thesis: ETS 1f, matK, and ndhF (Figure 1). These three regions were chosen not only because their rate of evolution is appropriate for addressing both higher (Dong *et al.* 2012) and lower level taxonomic problems (Dong *et al.* 2012; Logacheva *et al.* 2010), but also to account for the possibility of species hybridisation, which is often revealed by incongruent results from nuclear (ETS 1f) versus chloroplast loci (matK, ndhF) (Guibert 2008). The ETS 1f is a 5' external transcribed spacer fragment that is part of the nuclear ribosomal DNA locus (Figure 1; Starr *et al.* 2003), a series of genes and spacers that are tandemly repeated hundreds to thousands of times within each nucleus. Although the ribosomal genes (18S, 5.8S, 26S) of this locus are highly conserved, the transcribed spacers (ETS, ITS) typically display low intraspecific and high interspecific variation, a fact that makes them ideal for resolving species relationships and lower-level taxonomic problems such as the separation of sister species (Poczai & Hyvönen 2010). Unfortunately, paralogues may exist, often as non-functional “pseudogenes”, which are characterised by low GC contents as the selective pressure to maintain key secondary structures for splicing (i.e., GC stems) is relaxed. In order to deter the amplification of ETS 1f paralogs in this study, betaine was used during the amplification process. Betaine is believed to increase the likelihood of amplifying orthologs

with a high GC content during PCR by reducing melting temperature and also by increasing product specificity binding (Jensen et al. 2010). Although hundreds of plastids such as chloroplasts may occur in each plant cell and each plastid may have a genome that occurs in multiple copies, these genomic copies are almost always uniform within individuals and found in a single copy (Liang 1997). Variation tends to be very low within species and consequently the difficulty often associated with the use of nuclear markers, such as multiple alleles and the presence of paralogues are typically avoided with the use of plastid regions (Small *et al.* 2004, but see Liang 1997; Oxelman & Bremer 2000). The *matK* plastid region is the only chloroplast-encoded group II intron maturase that is required to splice four plastid regions that are not spliced by an imported nuclear maturase (Barthet 2006). The *matK* region is a rapidly evolving gene region that results in a high number of nucleotide substitutions making it an excellent region to resolve the relationships of both closely and even distantly related plants (Barthet 2006) and it can be particularly useful for this purpose in Cyperaceae (Starr et al. 2009 - BARCODES; Le Clerc-Blain et al. 2010). The *ndhF* plastid region forms the F-subunit of the plastid NAD(P)H dehydrogenase complex (Yen & Olmstead 2000). It is also a fast evolving gene region and has been shown to be very successful in separating Cyperaceae taxa in molecular analyses (Yen & Olmstead 2000).

This thesis has been divided into two separate studies (i.e., Chapters 2 & 3) that will help to clarify the taxonomic position of the intermediate species of *Scirpus* and *Eriophorum*, and to determine the phylogenetic relationships within the Cariceae + Dulichieae + Scirpeae clade. Each Chapter is preceded by an introduction that will clearly define the objective of each investigation as well as provide the details of the problem in greater depth.

The first study (Chapter 2) used morphological, embryological and DNA analyses (cpDNA and nuclear) to clarify the phylogenetic position and taxonomic status of the intermediate *Eriophorum crinigerum* as well as an hypothesized close sister species, *Scirpus maximowiczii*. Both nuclear (external transcribed spacer 1f, or ETS 1f; Figure 1) and chloroplast (*matK* and *ndhF*; Figure 1) DNA were used. The relationships presented by the molecular analysis were further supported through the morphological, anatomical and embryological analysis. The use of molecular analyses has been very successful in previous research endeavours for determining the positioning of Cyperaceae taxa within evolutionary trees (Chacon *et al.* 2006; Starr *et al.* 2009; Yen & Olmstead 2000). In keeping with the trends of previous research, I have determined that *E. crinigerum* is a new generic lineage, *Calliscirpus*, comprised of two species one of which is new to science (*Calliscirpus brachythrix*). *Scirpus maximowiczii* was found to be a true *Scirpus* using molecular DNA markers.

The second study (Chapter 3) examines the relationships of *Calliscirpus* species within Cariceae + Dulichieae + Scirpeae clade using molecular data and a much wider taxonomic sampling that includes additional intermediate taxa (*Karinia mexicana*, *Cypringlea* spp. and *Eriophorum comosum*). The known and recently discovered relationships found within the clade are examined and discussed with reference to molecular and morphological characteristics.

The final Chapter (Chapter 4) is a summary of the conclusions drawn from Chapter 2 & 3.

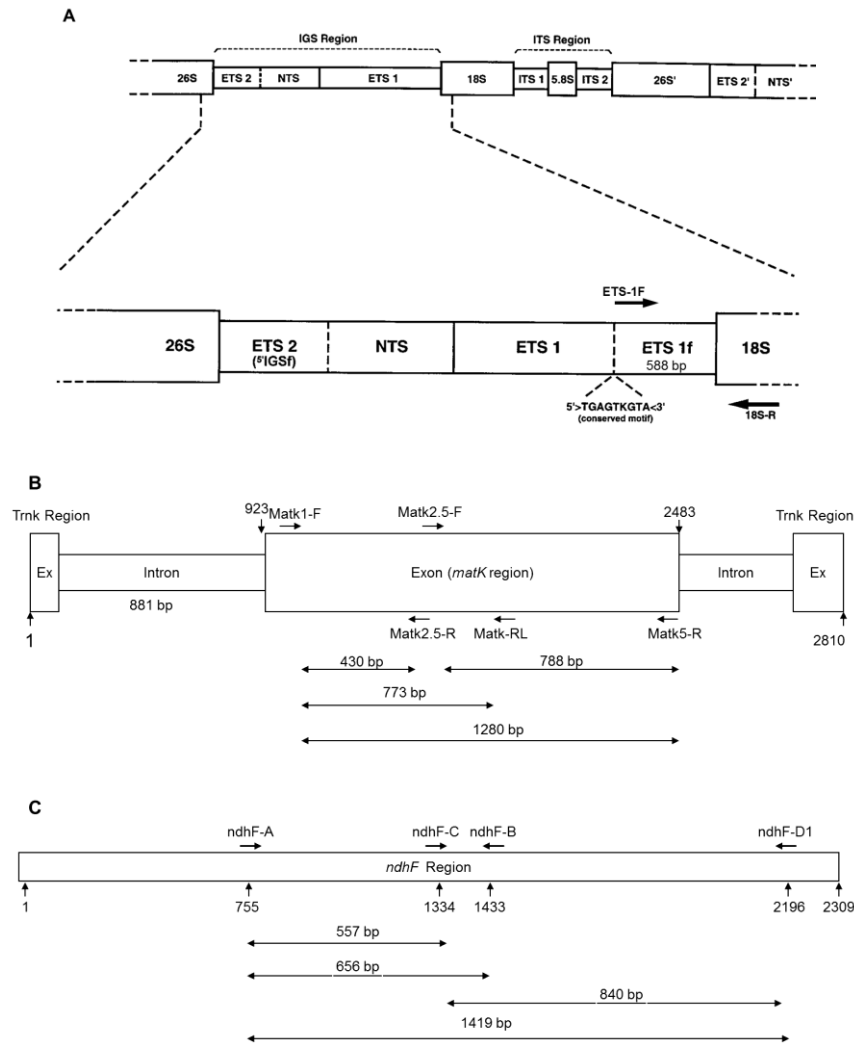


Figure 1. A) A single repeat of the nuclear ribosomal DNA region showing the external transcribed spacers (ETS 1 & 2), the 18S gene, the internal transcribed spacers (ITS 1 & 2), the 5.8S gene and the 26S gene (modified from Starr et al. 2003). The Intergenic spacer (IGS) containing the non-transcribed spacer (NTS) and the ETS 1 fragment (ETS 1f) used in this study with the primers used to amplify it are shown enlarged. B) Plastid *matK* gene region. The *matK* exon is located within the intron for the transfer RNA gene *trnK*. The primer positions and directions are shown with arrows for Matk1-F, Matk2.5-F, Matk2.5-R, Matk-RL and Matk5-R. The number of base pairs between primers and for the full amplified exon region (1280 bp) are shown. The reference base pairs (1, 923, 2483, and 2810) are in reference to the region seen in for the whole chloroplast genome of *Nicotiana tabacum*. C) Plastid *ndhF* gene region displaying the position and directions of the primers (ndhF-A, ndhF-B, ndhF-C, and ndhF-D1). The number of base pairs between primers and for the full amplified exon region (1419 bp) are shown. The reference base pair positions for the primers is shown below the gene region (755, 1334, 1433, 2196) for the whole chloroplast genome of *Nicotiana tabacum*.

Chapter 2: *Calliscirpus*, a new genus for two narrow endemics to the California Floristic Province, *C. criniger* and *C. brachythrix* sp. nov. (Cyperaceae)

INTRODUCTION

As conceived by Linnaeus (1753), the genus *Scirpus* L. s.l. consisted of species united by spirally arranged scales, bisexual flowers with or without perianth parts (i.e. bristles, etc.) and terete spikelets. These common characters drew together approximately 200 – 300 sedge (Cyperaceae) species, but many lines of evidence such as embryo types (Van der Veken 1965), fruit epidermal silica bodies (Schuyler 1971), and morphological (Goetghebeur 1986, 1998; Bruhl 1995) and molecular (Muasya *et al.* 1998, 2000; Muasya & Simpson 2002) phylogenies have shown that this concept for *Scirpus* s.l. was unnatural. The genus is now divided into more than 50 different genera, many of which are placed in distantly related tribes (Wilson 1981; Goetghebeur 1998).

Although *Scirpus* s.s. (tribe Scirpeae) now comprises only 64 Holarctic and Andean species (Jung & Choi 2010; World Checklist of Cyperaceae 2010; Muasya *et al.* 2012) distinguished by a *Fimbristylis*-/ *Schoenus*-type embryo, few to many-noded culms, and (0 –) 3 – 6 perianth parts in the form of bristles (Van der Veken 1965; Goetghebeur 1998; Dhooge 2005; Muasya *et al.* 2009b), its circumscription remains problematical. This is clearly illustrated by the recent discovery of the genus *Dracoscirpoides* Muasya, a distantly related *Scirpus* segregate now placed in tribe Cypereae (Muasya *et al.* 2012). However, the most difficult problems with its circumscription involve its close relatives. Within the Cariceae + Dulichieae + Scirpeae clade, the large monophyletic group to which *Scirpus* belongs (c. 2055

species; World Checklist of Cyperaceae 2010), two closely related segregate genera, *Zameioscirpus* Dhooge & Goetgh. and *Cypringlea* M. T. Strong, have been named since the modern circumscription of *Scirpus* s.s. by Goetghebeur in 1998. Moreover, the basic question as to whether the small (c. 18 species) Holarctic genus *Eriophorum* L. (the cottongrasses) can even be separated from *Scirpus* has yet to be resolved (Goetghebeur 1998). Although *Eriophorum* is typically delimited from *Scirpus* by the possession of 10 or more smooth, elongate bristles vs six or less short, serrulate bristles or the lack of bristles, some species cannot be easily placed in either genus (Goetghebeur 1998; Dhooge 2005).

One such species is *Eriophorum crinigerum* (A. Gray) Beetle (= *Scirpus criniger* A. Gray), a taxon endemic to the California Floristic Province, USA, a region that is rich in endemic species and genera (DellaSala *et al.* 1999; Myers *et al.* 1999; Thorne *et al.* 2009). Like *Scirpus*, the number of bristles in *E. crinigerum* is typically six and the bristles are serrulate, but similar to *Eriophorum*, some flowers may have as many as 12 bristles (pers. obs.; Beetle 1942) though bristle length is shorter than in any other species. *Eriophorum crinigerum* also differs from typical *Eriophorum* by inflorescence branches bearing more than one spikelet and by the lack of leaf-like bracts in a multi-spikelet species (Ball & Wujek 2002; pers. obs.), and yet its spikelet numbers and achene sizes are more in line with *Eriophorum* than with *Scirpus* (Beetle 1942; Ball & Wujek 2002; Whittemore & Schuyler 2002). These seemingly unique character combinations suggest that *E. crinigerum* may not be directly related to either *Scirpus* or *Eriophorum*, but may be best placed in a separate, but closely related genus.

Key to resolving this problem is *Scirpus maximowiczii* C.B. Clarke [= *Scirpus japonicus* (Maxim.) Kuntze, *Eriophorum japonicum* Maxim., *Eriophorum maximowiczii*

(C.B. Clarke) Beetle, another seemingly transitional species that has blurred the limits of *Scirpus* and *Eriophorum*. Like *E. crinigerum*, its six antrorsely serrulate bristles suggest an affinity to *Scirpus*, but its bristle lengths (3 to 4 times achene length) are more in line with the cottongrasses (Tucker & Miller 1990). Similarly, *S. maximowiczii* and *E. crinigerum* occur in *Eriophorum*-like habitats (wet alpine, montane or arctic meadows) and both have achenes that are the typical size and shape for *Eriophorum* s.s., features cited by Beetle (1942, 1946) when he transferred each species to *Eriophorum*. Other largely vegetative characteristics of *S. maximowiczii*, such as its large pendulous spikelets, scarious blackish scales, black spathaceous bracts and nearly bladeless blackish culm sheaths, either convinced authors that *S. maximowiczii* was an *Eriophorum* (Oteng-Yeboah 1974), that *Eriophorum* and *Scirpus* were inseparable (Koyama 1958) or that its unique character combinations warranted its treatment in the monotypic genus *Maximowiczella* (Maxim.) M.S. Novos. (Khokhrjakov 1985, 1989). The inclusion of *S. maximowiczii* in the current study is based on Ball & Wujek's (2002) contention that the floral structure of *E. crinigerum* was most similar to *S. maximowiczii* and the opinion of Dhooge (2005) that it was highly unlikely that either species belonged to *Eriophorum* or *Scirpus* given the unusual character combination of a 3 + 3 bristle arrangement and an *Eriophorum*-like vegetative habit.

In this paper, we present molecular, morphological and embryological evidence that *Eriophorum crinigerum* is not related to *S. maximowiczii*, nor to *Scirpus* s.s. or *Eriophorum* s.s., but represents a new lineage within the Cariceae + Dulichieae + Scirpeae clade, here recognised as the genus *Calliscirpus* C.N. Gilmour, J.R. Starr, & Naczi (tribe Scirpeae). Moreover, based on morphological and molecular data, we demonstrate that *Eriophorum crinigerum* consists of two allopatric species each confined to distinct regions of high

endemism: *Calliscirpus criniger* (A. Gray) C.N. Gilmour, J.R. Starr, & Naczi comb. nov., which is distributed throughout the Klamath-Siskiyou and North Coast mountain ranges of Oregon and California, and a new species, *Calliscirpus brachythrix* C.N. Gilmour, J.R. Starr, & Naczi that is unique to the Sierra Nevada mountain range of California.

MATERIALS AND METHODS

MOLECULAR DATA

Sampling, Molecular Marker Selection, & Outgroups

For analyses focused on the generic status of *Calliscirpus*, taxonomic sampling reflected the morphological and phylogenetic diversity of taxa within the Cariceae + Dulichieae + Scirpeae clade as determined in previous analyses (Dhooge & Goetghebeur 2004; Muasya *et al.* 2009a). Portions of two rapidly evolving chloroplast genes, *matK* and *ndhF* (Figure 1), were used in these analyses after a pilot study testing other commonly used chloroplast (*trnL-trnF*, *rps16*, *atpB*, *rbcL* and *psbA-trnH*) and nuclear markers (internal transcribed spacer and external transcribed spacer regions, i.e., ITS and ETS) either could not provide further resolution and support for trees or they could not be reliably amplified, sequenced, or aligned. Species of tribe Abildgaardieae were chosen as the outgroup on the basis of previous molecular analyses (Muasya *et al.* 1998, Muasya *et al.* 2009a) that placed the tribe in the sister group to the Cariceae + Dulichieae + Scirpeae clade.

For species-level analyses within *Calliscirpus*, individuals were selected with the aim of covering the complete distributional range of both putative species within the genus, *C. criniger* and *C. brachythrix*. The chloroplast gene *matK* and the nuclear ETS 1f region were

used in these analyses based on the results of the pilot study mentioned above (Figure 1). As these taxa are currently treated as unusual members of *Eriophorum*, a typical unispikelet (*E. callitrix* Cham. ex C. A. Mey.) and multispikelet (*E. viridicarinatum* (Engelm.) Fernald) species from the genus were used as outgroups to represent the breadth of morphological diversity in *Eriophorum* and to confirm their clear separation from *Eriophorum* s.s.

DNA Extraction, Amplification, & Sequencing

Whole genomic DNA was extracted from leaf tissue removed from herbarium specimens or silica-dried in the field following the silica-column based protocol of Alexander *et al.* (2007) as modified by Starr *et al.* (2009).

All primers used in the amplification and sequencing of *matK* and *ndhF* are given in Table 1. Amplifications for *matK* were typically conducted using the forward primer matK-1F and the reverse primer matK-5R, although amplifying smaller overlapping portions of this product with internal primers (i.e., matK-1F + matK-RL and matK-2.5F + matK-5R) was occasionally necessary when the larger product would not amplify. Similarly, *ndhF* was amplified using the forward primer ndhF-A and reverse primer ndhF-D1 or in overlapping portions using ndhF-A + ndhF-B and ndhF-C + ndhF-D1 when problems with amplification were encountered. The ETS 1f region was always amplified using the ETS-1F and 18S-R primers of Starr *et al.* (2003). For chloroplast regions, each PCR consisted of 1X reaction Buffer (Sigma Aldrich), 2mM MgCl₂ (Sigma Aldrich), 0.2 mM of each deoxynucleotide (dATP, dCTP, dTTP, and dGTP), 0.25 µM of each primer, 1.0 µg Bovine Serum Albumin (BioShop, Canada), 4 U of Hot Start (HS) *Taq* DNA Polymerase (BioShop, Canada) and 20-

30 mg genomic DNA, adjusted to an end volume of 15 μ L using nuclease-free ddH₂O. PCR products were produced on an Eppendorf© EPGradientS Mastercycler via a pretreatment of 94°C for 2 min, followed by 40 cycles of DNA denaturation at 94°C for 30s, primer annealing at 47°C for 60s, and DNA extension at 72°C for 90s (*matK*) or 120s (*ndhF*) with a final extension step at 72°C for 8 min. Minor adjustments to the PCR recipe and thermocycler conditions occasionally were required for problematic samples.

PCRs of the nuclear ETS 1f region were the same as for chloroplast reactions with the following minor adjustments: 2.5 mM MgCl₂, 0.3 mM of each deoxynucleotide, 0.4 μ M of each primer and with BSA being replaced with 1M Betaine (Sigma Aldrich). Thermal cycling conditions were set as follows: 94°C for 1 min, followed by 38 cycles consisting of 94°C for 30s, 49°C for 30s, and 72°C for 120s with a final extension step at 72°C for 7 min.

Successful PCR products were purified using an Exonuclease I and Shrimp Alkaline Phosphatase protocol (MJS Biolynx Inc., Canada) and then cycle sequenced with an ABI Prism™ Big Dye™ terminator kit version 3.1 (Applied Biosystems; Foster City, CA). Cycle-sequencing for the ETS 1f region was conducted with the same primers as used in PCR, but owing to their larger size, both cpDNA markers were sequenced using PCR primers plus two internal primers (Table 1). Cycle-sequencing products were then purified according to a sodium acetate/ethanol procedure (Applied Biosystems) and run on a 3130x1 Genetic Analyser. Sequenced data were assembled and edited using the program Sequencher 4.10 (Gene Codes Corporation, Ann Arbor, MI, USA) and all sequences used in analyses were subsequently submitted to Genbank (Table 2).

PHYLOGENETIC ANALYSIS

Two separate matrices were analysed: a matrix composed of *matK* + *ndhF* to evaluate the generic status of *Calliscirpus* within the Cariceae + Dulichieae + Scirpeae clade and a *matK* + ETS 1f matrix to determine whether two species exist within *Calliscirpus*. Both matrices were initially aligned in CLUSTAL X (Thompson *et al.* 1997) with minor adjustments made manually using parsimony as an objective criterion (see Starr *et al.* 2004).

Each data matrix was then analyzed in PAUP* vers. 4.0b10 (Swofford 2002) using a branch-and-bound search under the criterion of maximum parsimony (MP). When more than one tree was found, relationships were evaluated from a strict consensus tree produced in PAUP*. The number of unambiguous changes along branches of the single most parsimonious tree discovered in *matK* + ETS 1f analyses was determined in PAUP* using the DESCRIBETREES APOLIST=yes command. Clade support was assessed from bootstrap (BS; Felsenstein 1985) analyses as determined from 1,000,000 repetitions of a heuristic search with the MULTREES option off (DeBry & Olmstead 2000). The level of BS support was subjectively described as follows: strong 95-100% BS; very good 85-94% BS; good 75-84% BS; moderate 65-74% BS; weak 55-64% BS; and very weak <55% BS (Starr *et al.* 2004).

Both the *matK* + *ndhF* and *matK* + ETS 1f data matrices and all the most parsimonious trees obtained from their analyses were submitted to TreeBASE (<http://treebase.org/treebase-web>).

MORPHOLOGICAL, EMBRYOLOGICAL AND BIOGEOGRAPHICAL STUDIES

Morphology

Two hundred and thirteen specimens for morphological studies were received on loan from the following herbaria: A, CAN, CAS, CHSC, DAO, DOV, DS, JEPS, NY, OSC, PH, UC, US, WS. In order to determine which morphological characters could be the most informative for separating *C. brachythrix* from *C. criniger*, 121 herbarium specimens representing the entire geographical range of *Eriophorum crinigerum* were measured for 23 characters (Table 3). Ligule hair length could not be measured in 15 (7 *C. criniger*, 8 *C. brachythrix*) of the 121 specimens examined as the character was hidden during mounting. The statistical significance of morphological traits was determined by t-tests conducted in PAST (Hammer *et al.* 2001). A bivariate data plot of the two most significant characters for separating specimens of *C. brachythrix* from *C. criniger* (ligule hair length, floral scale width) was generated and representative micrographs of both characters were made to illustrate morphological differences. A third character, bristle scabrousness, appeared to show significant differences between species, but it could not be reliably measured under a stereomicroscope. It was therefore necessary to describe it qualitatively. To best illustrate this character, achenes and their bristles from each species were mounted onto aluminum stubs with adhesive carbon discs and micrographed in high vacuum mode with a Philips XL-30 environmental scanning electron microscope (ESEM) at an accelerating voltage of 4 kV.

Anatomy

Three specimens from *Calliscirpus brachythrix* and *C. criniger* were selected for cross-sectional examinations. For each specimen, a 1 cm long section was removed from the

medial portion of culm and from the medial portion of the most mature leaf associated with the culm. The tissue samples were soaked for 24 hours in distilled H₂O. Once the tissues were softened, they were thinly sectioned using a razor blade and foam matrix for support.

The sections were photographed using a stereomicroscope mounted with a digital camera. The drawings of the leaf and culm cross sections were made by camera lucida and edited into Adobe Illustrator CS2.

Embryology

Embryo morphology in Cyperaceae has proven to be an excellent tool for determining species' tribal and generic affiliations when used with other types of data (Van der Veken 1965; Goetghebeur 1986, 1998; Dhooge 2005). As embryo morphology has already been determined for *Scirpus maximowiczii* (Van der Veken 1965) and for all the closely related generic lineages to *Scirpus* and *Eriophorum* within the Cariceae + Dulichieae + Scirpeae clade (Van der Veken 1965; Goetghebeur 1986, 1998; Dhooge 2005), except *Khaosokia* D.A.Simpson, Chayam. & J.Parn. (see Simpson *et al.* 2005), only the embryos of *C. brachythrix* and *C. criniger* were examined in this study.

Mature achenes were removed from specimens and soaked for 24 hours in a 40% sodium hypochlorite (NaOCl) solution before being placed in lactophenol (10 g phenol dissolved in 10 mL dH₂O, 10 mL glycerine, 10 mL lactic acid) for 24 to 96 hours. The embryo was then dissected from the achene and cleared with NaOCl for 10 mins, rinsed with water for 5 mins, and transferred from a watch glass with a fine pipette tip to a microscope slide with coverslips set on each of its sides (Van der Veken 1965). The embryos could thus

be manipulated with a gentle movement of one or both coverslips to obtain a medial section photograph under a stereomicroscope.

Species Distribution, Associates, & Habitat Descriptions

Specimen localities were only mapped when the precise latitude and longitude could be extracted directly from label data or when the specimen could be georeferenced based on a locality description with a 1 km area of uncertainty. Separate data points for *Calliscirpus criniger* and *C. brachythrix* were plotted on a basemap of the contiguous United States using DIVA-GIS 7.5 (Hijmans *et al.* 2002) that was cropped to highlight the California Floristic Province. The California Floristic Province and the Klamath-Siskiyou, North Coast, and Sierra Nevada mountain ranges were defined according to Irwin (1966), Walker & MacLeod (1991), Hickman (1993), Miles & Goudey (1997), and Bunn *et al.* (2007). The habitat descriptors used in the Taxonomic Treatment were present on the majority of labels of the specimens examined as well as through photographs obtained during field work. Species associates were listed in the Taxonomic Treatment only when they occurred on at least two separate labels.

RESULTS

PHYLOGENETIC ANALYSIS

The alignment of *matK* and *ndhF* sequences for 21 taxa used to evaluate the generic status of *Calliscirpus* within the Cariceae + Dulichieae + Scirpeae clade produced a matrix of

2,184 characters (*matK*, 1,120 bp; *ndhF*, 1,064 bp), of which 1,658 were constant and 259 were parsimony-informative. Branch and bound searches recovered two most parsimonious trees, 770 steps long with consistency and retention indices of 0.78 and 0.71. The strict consensus tree (Figure 2) resolves a strongly supported, monophyletic tribe Dulichieae as sister to all other members of the Cariceae + Dulichieae + Scirpeae clade and it positions the monotypic genus *Khaosokia* as sister to a very weakly supported clade consisting of two major lineages. The first, Clade A, is a very weak group comprising three strongly supported clades: tribe Cariceae (100% BS), a *Cypringlea* + *Oreobolopsis* T.Koyama & Guagl. + *Trichophorum* Pers. clade (100% BS), and a *Calliscirpus* species clade (99% BS). *Calliscirpus* is sister to Cariceae, but the relationship is very weakly supported. The second major lineage, Clade B, is a weakly supported clade of two monophyletic groups, a strongly supported *Eriophorum* s.s. + *Scirpus* s.s. clade and a weak clade comprising the genera *Zameioscirpus* + *Amphiscirpus* Oteng-Yeb. + *Phylloscirpus* C. B. Clarke. The enigmatic *Scirpus maximowiczii* is strongly positioned (100% BS) within the *Eriophorum* s.s. + *Scirpus* s.s. clade and part of a paraphyletic *Scirpus* grade that includes a monophyletic *Eriophorum* (100% BS).

The alignment of *matK* and ETS 1f sequences for species-level circumscription within *Calliscirpus* (18 *Calliscirpus* individuals and two outgroups) produced a matrix of 1,655 characters (*matK*, 1,136 bp; ETS 1f, 519 bp), of which 1,529 were constant and 105 were parsimony-informative. Branch and bound searches recovered a single most parsimonious tree, 146 steps long with consistency and retention indices of 0.91 and 0.95 (Figure 3). Analyses divide *Calliscirpus* into two strongly supported clades (96 – 97 % BS) that are entirely consistent with morphology in suggesting that *Calliscirpus* consists of two distinct species, *C. brachythrix*, a Sierra Nevadan endemic, and *C. criniger*, a Klamath-

Siskiyou and North Coast mountain ranges endemic. Although there is considerable resolution amongst individuals within each species, no clade receives more than moderate support and no phylogeographic pattern is evident.

ANATOMY

Although differences in culm and leaf morphology are presented in Figure 11, such as an acute keel in *C. criniger* and bundles extending to the epidermis in *C. brachytrix*, no significant differences were found due to too small of a sample size.

EMBRYOLOGY

All embryos of *C. criniger* and *C. brachytrix* were top-shaped in outline, and possessed a basally positioned root cap and a laterally positioned first leaf (Figure 4). These characteristics clearly identify their embryos as the *Carex*-type (Van der Veken 1965, Goetghebeur 1986; Dhooge 2005).

MORPHOLOGY

Of the 23 characters examined in morphological studies, three characters, ligule hair length, floral scale width, and bristle scabrousness clearly separate *C. brachytrix* from *C. criniger*. The two most important characters, ligule hair length and floral scale width, were found to be statistically significant ($P < 0.001$; Table 4). Ligule hair length completely separates *C. brachytrix* (short hairs) from *C. criniger* (long hairs), whereas floral scale

width (usually wider in *C. criniger*) can distinguish each species 88% of the time (Figures 5, 6). A third important character for separating these species, bristle scabrousness, could not be measured reliably under a stereomicroscope. Species differences are therefore described qualitatively as follows. *Calliscirpus brachythrix* possesses thinner, shorter, and less dense bristle barbs than the thicker, longer, and denser bristle barbs of *C. criniger* (Figure 7). Consequently, the bristle barbs of *C. brachythrix* are poorly visible at high magnification under a stereomicroscope whereas those of *C. criniger* are clearly visible.

SPECIES DISTRIBUTIONS, ASSOCIATES AND HABITAT DESCRIPTIONS

All 155 specimens that could be accurately georeferenced were confined to the Sierra Nevada, Klamath-Siskiyou and North Coast mountain ranges that are part of the California Floristic Province (Figure 8). All 74 *C. brachythrix* specimens were restricted to the Sierra Nevada mountain range of California, whereas all 81 *C. criniger* specimens were distributed within the Klamath-Siskiyou mountain range of California and Oregon, with the exception of four specimens from the North Coast mountain range of Sonoma and Mendocino counties, California (Sonoma: *Raiche* 20425, JEPS; Mendocino: *Gonkin, Hildreth, Knight & Knight* 2705, CAS; *McMurphy* 595, DS; *Raiche, Forbes & Zadnik* 132, JEPS; *Smith* 6877, CAS). Of the 213 total specimens of *C. brachythrix* and *C. criniger* examined, only one collection of *C. criniger* (Modoc Co., 14 June 1879, *Plummer* s.n., A) was found in a county outside the California Floristic Province. This specimen may represent a labelling error (see Notes for *C. criniger* in Taxonomic Treatment). Although no *C. brachythrix* specimens cite the presence of serpentine, 21 of 116 labels for *C. cringer* do mention the presence of serpentine or related rock types, including two of the four specimens from Sonoma and Mendocino counties

mentioned above (*Raiche* 20425, JEPS; *Raiche, Forbes & Zadnik* 132, JEPS). Note that only 60% of specimen labels cite details beyond county. Species lists of *C. criniger* and *C. brachythrix* associates including habitat descriptions based on label data are presented in the Taxonomic Treatment under the description of each *Calliscirpus* species.

From an observational viewpoint the habitats of *Calliscirpus brachythrix* and *C. criniger* are very different (Figure 10). The habitat of *Calliscirpus brachythrix* is commonly a red fir forest meadow that is lushly vegetated with a local source of water whether stream, creek or seep. The habitat of *Calliscirpus criniger* is a much more sparsely vegetated habitat that is open and drier than that of *C. brachythrix*.

DISCUSSION

CALLISCIRPUS, A NEW SEDGE GENUS FOR TRIBE SCIRPEAE

Molecular, embryological, and morphological data are congruent in indicating that the enigmatic taxon *Eriophorum crinigerum*, whose treatment as either a *Scirpus* or an *Eriophorum* has blurred the limits of both genera, is in fact a separate generic lineage sister to tribe Cariceae and distantly related to *Eriophorum* s.s. or *Scirpus* s.s. Although support along the backbone of our molecular tree was poor, our topology is consistent with previous molecular analyses in strongly supporting a monophyletic Dulichieae often sister to all other members of the Cariceae + Dulichieae + Scirpeae clade, a monotypic *Khaosokia* lineage, and four primary, often strongly supported clades, consisting of tribe Cariceae, the *Cypringlea* +

Oreobolopsis + *Trichophorum* clade, the *Eriophorum* s.s. + *Scirpus* s.s. clade, and the *Zameioscirpus* + *Amphiscirpus* + *Phylloscirpus* clade (Dhooge 2005; Simpson *et al.* 2005; Muasya *et al.* 2009a). Moreover, our treatment of *Eriophorum crinigerum* and its new sister species (see below) in the genus *Calliscirpus* and our tree topology are strongly supported by embryology, a character source of major taxonomic value for generic circumscription when correlated with morphological and molecular results (Van der Veken 1966; Goetghebeur 1986, 1998; Dhooge 2005).

In this analysis, *Calliscirpus* was found to have a *Carex*-type embryo, which is characterised by a basal rootcap and a lateral first leaf (Goetghebeur 1986). This embryo type is also known to occur in *Carex* L., *Kobresia* Willd., *Trichophorum*, *Cypringlea*, and *Oreobolopsis*, which form Clade A with *Calliscirpus*, and in tribe Dulichieae (Van der Veken 1965; Strong 2003; Dhooge 2005) at the base of the tree. In contrast, all the members of Clade B are known to possess a hybrid *Fimbristylis*-/*Schoenus*- (*Scirpus*) or *Schoenus*- type embryo (*Amphiscirpus*, *Phylloscirpus*, *Zameioscirpus*), or an embryo with a transitional morphology between these two types (*Eriophorum*) (Van der Veken 1965; Dhooge 2005; Goetghebeur 1986). These embryo types share a sublateral position of the first leaf and root cap and are believed to represent the derived state relative to the plesiomorphic *Carex*-type condition (Dhooge 2005; Goetghebeur 1986). This is fully consistent with the placement of tribe Dulichieae as sister to all other members of the Cariceae + Dulichieae + Scirpeae clade in our trees. The fact that the *Zameioscirpus* + *Amphiscirpus* + *Phylloscirpus* clade and its sister *Scirpus* s.s. + *Eriophorum* s.s. clade possess these derived embryo types whilst *Calliscirpus* does not is strong evidence that *Calliscirpus* is not closely allied to this clade or *Scirpus* and *Eriophorum*, but constitutes a separate generic lineage.

Despite perceived similarities in floral structure between *Scirpus maximowiczii* and *Calliscirpus* species (Ball & Wujek 2002) and Beetle's (1946) statement that he transferred *S. maximowiczii* and *S. criniger* to *Eriophorum* for the same reasons, our analysis strongly positions *S. maximowiczii* in the *Scirpus* s.s. + *Eriophorum* s.s. clade. This result is consistent with the fact that *S. maximowiczii* shares a hybrid *Fimbristylis*-/ *Schoenus*-type embryo (Van der Veken 1965) with species in the *Scirpus* s.s. + *Eriophorum* s.s. clade and with the fact that the size, colouration and shape of its achenes are typical for *Scirpus*, but not for *Calliscirpus* (see Tucker & Miller 1990). Our results clearly indicate that *Scirpus maximowiczii* and the genus *Calliscirpus* are not closely related and any perceived similarities between these taxa are superficial. Nonetheless, the question as to whether *S. maximowiczii* could represent a transitional species suggesting that *Eriophorum* and *Scirpus* should be merged (Koyama 1958) or whether it should be treated as a separate genus *Maximowiczella* (Khokhrjakov 1985, 1989), cannot be resolved by our analyses. Although *Scirpus atrovirens* Willd. was sister to a monophyletic *Eriophorum*, support was weak, and this clade formed a polytomy with *S. maximowiczii* and *S. cyperinus* (L.) Kunth. Therefore, *Scirpus* s.s. could still form a natural genus sister to *Eriophorum* s.s. with or without *S. maximowiczii*.

The discovery that a well-known species of *Eriophorum* should be treated as a separate genus is not surprising because the unique character combination in *Calliscirpus* of six or more, medium length, antrorsely serrulate bristles, more than one spikelet per inflorescence branch, an absence of leaf-like bracts, and a *Carex*-type embryo does not fit into any currently recognised genus. As *Calliscirpus*, *Scirpus maximowiczii*, and the recent segregation of multiple closely (*Zameioscirpus*, Dhooze *et al.* 2003; *Cypringlea*, Strong

2003) and distantly (*Dracoscirpoides*, Muasya *et al.* 2012) related new genera from *Scirpus* demonstrate, the circumscription of *Scirpus* and *Eriophorum* remains incomplete. The continued study of these two genera may confirm their paraphyly and it could lead to the discovery of other new generic lineages.

CALLISCIRPUS, A GENUS OF TWO SPECIES ENDEMIC TO THE CALIFORNIA FLORISTIC PROVINCE

Based on the results of our morphological and molecular analyses, *Calliscirpus* consists of two allopatric species, *Calliscirpus criniger*, which is distributed throughout the Klamath-Siskiyou and North Coast mountain ranges of Oregon and California, and a new species, *C. brachythrix* that is unique to the Sierra Nevada mountain range of California. Both of these species fall entirely within the California Floristic Province (one anomaly – see below) and in mountain ranges that are well-known for their high levels of species and generic endemism (DellaSala *et al.* 1999; Myers *et al.* 1999; Thorne *et al.* 2009).

Molecular data strongly support (>96% BS) the recognition of two species in *Calliscirpus* with at least 13 synapomorphies supporting the monophyly of *C. brachythrix* and six synapomorphies supporting the monophyly of *C. criniger*. In addition, *C. brachythrix* and *C. criniger* can be separated by two statistically significant quantitative characters: ligule hair length, which displays no overlap in measurements between species and is much shorter in *C. brachythrix*, and floral scale width, which is much wider in *C. criniger*, although 12% of the specimens cannot be definitively identified by this character alone. In addition, one qualitative character, the degree of bristle scabrousness, can also be used and is clearly visible under a stereomicroscope. *Calliscirpus brachythrix* possesses thinner, shorter, and

less dense bristle barbs than the thicker, longer, and denser bristle barbs of *C. criniger*. Even though a combination of scale width and bristle scabrousness is sufficient to distinguish species, the best character for identification is ligule hair length. When pressing and mounting specimens, it is important that the ligule is visible. This character could not be measured in approximately 12% of the specimens examined as it was hidden from view.

Although the treatments for *Eriophorum crinigerum* in the Jepson Manual (Baldwin *et al.* 2012) and the Flora of North America (Ball & Wujek 2002) suggest that *Calliscirpus* species should be commonly found on serpentine substrates, label data suggest that only *C. cringer* is common to such habitats. Whereas 21 of 116 specimens of *C. cringer* mention the presence of serpentine and it is commonly cited as growing on serpentine in vegetation surveys in the Klamath-Siskiyou mountain range (e.g., Sikes & Muir 2009; Scott 2010; Schuller *et al.* 2010), no mention of serpentine is found on any *C. brachythrix* labels or in vegetation surveys where the species is documented despite the widespread presence of serpentine in the Sierra Nevada mountain range. In addition, the most common associate listed on labels for *C. criniger* was *Pinus jeffreyi* A. Murray (five times), a strong serpentine indicator (Safford *et al.* 2005), whereas the most common associate for *C. brachythrix* was *Pinus contorta* Douglas (four times). Like *Eriophorum crinigerum*, *Pinus contorta* was not evaluated by Safford *et al.* (2005) in their paper on Californian serpentine endemism as the accessed resources suggested its serpentine affinity was not worth ranking (H. Safford, pers. comm.). Given the discovery of *C. brachythrix*, the serpentine affinity of *C. criniger* should be re-evaluated.

TAXONOMIC TREATMENT

Key to the genera of tribe Scirpeae

- 1 Perianth of scale-like tepals*Oreobolopsis*
 Perianth of bristles or perianth absent2
- 2 Perianth bristles ≥ 6 , greatly elongating after anthesis and often forming white or
 pigmented cottony masses around spikelets.....3
 Perianth bristles 3 to 6, short or reduced, sometimes rudimentary or absent, rarely
 elongating and forming white or brown wooly masses around spikelets.....5
- 3 Perianth bristles (8) 10 or more.....*Eriophorum*
 Perianth bristles usually 6 (–12).....4
- 4 Perianth bristles smooth.....*Trichophorum* (in part)
 Perianth bristles barbed.....*Calliscirpus*
- 5 Perianth bristles with retrorsely or antrorsely set spine-like barbs at apex or perianth
 lacking; involucre of leaf-like or spathe-like bracts or involucre lacking.....6
 Perianth bristles with antrorsely or divergently set hairs or blunt barbs, rarely smooth,
 sometimes rudimentary; involucre of scale-like or setaceous bracts.....9
- 6 Inflorescence compound, anthelate with elongate branches; spikelets
 numerous.....*Scirpus*
 Inflorescence a simple head of 1 to few spikelets at the summit of the culm.....7
- 7 Style 2-branched.....*Amphiscirpus*
 Style 3-branched.....8

- 8 Perianth absent; ligule present.....*Zameioscirpus*
 Perianth present; ligule absent.....*Phylloscirpus*
- 9 Leaf blades reduced, often with rounded or thickened apex; achenes
 beaked.....*Trichophorum* (in part)
 Leaf blades well-developed, attenuate to triquetrous apex; achenes
 unbeaked.....*Cypringlea*
-

Calliscirpus *C. N. Gilmour, J. R. Starr, & Naczi* **gen. nov.** Type: *Calliscirpus criniger* (A. Gray) C.N. Gilmour, J.R. Starr, & Naczi.

Herb caespitose, short-rhizomatous, perennial. *Culms* trigonous, (10–) 19 – 110 cm x 0.6 – 3 mm, occasionally dentate near apex. *Leaves* 3 – 6, basal and cauline, striate, 5 – 85 cm x 1 – 6 mm, distal leaf longer than sheath; leaf sheaths green or light brown at base, filamentose, dry and persistent. *Ligule* fimbriate with hairs ≤ 0.6 mm. *Inflorescences* capitate; bracts 2 – 5, sheathless, scale-like, rarely green and large, sometimes mucronate; spikelets 5 - 30 or more, oblong-lanceoloid, in a dense ovoid to hemispheric head of 1 or more inconspicuous branches; floral scales brown, with pale green or brown, sometimes red-spotted, 1 – 3-ribbed centre, ovate, apex acute; flowers bisexual; perianth bristles 6 (–12), 3 – 10 mm, dull white at anthesis, antrorsely barbed, deciduous at fruit maturity; stamens 3; anthers 1.2 – 2 mm long, white at anthesis; style deciduous, linear, 3-fid. *Achenes* 1.3 – 3 x 0.7 – 1.1 mm, dark brown, oblong-obovoid; embryo *Carex*-type.

RECOGNITION. New genus resembling *Eriophorum* L., but differing by its fewer, barbed bristles, *Carex*-type embryo, and inflorescence branches bearing more than one spikelet. Also similar to *Trichophorum* Pers., but differing by the presence of six or more bristles, well-developed leaves and more numerous spikelets.

DISTRIBUTION. A genus of two species endemic to the Klamath-Siskiyou, North Coast, and Sierra Nevada mountain ranges of California and Oregon, USA that are part of the California Floristic Province.

NOTES. Although the two species of *Calliscirpus* can be distinguished on the basis of scale width and bristle scabrousness, the best character for separating species is ligule hair length. Therefore, when pressing and mounting specimens, it is important that the ligule is visible as this character could not be measured in approximately 12% of the specimens examined in this study. Couplet 8 of the generic key for Cyperaceae in the Flora of North America (Ball *et al.* 2002) which separates “*Trichophorum* (in part)” from “*Eriophorum* (in part)” is used to distinguish the long- and smooth-bristled *Trichophorum alpinum* (L.) Pers. from *Calliscirpus* species. The genus is a member of tribe Scirpeae sensu Goetghebeur (1998) based on its spirally arranged and fertile scales, hypogynous perianth parts, and *Carex*-type embryo.

ETYMOLOGY. The Greek prefix *Calli-* means “beautiful”. When combined with the generic name *-scirpus* (the classical Latin name for *Schoenoplectus lacustris* of unknown

derivation) it distinguishes *Calliscirpus* species as among the most beautiful and striking to be segregated from *Scirpus*.

Key to the species of the genus *Calliscirpus*

1. Ligule hairs few, ≤ 0.2 mm long (often so short that ligule appears entire). Floral scales lanceolate, ≤ 1.3 mm wide. Perianth bristles sparsely scabridulous.....**1. *Calliscirpus brachythrix* sp. nov.**
2. Ligule hairs numerous, ≥ 0.25 mm long. Floral scales ovate, ≥ 1.2 mm wide. Perianth bristles densely scabridulous.....**2. *Calliscirpus criniger***

1. ***Calliscirpus brachythrix*** C.N. Gilmour, J.R. Starr, & Naczi **sp. nov.** Type: United States of America, California, Alpine County, Winnemucca Lake Trail, near outlet of Winnemucca Lake, [38° 40' 7.68" N, 119° 59' 54.384" W], 2763 m, 16 July 2007, J. R. Starr 07-039 & J. Thibeault (holotype, CAN; isotypes, K, NY).

Herb caespitose, short-rhizomatous, perennial. *Culms* 19 – 85 cm x 1.1 – 1.9 mm, prominently striate, occasionally scabridulous near the apex. *Leaves* 3 – 6, basal and cauline, striate, 10 – 85 cm x 1 – 5 mm, distal leaf longer than sheath; leaf sheaths green or light brown at base, filamentose, dry and persistent. *Ligule* fimbriate with hairs 0.05 – 0.2 mm. *Inflorescences* capitate; bracts 2 – 5, sheathless, scale-like, 0.35 – 2 cm, rarely green and large, sometimes mucronate; spikelets 5 - 30 or more, oblong-lanceoloid, 5 - 14 mm in

flower and fruit, in a dense ovoid to hemispheric head of 1 or more inconspicuous branches; floral scales brown, with pale green or brown, sometimes red-spotted, 1 – 3-ribbed centre, ovate-oblong, 1.2 – 5.7 x 0.6 – 1.3 mm, apex acute; perianth bristles 6 (–12), 4 – 10 mm long, antrorsely barbed, elongate, straight; barbs thin, short, sparse; anthers 1.2 – 2 mm long; style deciduous, linear, 3-fid. *Achenes* 1.3 – 3 x 0.6 – 1.1 mm, dark brown, usually dull, smooth, beak short, trigonous, oblong; embryo *Carex*-type (Figure 9; Figure 12).

RECOGNITION. This species differs from *Calliscirpus criniger* (A. Gray) C. N. Gilmour, J. R. Starr, & Naczi by its shorter ligule hairs, narrower floral scales, and less dense bristle barbs.

DISTRIBUTION. USA: California. Alpine, El Dorado, Fresno, Inyo, Madera, Mariposa, Mono, Nevada, Placer, Plumas, Sierra, Tulare and Tuolumne counties. Sierra Nevada mountain range.

SPECIMENS EXAMINED (all cited specimens have been seen by the author; specimens with an asterisk “*” were used in molecular analyses; specimens marked with a dagger “+” were used in morphological analyses). USA. California: Alpine Co.: 19 km WSW of Markleeville, 6 Aug. 2006, *Naczi* 11535 (DOV); Winnemucca Lk Trail, 16 July 2007, *Starr* 07-039 (holotype CAN*⁺; isotype K, NY); Winnemucca Lk Trail, 16 July 2007, *Starr* 07-038 (CAN)*⁺. El Dorado Co.: Trail to Agora Lks, 21 July 1906, *Eastwood* 1220

(CAS)⁺; Heather Lk, 18 Aug. 1909, *McGregor* 100 (CAS, NY); Lk Susie, 29 July, 1911, *Abrams* 4864 (CAS, NY); Desolation Valley, July 1915, *van Dyke* s.n. (CAS); Heather Lk, 28 Aug. 1918, *Jepson* 8175 (JEPS); Fallen Leaf Lk, 3 July 1920, *Ottley* 981 (NY); Desolation Valley, 15 Aug. 1930, *Abrams* 12734 (A, JEPS, NY, UC); S side of Upper Echo Lk, 16 July 1945, *Beetle*, *Beetle & Hansen* 4053 (A, NY); Desolation Valley Wilderness Area, 28 July 1946, *Grant & Grant* 7802 (UC, CAS); Grouse Lk, 18 July 1972, *Smith* 3560 (JEPS)⁺. Fresno Co.: Charlotte Lk, 25 July 1910, *Clemens* 18 (CAS, NY, UC); Granite Basin, 30 July 1910, *Clemens* s.n. (NY); Kearsange Lakes, 14 Aug. 1937, *Kerr* 411 (CAS); Kaiser Park, 19 July 1943, *Pollard* s.n.(CAS); Granite Basin, 16 Aug. 1946, *Leschke* s.n. (CAS); Bubbs Crk Canyon, 22 July 1948, *Howell* 24871 (CAS)⁺; Edge of Laurel Crk, 26 July 1951, *Robbins* 3394 (CAS)⁺; Laurel Creek Basin, 26 July 1951, *Robbins* 972350 (UC); South Dinkey Lk, 12 July 1952, *Quibell* 1003 (CAS); Main Dinkey Lk, 14 July 1952, *Quibell* 1025 (CAS); McGee Crk, 6 Aug. 1952, *Raven* 4951 (CAS); 23 Aug. 1954, *Quibell* 4538 (DAO*⁺, JEPS); Above Muir trail, NW of outlet of Evolution Lk, 31 July 1957, *Quibell* 6493 (UC); SW side South Dinkey Lk, 20 Aug. 1958, *Bacigalupi & Quibell* 6732 (JEPS); Bordering Finger Lk, 28 July 1962, *Kaune* 551 (CAS, NY); Little Kern River, 31 Aug. 1975, *Shevock* s.n. (CAS)⁺; 64 Km NE of Fresno, 18 July 1998, *York & Shevock* 2197 (CAS)*⁺. Inyo Co.: Vidette meadows, Mt Whitney, 25 July 1916, *Campbell* s.n. (A); Rock Crk Lk Basin, 7 Aug. 1933, *Peirson* s.n. (UC); Rock Crk Lk Basin, 16 July 1946, *Howell* 22456 (CAS)⁺; Ridge W of Dingleberry above Lake Sabrina, 4 Aug. 1950, *Raven & Stebbins* 148 (CAS⁺, UC); NE of Dingleberry Lake, 11 Aug. 1950, *Raven* 299 (CAS)⁺; W of Long Lk., 7 July 1973 *Papenhagen* 800 (CAS). Madera Co.: S of Givens Lk Trail junction, 7 July 1941, *Mason* 12509 (UC); Near Garnet Lk,

2 Aug. 1941, *Howell* 16691 (CAS)⁺; Near Isberg Lk, 12 Aug. 1958, *Howell* 34317 (CAS)⁺; Shadow Crk, 15 Aug. 1967, *Buick* 67-02 (CAS)⁺; 13.5 km NE of Oakhurst, 27 July 2004, *Naczi* 10594 (DOV); 37° 23' 59.1714"N 119° 31' 16.6434"W, 2006, *Starr* 06-020 (CAN)⁺; Clark Range at the headwaters of Merced Peak Fork, 4 Aug. 2007, *Grossenbacher, Moore & Moore* 661 (UC). Mariposa Co.: Peregoy meadow, 2 July 1911, *Jepson* 4331 (JEPS)⁺; Mt Donahue, 23 July 1935, *Christensen* 2074 (UC); 25 July 1935, *Schreiber* 1816 (UC); Snow Flat, 13 Aug. 1938, *Howell* 14571 (CAS)⁺. Mono Co.: Slate Crk Valley near Split Lk, 29 July 1932, *Clausen* 534 (CAS⁺, DS⁺); Above Saddlebag Lk, 22 July 1933, *Sharsmith* 175 (JEPS); Slate Creek Basin, 26 Aug. 1937, *Kreck* 4587 (DS)⁺; Mt Dana, Glacier Canyon, 9 July 1940, *Clausen & Trapido* 4850 (PH, UC, A, NY); Mono Lk Basin, 9 July 1940 *Clausen & Trapido* 4850 (A); Along Cabin Crk, 17 Aug. 1941, *Kruekeberg* 1484 (CAS); Slate Crk, 16 Aug. 1954, *Munz* 19915 (CAS⁺, NY); Head of Lk Mildred, 30 Aug. 1962, *Major & Bamberg* 1490 (UC, CAS⁺); Slate Crk Meadow, 17 Aug. 1971, *Zufelt* 116 (CAS)⁺; Horse Crk S of Twin Lks, 7 Aug. 1980, *Lavin & Smith* SW114AA (CAS)⁺; T6NR21E Sec 36, 20 Aug. 1988, *Hardham* s.n. (UC). Nevada co.: Frog Lk, 24 July 1943, *Howell* 18782 (A, CAS⁺); Red Mt above Culbertson Lk, 9 July 1967, *Mott* 6768 (CAS)⁺; 4 km ESE of Graniteville, 28 July 2004, *Naczi* 10621 (DOV). Placer Co.; Aug. 1892, *Carpenter* s.n. (UC); 3.8 km N of Barker Pass, 18 Sept. 1998, *Janeway* 6344 (CHSC)⁺; Near Salmon Lks, 10 Aug. 1926, *Smith* 2004 (CAS)⁺. Plumas Co.: 1846, *Austin* s.n. (NY); 1870, *Austin* s.n. (A); 1879, *Austin* s.n. (DOV); 6.4 km SW of Bucks Lk, 12 July 1995, *Durlo, Castro & O'Connell* s.n. (CHSC)⁺; Meadow at head of E branch of Coldwater Crk, 16 July 2000, *Janeway* 6916 (CHSC)⁺. Sierra co.: SSE of Downieville, 12 July 1992, *Ahart & Oswald* 5099 (CHSC)⁺; 11.3 km NE of Alleghany, 13 July 2000, *Ahart*

8591 (CHSC⁺, JEPS). Tulare Co.: Monarch Crk, Mineral King, Aug. 1904, *Hall & Babcock* 5699 (A, UC); S slope of Reflection Lk, 2 Aug. 1940, *Howell* 15887 (CAS)⁺; Little Five Lks Basin, 29 July 1942, *Howell* 17406 (CAS)⁺; E side of Franklin Pass, 13 July 1948, *Bailey & Bailey* 2248 (UC); White Chief Region, 21 July 1951, *Howell* 28079 (CAS⁺, NY); 1.6 km above Silver City, 26 Sept. 1968, *Twisselman & McMillan* 14993 (CAS⁺, NY); Casa Vieja Meadow, 2 Sept. 1971, *Twisselman, McMillan, Nathan & Burkhardt* 18434 (NY); Little Kern River Basin, 31 Aug. 1975, *Shevock* 4783 (CAS)⁺; Jennie Lks Wilderness, 29 July 1980, *Shevock* 7832 (CAS); Jennie Lks Wilderness, 29 July, 1980, *Shevock* 9760 (CAS)⁺; Kern Plateau, 30 Aug. 1981, *Shevock* 9019 (CAS)⁺. Tuolumne Co.: Soda Springs Canyon, Kennedy's Lk, 13 Sept. 1915, *Grant* 508 (JEPS, NY); Elizabeth Lk, 15 Aug. 1916, *Smiley* 800 (A); N of Tuolumne Meadow, 22 July 1936, *Mason* 11263 (UC); By Elizabeth Lk, 24 July 1936, *Lee* 2309 (JEPS); Ridge S of Elizabeth Lk, 8 Aug. 1944, *Howell* 20124 (CAS)⁺; Young Lk, 15 Aug. 1944, *Howell* 20513 (CAS)⁺; Stubblefield Canyon, 12 Aug. 1982, *Botti* 165 (CAS)⁺.

HABITAT. Alpine and montane meadows and seepage slopes surrounded by conifers. Often near streams, creeks or with underground seeps at elevations ranging from 1250 to 3600 m. Common associates of *Calliscirpus brachythrix* include *Abies magnifica* A. Murray, *Arnica* L. spp., *Carex scopulorum* Holm, *Carex spectabilis* Dewey, *Darlingtonia californica* Torr., *Dodecatheon* L. spp., *Eleocharis acicularis* (L.) Roem. & Schult., *Gentiana newberryi* A. Gray, *Juncus mertensianus* Bong., *Kalmia* L. spp., *Lilium parvum* Kellogg, *Parnassia palustris* L., *Platanthera dilatata* var. *leucostachys* (Lindl.) Luer, *Pinus contorta* Douglas ex

Loudon, *P. monticola* Douglas ex. D. Don, *Rhododendron* L. spp., *Spiranthes* Rich. spp., *Sisyrinchium* L. spp., *Tsuga* Carrière spp., *Vaccinium* L. spp.

CONSERVATION STATUS. Least Concern (LC) category of IUCN (2001). The number of known populations is relatively large. Extent of Occurrence is c. 24,000 km², above the threshold (20,000 km²) for Vulnerable. In addition, most of the known occurrences are in protected areas (several national forests). Thus, despite being endemic to the Sierra Nevada mountain range in California, the future appears secure for *Calliscirpus brachythrix*.

ETYMOLOGY. The specific epithet *brachythrix* combines the Greek prefix *brachy-* meaning “short” with the Greek root *-thrix* meaning “hair” to highlight the defining character of short ligule hairs that clearly separates *Calliscirpus brachythrix* from its sister species, *C. criniger*.

NOTES. Distinguished by its short ligule hairs, narrow floral scales, and less densely scabridulous bristles. Localities for this species are all found within the Sierra Nevada mountain range. Although the habitat for *Eriophorum crinigerum* (A. Gray) Beetle s.l. has often been cited as being commonly associated with serpentine substrates (Ball & Wujek 2002; Baldwin *et al.* 2012), no labels for *Calliscirpus brachythrix* make any mention of serpentine or related rock formations despite their presence in the Sierra Nevadas.

2. **Calliscirpus criniger** (A. Gray) C. N. Gilmour, J. R. Starr, & Naczi **comb. nov.** Type: California, Humboldt County, Red Mountain, 1866, *H.N. Bolander* 6475 (holotype, GH!; isotypes GH!, NY!, UC!)

Scirpus criniger A. Gray, *Proc. Amer. Acad. Arts* 7: 392 (1868).

Eriophorum crinigerum (A. Gray) Beetle (1942: 165).

Herb caespitose, short-rhizomatous, perennial. *Culms* (10–) 20 – 110 cm x 1 – 3 mm, prominently striate, occasionally scabridulous near the apex. *Leaves* 3 – 6, basal and cauline, striate, 5 – 45 cm x 1 – 6 mm, distal leaf longer than sheath; leaf sheaths green or light brown at base, filamentose, dry and persistent. *Ligule* fimbriate with hairs 0.25 – 0.6 mm.

Inflorescences capitate; bracts 2 – 5, sheathless, scale-like, 0.3 – 1.0 (–1.3) cm, rarely green and large, sometimes mucronate; spikelets 5 - 30 or more, oblong-lanceoloid, 5 - 15 mm in flower and fruit, in a dense ovoid to hemispheric head of 1 or more inconspicuous branches; floral scales brown, with pale green or brown, sometimes red-spotted, 1 – 3-ribbed centre, ovate-oblong, 0.7 – 5.2 x 1.2 – 2.0 mm, apex acute; perianth bristles 6 (–12), 4.5 – 9 mm, antrorsely barbed, elongate, straight; barbs thick, long, dense; stamens with anthers 1.2 – 2 mm long; style deciduous, linear, 3-fid. *Achenes* 1.3 – 3 x 0.7 – 1.1 mm, dark brown, usually dull, smooth, beak short, trigonous, oblong; embryo *Carex*-type (Figure 13).

DISTRIBUTION. United States of America: California. Del Norte, Humboldt, Mendocino, Modoc, Shasta, Siskiyou, Sonoma and Trinity counties; Oregon. Coos, Curry and Josephine counties. Klamath-Siskiyou and North Coast mountain ranges (one anomaly, see Notes).

SPECIMENS EXAMINED (all cited specimens have been seen by the author; specimens with an asterisk “*” were used in molecular analyses; specimens marked with a dagger “†” were used in morphological analyses). USA. California: Del Norte Co.: Gasquet to Patrick, 27 June 1922, *Abrams* 8543 (DS)⁺; Near Douglas Park, 5 June 1928, *Thompson* 4531 (DS, PH); Gasquet, 10 May 1931, *Tracy* 9380 (DAO)*⁺; 8 km E of Gasquet, 13 July 1933, *Parks & Tracy* 12719 (A, DS, JEPS, UC); along old highway over Oregon Mt, 6 Aug. 1935, *Thompson* 12531 (NY); Oregon Mt, 2 July 1936, *Eastwood & Howell* 3711 (DS); 17 July 1938, *van Deventer* 282 (JEPS); 1.6 km N of Gasquet, 10 Aug. 1949, *Nobs & Smith* 1276 (UC); Between French Hill and Gordon Mt, 26 June 1952, *Munz* 17786 (NY); 12 July 1952, *Howell* 28841 (CAS⁺, DAO*⁺); N bank of Smith River, 8 June 1962, *Breedlove* 3097 (CAS)⁺; along Old Gasquet Toll Rd, 20 Sept. 1965, *Thorne* 35501 (PH); Gasquet, 28 June 1982, *Lenihan & Becking* 4013 (DS). Humboldt Co.: Red Mt, 1866, *Bolander* 6475 (holotype GH; isotype NY, UC, GH); Red Mt, Aug 1869, *Bolander & Kellogg* s.n. (UC); Horse Mt, 20 June 1926, *Tracy* 7637 (UC). Mendocino Co.: Red Mt, 14 July 1969, *Gonkin, Hildreth, Knight & Knight* 2705 (CAS)⁺; Red Mt, 29 July 1909, *McMurphy* 595 (DS)⁺; Red Mt, 12 June 1991, *Raiche, Forbes & Zadnik* 132 (JEPS)⁺; Cabin Bog, 7 July 1981, *Smith* 6877 (CAS)⁺. Modoc Co.: [no additional locality data], 14 June 1879, *Plummer* s.n. (A). Shasta Co.: Trinton Canon, 23 June 1893, *Dudley* (DS)⁺; Pit River Canyon, 15 June 1996,

Taylor 15691 (JEPS)⁺; 4.8 Km N of Pony Buck Peak, 8 July 2001, *Janeway* 7272 (CHSC)⁺.
 Siskiyou Co.: Mt Eddy, 18 Aug. 1903, *Copeland* 3902 (A); Log Lk, 9 July 1910, *Butler* 1703
 (UC); 1 July 1928, *Kildale* 5333 (DS)⁺; Elk Lick Ridge, 21 July 1929, *Kildale* 8739 (DS)⁺;
 Caribou Basin, 24 July 1937, *Howell* 13411 (DS)⁺; Taylor Lk, 1 Sept. 1938, *Harris &*
Leland 5445 (A); On shore of Castle Lk, 6 June 1940, *Stacey* s.n. (NY); Shores of Castle Lk,
 6 June 1940, *Cooke* 15054 (CAS)⁺; Caribou Lk, 27 July 1955, *Wiggins* 13553 (DS, NY,
 UC⁺); Ridge S of Mt Eddy, 4 Sept. 1956, *Raven* 10383 (DS)⁺; Gumboot Lk, 26 June 1957,
Bacigalupi & Smith 5953 (JEPS, UC); Near Diamond Lk, 2 Aug. 1968, *Oettinger* 350 (UC);
 N of Tobacco Lk, 7 Sept. 1968, *Oettinger* 767 (UC); S of Lily Pad Lk, 17 July 1986,
Heckard & Hickman 83994 (JEPS)⁺; W side of Cedar Lk, 30 Aug. 1993, *Janeway* 4567
 (CHSC)^{*+}. Sonoma Co.: Head of Austin Crk, 25 June 1983, *Raiche* 20425 (JEPS)^{*+}. Trinity
 Co.: At head of White's Crk, 7 Aug. 1935, *Tracy* 14601 (A⁺, UC); Upper Canyon Crk Lk, 22
 Aug. 1948, *Alexander & Kellogg* 5531 (A, JEPS⁺); W end of Gumboot Lk, 26 June 1957,
Oswald & Ahart 6424 (JEPS)⁺; along Dead Fall Crk, 13 Aug 1983, *Raiche* 30601 (JEPS);
 Slope NE of Picayune Lk, 30 Aug. 1993, *Janeway* 4559 (CHSC)⁺; Meadow adjacent to Scott
 Summit campground, 22 June 1997, *Janeway* 881 (CHSC)⁺; SE of Callahan, 5 July 1999,
Oswald & Ahart 9829 (JEPS)⁺; 1.3 Km S of Red Mt at head of Red Mt Crk, 15 July 2001,
Janeway 7317 (CHSC)⁺; 0.3 Km SW of crossing of E fork Smoky Crk, 15 July 2001,
Janeway 7337 (CHSC)⁺; Shasta National Forest, W of Picayune Crk, 7 Aug 2010, *Starr* 10S-
 055 (CAN)^{*+}. Oregon: Coos Co.: Iron Mt, 15 June 1948, *Baker* 5451 (CAS⁺, OSC^{*+}). Curry
 Co.: Near Snow Camp Mt, 22 June 1936, *Thompson* 12849 (A, CAS⁺, PH, NY); Snow Camp
 Mt, 8 July 1973, *Denton* 3105 (WS)⁺; 10 m SE of Squaw Lk, 9 July 1973, *Denton* 3143

(WS)⁺; 10 Km E of Gold Beach, 28 July 1980, *Sundberg* 1109 (OSC)^{*+}; Head of Little Cedar Crk, 20 June 1981, *Greenleaf* 574 (OSC)⁺; Snow Camp Meadow, 11 July 1981, *Greenleaf* 892 (OSC)⁺; 0.4 km S of Vulcan Lk, 11 July 1981, *Greenleaf* 957 (OSC)⁺; 0.8 km S of Vulcan Lk, 11 July 1981, *Greenleaf* 981 (OSC)⁺; Siskiyou Mts, 11 July 1981, *Greenleaf* 937 (OSC)⁺; 30 June 1982, *Becking & Lenihan* 4408 (OSC)⁺; Chetco Peak, 30 June 1982, *Becking & Lenihan* 4610 (CAS)⁺; Hunter Crk Bog, 19 June 1997, *Wilson, Stansell & Zika* 8481 (CHSC)⁺. Josephine Co.: Near Kerby, 30 May 1884, *Howell* s.n. (NY⁺); Near Waldo, 1887, *Howell* 3893 (NY)⁺; Near Waldo, July 1888, *Howell* s.n. (NY)⁺; Eight Dollar Mt, 12 June 1904, *Piper* 1744 (WS)⁺; 12.9 km S of Waldo, 14 June 1904, *Piper* 6738 (A); 4 Aug. 1913, *Peck* 4624 (NY⁺, OSC⁺); Grants Pass 16 km W of Waldo, 4 Aug. 1913, *Peck* 8390 (A, PH); 4.8 km SE of Oregon Caves, 16 July 1918, *Peck* 8314 (OSC)^{*+}; Whiskey Crk, 1 July 1922, *Abrams* 8610 (DS)⁺; Eight Dollar Mt, near Selma, 16 April 1926, *Henderson* 5928 (A⁺, CAS⁺); 16 km SW of Waldo, 7 June 1928, *Thompson* 4585 (DS⁺, PH); Oregon Mt, 5 June 1930, *Leach* 2858 (OSC)⁺; Bigelow Lks, 31 July 1935, *Thompson* 12443 (WS)⁺; 3.2 km SW of O'Brien, 19 June 1936, *Yates* 5779 (UC); 6.4 km SE of Takilma, 14 July 1946, *Keck* 5647 (DS)⁺; Fiddler Mt, Road above Josephine Crk, 11 May 1974, *Chambers* 3953 (OSC)⁺. Mud Springs, 6.4 km above Rough and Ready Crk, 26 June 1950, *Kruckeberg* 1979 (UC, WS⁺); 30 Aug. 1970, *Chambers* 2973 (A⁺, DAO^{*+}, NY); Near Day's Gulch, 13 June 1981, *Greenleaf* 401 (OSC)⁺; S slope of Eight Dollar Mt, 14 June 1981, *Greenleaf* 449 (OSC)⁺; 4.8 km NW of Kerby, 30 June 1986, *Ertter & Vorobik*, 6490 (NY); Between Chetco Pass and Pearsoll Peak, 28 Jun 1998, *Olmstead* 98-36 (A⁺, WS⁺); Siskiyou National Forest, 2010, *Starr* 10S-056 (CAN)^{*+}.

HABITAT. Common on serpentine substrates in open, fairly sparsely vegetated areas adjacent to streams or on slopes with underground seepage at elevations from 200 m to 2250 m. Commonly cited associates include *Abies magnifica* A. Murray, *Arbutus* L. spp., *Calocedrus decurrens* (Torr.) Florin, *Chamaecyparis lawsoniana* (A. Murray) Parl., *Darlingtonia californica* Torr., *Dodecatheon jeffreyi* Van Houtte, *Pinus jeffreyi* Balf., *P. lambertiana* Douglas, *Poa* L. spp., *Dasiphora fruticosa* (L.) Rydb., *Pseudotsuga menziesii* (Mirb.) Franco, *Salix* L. spp., and *Viola mackloskeyi* F.E. Lloyd.

CONSERVATION STATUS. Least Concern (LC) category of IUCN (2001). The number of known populations is relatively large. Extent of Occurrence is at least 33,000 km², well above the threshold (20,000 km²) for Vulnerable. In addition, many of the known occurrences are in protected areas (including several national forests). Thus, despite being endemic to the Klamath-Siskiyou and North Coast mountain ranges, the future appears secure for *Calliscirpus criniger*.

ETYMOLOGY. The specific epithet *criniger* is a Latin adjective meaning “long-haired” and refers to the fact that when this species was originally named as *Scirpus criniger* by Asa Gray (1867), it possessed among the longest known bristles for *Scirpus*. Conveniently, *criniger* also aptly describes the best character for differentiating the two species in *Calliscirpus*, namely the “long-haired” ligules of *C. criniger* versus the “short-haired” ligules of *C. brachythrix*.

NOTES. Distinguished by its long ligule hairs, wide floral scales, and strongly scabrous bristles, localities for this species are entirely found within the Klamath-Siskiyou mountain range with the exception of five specimens. Four of these collections are from the North Coast mountain range of Sonoma (*Raiche* 20425, JEPS) and Mendocino (*Gonkin, Hildreth, Knight & Knight* 2705, CAS; *McMurphy* 595, DS; *Raiche, Forbes & Zadnik* 132, JEPS; *Smith* 6877, CAS) counties, California. Two of these specimens (*Raiche* 20425 and *Raiche, Forbes & Zadnik* 132) indicate the plants were growing on serpentine substrates, a fact cited on approximately 20% of *C. criniger* labels from the Klamath-Siskiyou Range and suggesting an association between this species and serpentine soils (note that only 60% of labels cite details beyond county). However, the fifth specimen, a Modoc County collection by S. A. Plummer in June 1879 (*Plummer* s.n., A) is anomalous as it is the only specimen of *C. criniger* or *C. brachythrix* seen outside the California Floristic Province. Other S. A. Plummer specimens from the summer of 1879 indicate that she was collecting in counties where *C. criniger* is common. Given that the label for this specimen is in Asa Gray's hand and it appears that the date might have been added later (a lighter shade of ink), this collection may represent a labelling error.

There is some confusion regarding the type locality for *C. criniger* as there is a Red Mountain in both Humboldt and Mendocino counties, and the type has been cited as being collected from one or the other locality (Beetle 1942; Purdy 1931). One isotype (UC 2028) has conflicting labels citing Mendocino on one and Humboldt County on the other, but it is clear from the label on the holotype (GH), "*Scirpus (Trichophorum) criniger*, n. sp., H. N.

Bolander 6475”, written in Asa Gray’s hand that the type locality is “Red Mt., Humboldt Co.”. Moreover, Purdy (1931) notes that although Bolander travelled to Mendocino County, he was never near its Red Mountain.

Note that the holotype sheet at GH has a later collection, “Plumas Co., Mrs. R. M. Austin, 1876”, affixed to the left side of the sheet. This is an individual of *C. brachythrix*.

TABLES AND FIGURES

Table 1. Primer sequences used to amplify and sequence the *matK* and *ndhF* gene regions. An asterisk (*) denotes a primer used for sequencing, but not for amplification.

Primer Name	Primer Region	Primer Sequence	Primer Designer
<i>matK</i> -1F	<i>matK</i>	5'-CGTCAACAACAATGCTTATATCC-3'	Starr, unpublished
<i>matK</i> -RL	<i>matK</i>	5'-GCTTTGCCTTGATATCGAAC-3'	Starr, unpublished
<i>matK</i> -2.5F	<i>matK</i>	5'-TCAATGCTGGRTCCAAGATA-3'	Starr, unpublished
<i>matK</i> -2.5R*	<i>matK</i>	5'-ATATCTTGGARCCAGCATTG-3'	Starr, unpublished
<i>matK</i> -5R	<i>matK</i>	5'-TTTATGTTTACGAGCCAAAG-3'	Starr, unpublished
<i>ndhF</i> -A	<i>ndhF</i>	5'-TATGGTTACCTGATGCCATGGA-3'	Starr, unpublished
<i>ndhF</i> -B	<i>ndhF</i>	5'-CCCCATAGAGATATTGAAT-3'	Starr, unpublished
<i>ndhF</i> -C	<i>ndhF</i>	5'-TAACAGCATTTTATATGTTTCG-3'	Starr, unpublished
<i>ndhF</i> -D1	<i>ndhF</i>	5'-CTATRTAACCRCGATTATATGACCAA-3'	Starr, unpublished

Table 2. Specimens used in molecular analyses with corresponding Genbank Accession numbers for the three DNA regions (*matK*, *ndhF*, and ETS 1f) sequenced in this study.

Taxa	Collection Number	Chloroplast DNA		Nuclear DNA
		<i>matK</i>	<i>ndhF</i>	ETS-1F
<i>Amphiscirpus nevadensis</i> (S.Watson) Oteng-Yeb.	Hudson 5177, Sask., Canada (CAN)	JX065075	JX074631	-
<i>Blysmus rufus</i> (Huds.) Link	Jokela (CAN)	JX065076	JX074632	-
<i>Bulbostylis hispidula</i> (Vahl) R.W.Haines	Muasya 1025, Kenya (EA, K)	JX065077	JX074633	-
<i>Calliscirpus brachythrix</i> C.N.Gilmour, J.R. Starr, & Naczi	Ahart & Oswald 5099, Sierra County, California (CHSC)	JX065078	JX074634	JX065112
	Quibell 4538, Fresno County, California (DAO)	JX074656	-	JX065100
	Starr & Thibeault 07-039, Alpine County, California (CAN)	JX074659	-	JX065103
	Starr & Thibeault 06-020, Madera County, California (CAN)	JX074660	-	JX065104
	Starr & Thibeault 07-038, Alpine County, California (CAN)	JX074661	-	JX065105
	York & Shevock 2197, Fresno County, California (CAS)	JX074665	-	JX065109
	Janeway 6344, Placer County, California (CHSC)	JX074667	-	JX065111
	Janeway 6916, Plumas County, California (CHSC)	JX074671	-	JX065115

*Calliscipus
criniger*
(A.Gray)
C.N.Gilmour,
J.R.Starr, &
Naczi

Starr 105-056, Josephine County, Oregon (CAN)	JX065079	JX074635	JX065106
Tracy 9380, Del Norte County, California (UC)	JX074654	-	JX065098
Chambers 2973, Josephine County, Oregon (DAO)	JX074655	-	JX065099
Howell 28841, Del Norte, California (DAO)	JX074657	-	JX065101
Starr 105-055 & Villaverde, Trinity County, California (CAN)	JX074658	-	JX065102
Peck 8314, Josephine County, Oregon (OSC)	JX074663	-	JX065107
Baker 5451, Coos County, Oregon (OSC)	JX074664	-	JX065108
Raiche 20425, Sonoma County, California (JEPS)	JX074666	-	JX065110
Sundberg 1109, Curry County, Oregon (OSC)	JX074669	-	JX065113
Janeway 4567, Siskiyou County, California (CHSC)	JX074670	-	JX065114
Starr & Thibeault 06- 016, Butte County, California, USA (CAN)	JX065080	JX074636	-
Porsild 8828, Greenland, Denmark (CAN)	JX065081	JX074637	-

Carex capitata
L.

Carex ursina
Dewey

<i>Cypringlea evadens</i> (C.D.Adams) Reznicek & S.González	Rawlins & Sholes 2830, Guerro, Mexico (MICH)	JX065082	JX074638	-
<i>Dulichium arundinaceum</i> (L.) Britton	Ford et al. 94233, Manitoba, Canada (FHO)	JX065083	JX074639	-
<i>Eriophorum callitrix</i> Cham. ex C.A.Mey	Porsild & Porsild 4753, Northwest Territories, Canada (CAN)	JX074653	JX074641	JX065097
<i>Eriophorum viridicarinatum</i> (Engelm.) Fernald	Shea 11351, Ontario, Canada (CAN)	JX074652	JX074640	JX065096
<i>Fimbristylis dichotoma</i> (L.) Vahl	Muasya 1006, Kenya (EA, K)	JX065086	JX074642	-
<i>Khaosokia caricoides</i> D.A.Simpson	Middleton et al. 4071, Surat Thani, Thailand (MICH)	JX065087	JX074643	-
<i>Kobresia simpliciuscula</i> (Wahlenb.) Mack.	Porsild 1825, Yukon Territory, Canada (CAN)	JX065088	JX074644	-
<i>Oreobolopsis tepalifera</i> T.Koyama & Guagl.	Wood 10463, Cochabamba, Bolivia (CAN)	JX065089	JX074645	-
<i>Phylloscirpus deserticola</i> (Phil.) Dhooge & Goetgh.	Ru 9797, Argentina (US)	JX065090	JX074646	-
<i>Scirpus atrovirens</i> Willd.	Stuckey 6020, Paulding County, Ohio, USA (CAN)	JX065091	JX074647	-
<i>Scirpus cyperinus</i> (L.) Kunth	Lindsay 1025, Ontario, Canada (CAN)	JX065092	JX074648	-

<i>Trichophorum alpinum</i> (L.) Pers.	Spetzman 4941, Alaska, USA (CAN)	JX065093	JX074649	-
<i>Trichophorum cespitosum</i> (L.) Hartm.	Saarela & Percy 1219, BC, Canada (CAN)	JX065094	JX074650	-
<i>Zameioscirpus atacamensis</i> (Phil.) Dhooge & Goetgh.	Ru 9884, Argentina (US)	JX065095	JX074651	-

Table 3. The 23 morphological characters measured for *Calliscirpus brachythrix* and *C. criniger* with brief descriptions.

Morphological Character	Character Description
Achene length	Length of achene measured from the base to the apex
Achene width	Widest point
Bract length	Base to apex
Bract width	Widest point
Culm length	From base where roots begin to the base of inflorescence
Culm sheath length	Length of culm sheath from base to apex
Culm width	Width of culm at widest point
Leaf length	Most mature leaf length from proximal to distal end

Leaf width	Most mature leaf width measured at widest portion
Length of longest bristle	Longest bristle from proximal to distal end
Ligule hair length	Ligule hair length from base (attached to ligule) to apex
Number of anthers	Counted in mature flower
Number of bristles	Counted in mature flowers
Number of leaves	Basal and cauline leaves counted
Number of spikelets	Spikelets counted
Scale length	Mature, complete scale removed and length measured from base to apex
Scale midrib length	Midrib length of removed mature scale from base to apex
Scale midrib width	Width of midrib at widest point on removed mature scale
Scale width	Width of removed mature scale at widest point
Spike length	Length of spike from proximal to most distal point
Spike width	Width of spike at widest point
Spikelet length	Largest spikelet's length from base to apex
Spikelet width	Largest spikelet's width widest point

Table 4. Means \pm 1 SD and ranges (in parentheses) for the two best morphological characters for separating *Calliscirpus criniger* and *C. brachythrix*. All measurements are in millimetres. *N* = sample size. Unpaired t-tests were used to determine the statistical significance of characters.

Character	<i>Calliscirpus criniger</i>	<i>Calliscirpus brachythrix</i>	p-value
	N=66	N=54	
Floral Scale Width	1.36 \pm 0.156 (1.2-2)	0.96 \pm 0.163 (0.7-1.3)	<0.001
Ligule Hair Length	0.35 \pm 0.097 (0.25-0.6)	0.11 \pm 0.037 (0.05-0.2)	<0.001

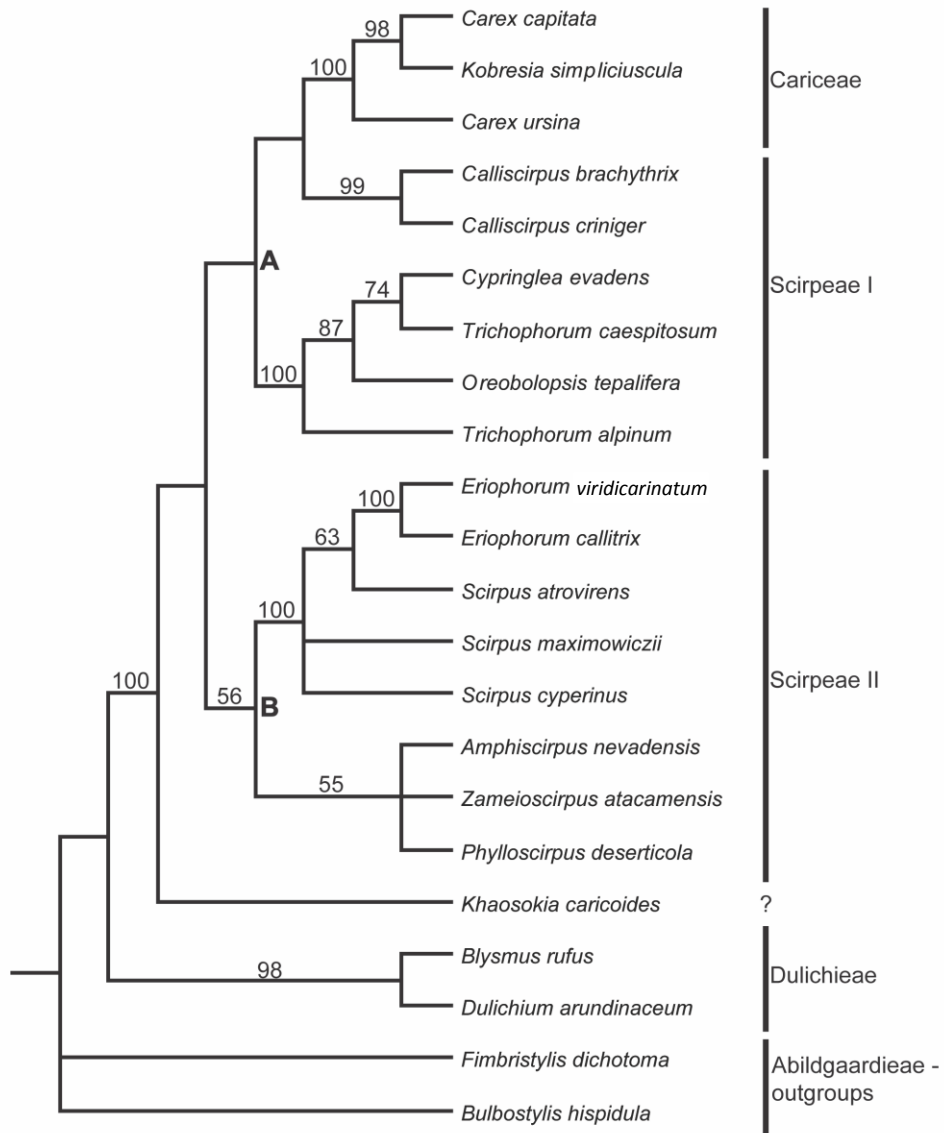


Figure 2. The strict consensus of two trees recovered during the combined cpDNA (*matK* + *ndhF*) parsimony analysis of the Cariceae + Dulichieae + Scirpeae clade. Bootstrap support values >50% are given above branches. The labels A and B highlight clades described in the text. Genera are assigned to tribes according to Goetghebeur (1998) and Dhooge (2005).

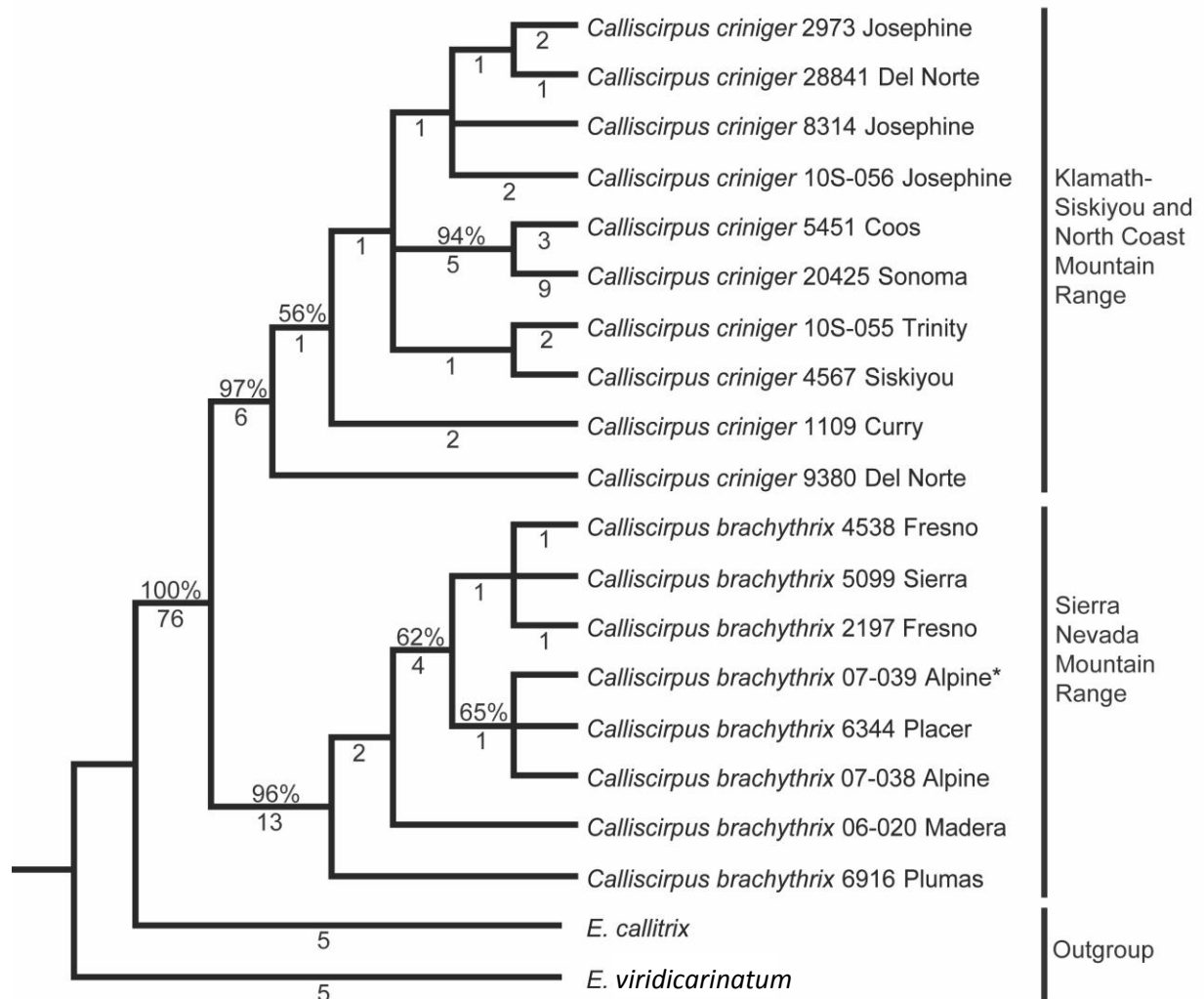


Figure 3. The single most parsimonious tree recovered in the combined parsimony analysis of *matK* (cpDNA) and ETS 1f (nDNA). Specific epithets are followed by collection numbers and the county in which the voucher was collected. Bootstrap values >50% are given above the branches with unambiguous character changes given below. The holotype for *C. brachythrix* is distinguished by an asterisk (*).

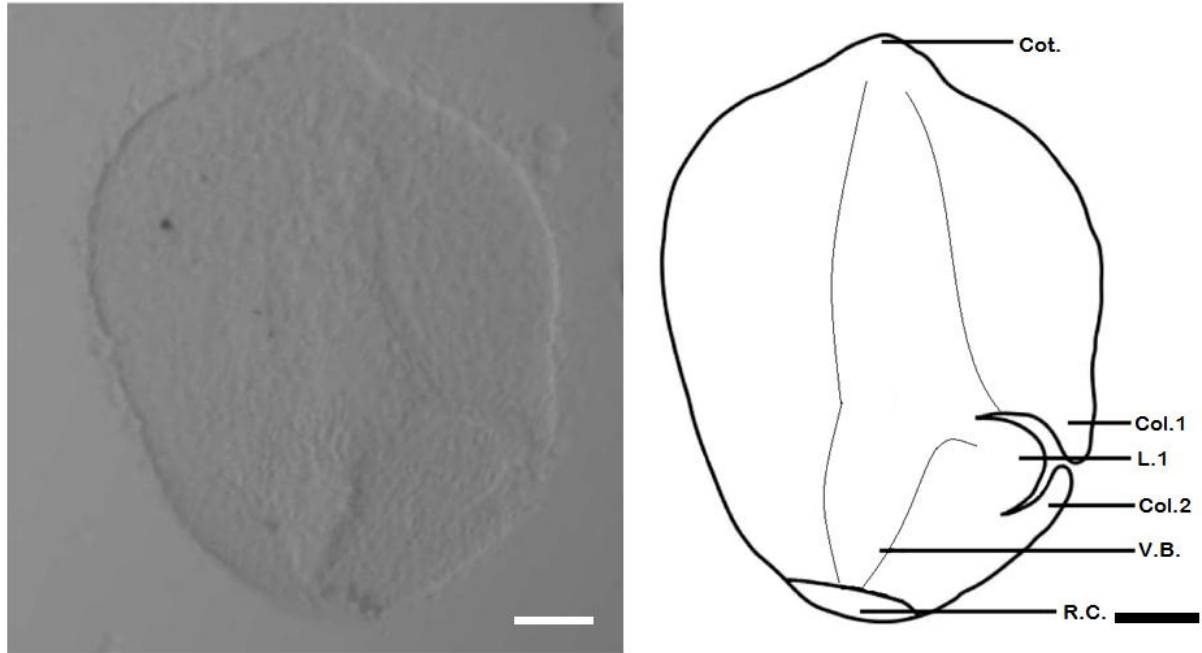


Figure 4. Median-sagittal view of the *Carex*-type embryo of *Calliscirpus brachythrix* (Starr 06-020, CAN). Scale bar = 0.05 mm. Col.1 = first coleoptile lip; Col.2 = second coleoptile lip; Cot. = cotyledon; L.1 = first leaf; R.C. = root cap, V.B. = vascular bundle.

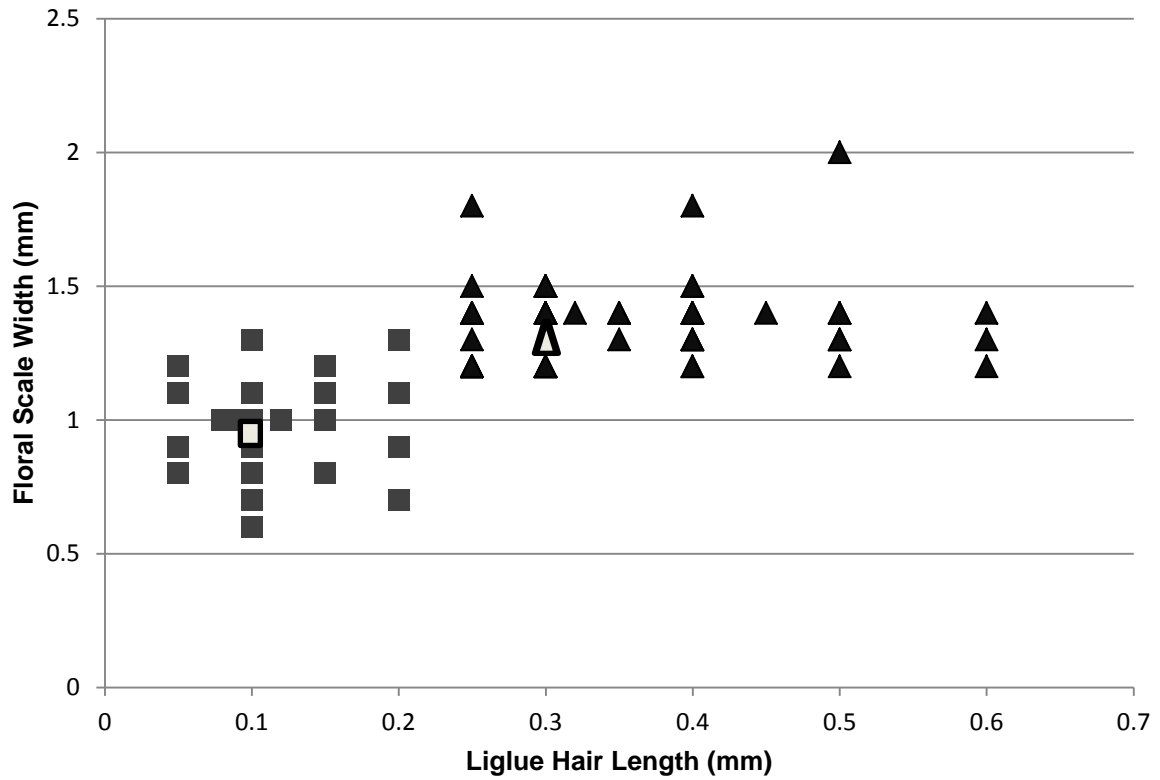


Figure 5. Bivariate plot of the two quantitative variables that best separate *Calliscirpus* species, ligule hair length and floral scale width. Black triangles represent *C. criniger* and the grey squares represent *C. brachythrix*. The type specimens for *C. brachythrix* and *C. criniger* are distinguished by an open square and open triangle, respectively.

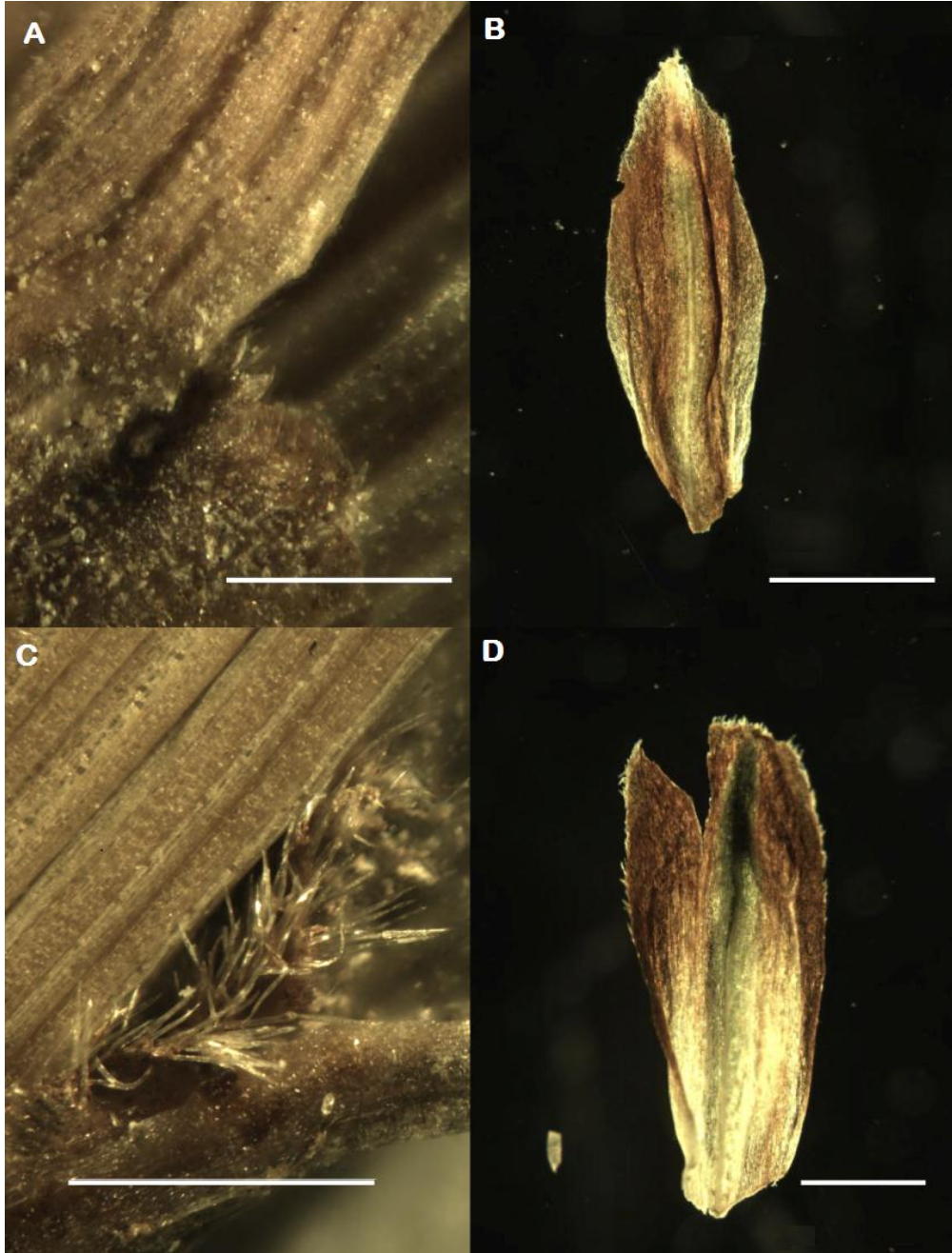


Figure 6. Photographs of ligule hairs and floral scales. **A** *Calliscirpus brachythrix* (Starr 06-020, CAN), ligule hairs; **B** *C. brachythrix* (Starr 06-020, CAN), floral scale. **C** *C. criniger* (Starr 10S-055, CAN), ligule hairs; **D** *C. criniger* (Starr 10S-055, CAN), floral scale. Scale bar = 1.0 mm.

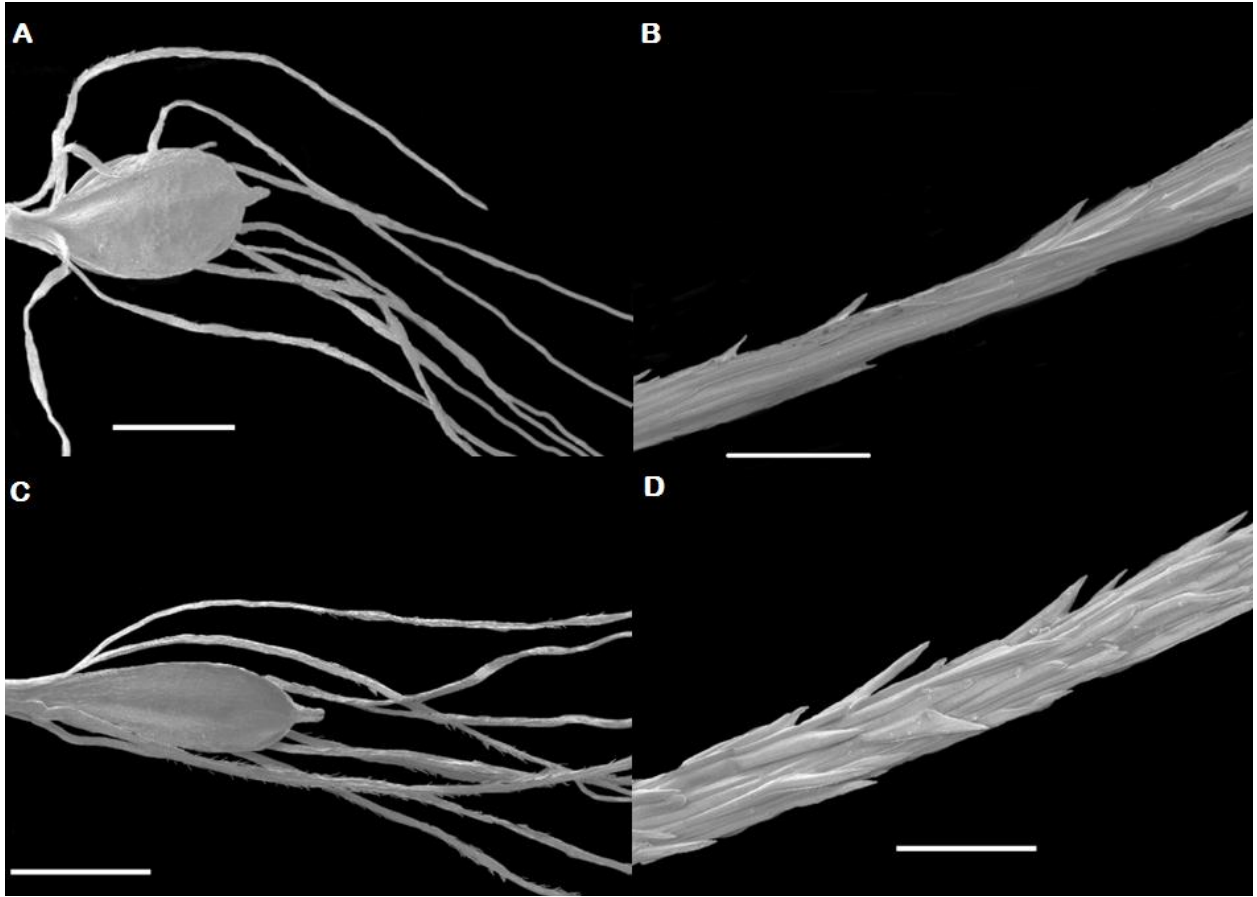


Figure 7. Environmental scanning electron micrographs of achenes and bristles. **A** *Calliscirpus brachythrix* (Shevock 9019, CAS), achene with bristles and filaments (three smooth structures); **B** *C. brachythrix* (Shevock 9019, CAS), bristle enlarged; **C** *C. criniger* (Keck 5647, CAS), achene with bristles and filaments (three smooth structures); **D** *C. criniger* (Keck 5647, CAS), bristle enlarged. Scale bar for **A** & **C** = 100 μm . Scale bar for **B** & **D** = 1000 μm .

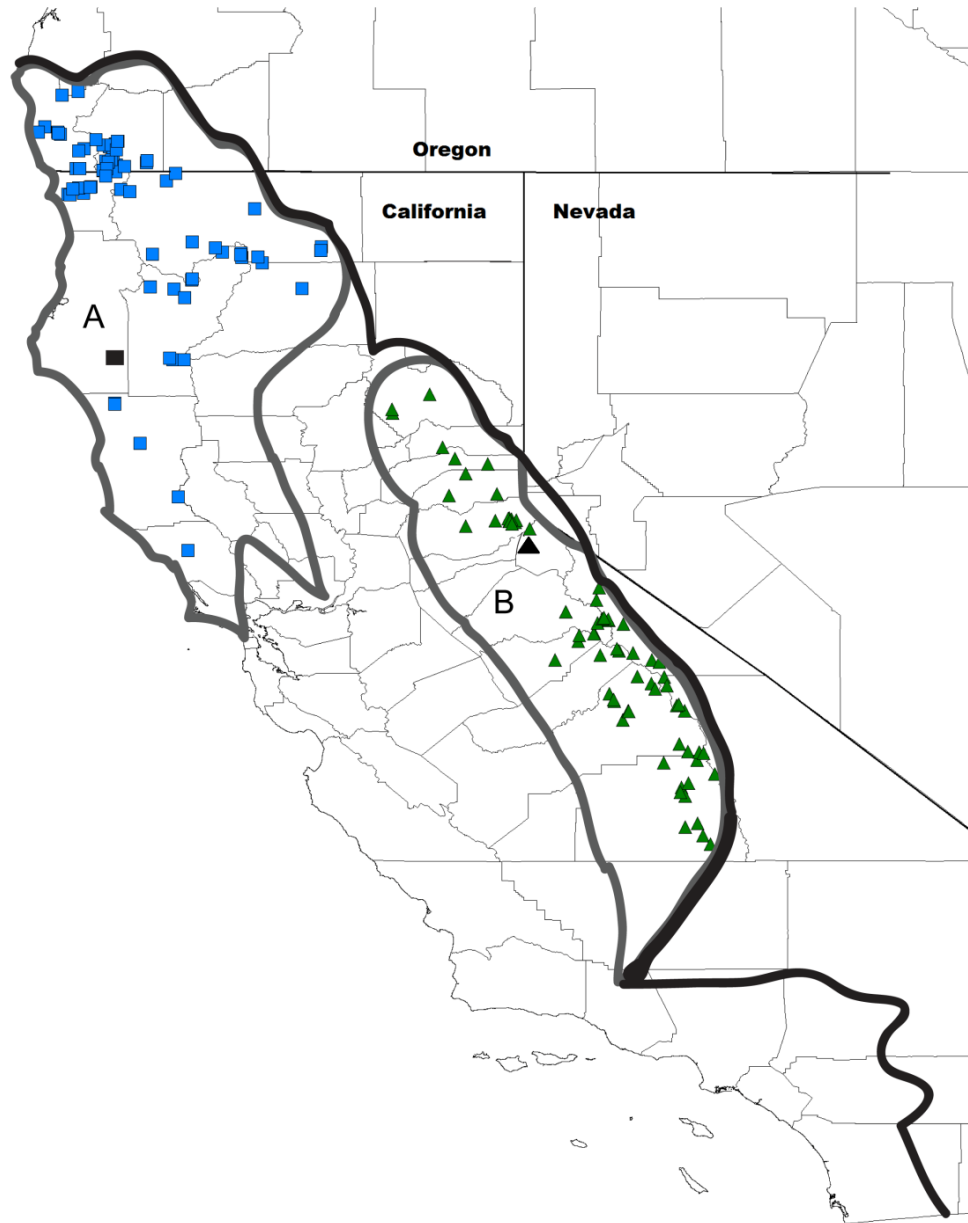


Figure 8. A map of the California Floristic Province, USA, as outlined in black. The outlined area marked “A” is the Klamath-Siskiyou and North Coast mountain ranges and the outlined area marked “B” represents the Sierra Nevada mountain range. Georeferenced specimens of *Calliscirpus criniger* are marked by squares with the type specimen being black. *Calliscirpus brachythrix* specimens are marked by triangles with the type specimen being black.

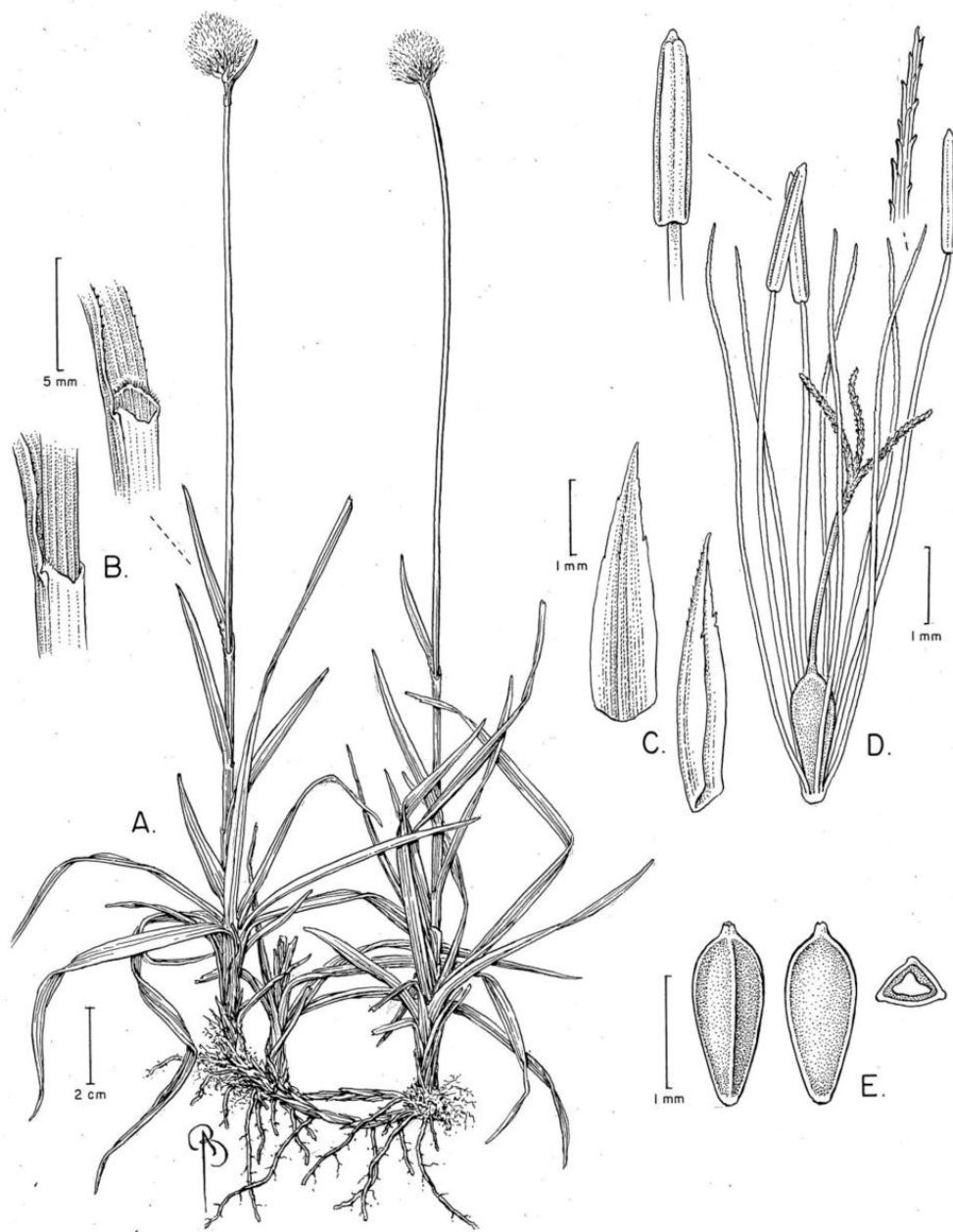


Figure 9. *Calliscirpus brachythrix*. **A** habit; **B** sheath-blade junction, with culm present (left) and removed (right) to reveal fimbriate ligule; **C** Removed floral scales; **D** Floral structures including perianth, pistil, stamen and achene, with closeup of anther and distally scabridulous perianth bristle; **E** Mature achene. From J.R. Starr 07-039 & J. Thibeault (holotype, CAN). Drawn by Bobbi Angell.

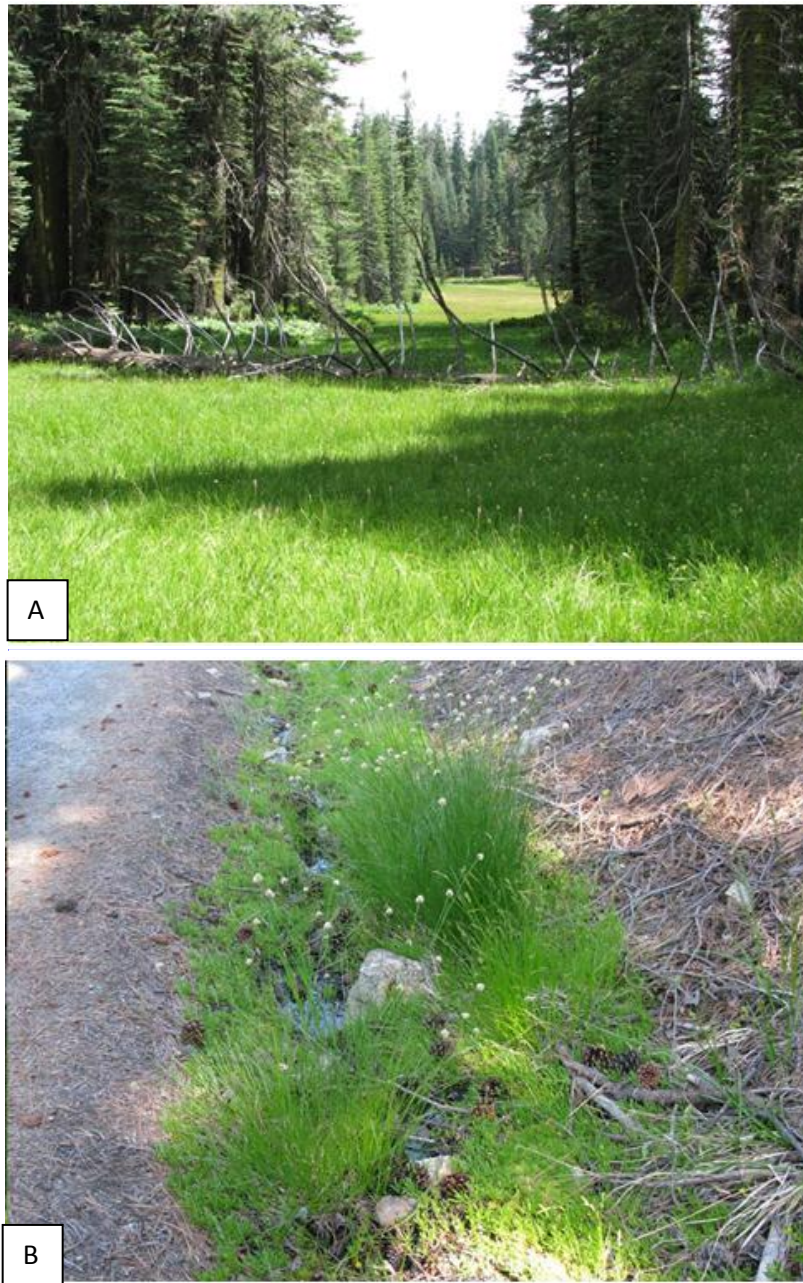


Figure 10. Photographs of a typical habitat of **A:** *Calliscirpus brachythrix* (Starr 07-039 & Thibeault) in Alpine County, California, and **B:** *Calliscirpus criniger* (Starr 105-055 & Villaverde) in Trinity County, California, taken during field collections.

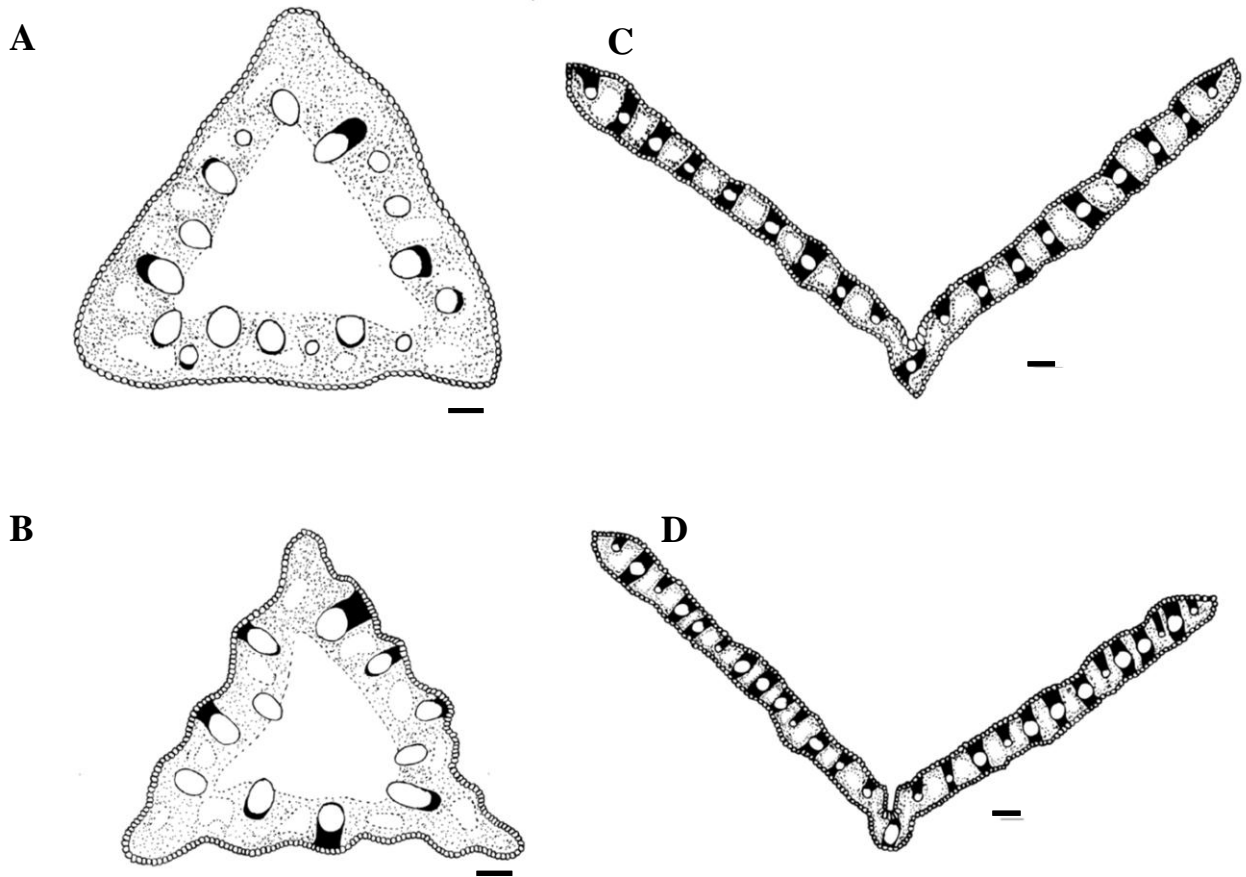


Figure 11. Anatomical drawings of culm and leaf cross sections. **A** *Calliscirpus criniger* (Starr 105-055, CAN), culm cross section; **B** *C. criniger* (Starr 105-055), leaf cross section; **C** *C. brachythrix* (Starr 06-020), culm cross section; **D** *C. brachythrix* (Starr 06-020), leaf cross section. Darkened areas are sclerenchyma, stippled areas are chlorenchyma, stippled bordered spaces are air cavities, continuous circles are vascular bundles, and the dashed line separates the chlorenchyma tissue from the central ground tissue. Scale bar = 100 μm .



Figure 12. Holotype scan of *Calliscirpus brachythrix* before new genus and species was published, hence *Eriophorum* sp. nov. on voucher label.



Figure 13. Holotype scan of *Calliscirpus criniger* specimen, prior to addition of new *Calliscirpus criniger* label. Note the plant and label affixed to the left is a later collection, “Plumas Co., Mrs. R. M. Austin, 1876”. This is an individual of *C. brachythrix*.

Chapter 3: Phylogenetic relationships within the Cariceae + Dulichieae + Scirpeae clade with emphasis on the recently discovered genus *Calliscirpus*

INTRODUCTION

Even within such a diverse and cosmopolitan family as Cyperaceae (ca 5000 spp.), the clade that comprises tribes Cariceae, Dulichieae and Scirpeae is remarkable for its extraordinary diversity (2055 spp.; Govaerts *et al.* 2007) and its nearly global distribution with species common on all continents, except Antarctica. It is also remarkable because almost all its species are found within a single tribe (Cariceae; 1942 spp.), and even within this tribe almost all species are placed in *Carex* (1781 spp.), one of the largest and ecologically significant angiosperm genera (World Checklist of Cyperaceae 2010). Outside of Cariceae, all remaining species in the clade are either distributed among eleven small genera each consisting of a handful of species (1-10) or they are placed in *Eriophorum* (cottongrasses; 17 spp.) or *Scirpus* (club rushes and bulrushes; 66 spp.) (Govaerts *et al.* 2007). Of the three tribes that compose the clade, Dulichieae and Cariceae can be easily defined by morphology (Goetghebeur 1998; Ball & Wujek 2002; Dhooge 2005) and all molecular analyses to date suggest they are monophyletic (Muasya *et al.* 1998; Starr *et al.* 2003; Dhooge 2005; Simpson *et al.* 2007; Muasya *et al.* 2009a, 2009b). Dulichieae can be clearly recognised by its fertile spikelet prophylls and cladoprophylls that bear bisexual flowers, whereas Cariceae is distinguished by its unisexual flowers and perigynia, sac-like prophylls that surround gynoecia (Goetghebeur 1998). In contrast, Scirpeae is much harder to define and appears to be based upon a series of plesiomorphic characters that are common

throughout the family (Goetghebeur 1998; Dhooge 2005). This explains in part why *Scirpus* s.l., a genus previously comprising >200 species has now been divided into more than 50 different genera (Goetghebeur 1998), some of which are placed in distantly related tribes (World Checklist of Cyperaceae 2010; Muasya *et al.* 2012). Although the modern circumscription of *Scirpus* now comprises only 64 species (World Checklist of Cyperaceae 2010; Muasya *et al.* 2012) the recent discovery of *Dracoscirpoides* (Muasya *et al.* 2012), a distantly related South African genus for two former *Scirpus* species (*S. falsus* and *S. ficinioides*) now placed in tribe Cyperaceae, starkly demonstrates that the limits of *Scirpus* are still poorly known. With the exception of *Khaosokia* D.A.Simpson & Chayam, all new genera (*Calliscirpus* C.N.Gilmour, J.R.Starr & Naczi, *Cypringlea* M.T.Strong, and *Zameioscirpus* Dhooge & Goetgh.) discovered within the Cariceae + Dulichieae + Scirpeae clade during past 10 years were segregated from *Scirpus*, and the most difficult problem, the delimitation of *Scirpus* from its possible sister genus *Eriophorum* has yet to be resolved (Dhooge *et al.* 2003; Strong 2003; Simpson *et al.* 2005; Gilmour *et al.* 2013).

Part of the difficulty with circumscribing *Scirpus* is the fact that many intermediate taxa blur the limits of what were once thought to be well understood genera. This is particularly true for *Scirpus* and *Eriophorum*, two genera that can only be separated by their perianth parts. *Scirpus* displays 6 or fewer perianth parts, whereas *Eriophorum* displays 10 or more perianth parts (Vrijdaghs *et al.* 2005; Goetghebeur 1998). In Chapter 2, I examined the morphology, embryology and phylogenetic position of two species that could not be clearly placed in either *Scirpus* or *Eriophorum*, *Scirpus maximowiczii* and *E. crinigerum*. These two taxa were classified as intermediates because of their bristle characteristics. Both taxa display 6 bristles that are barbed, a *Scirpus* character, but both taxa were placed in their

respective genera according to their achene characteristics (Beetle 1942, 1946). However, on the basis of molecular, morphological and embryological evidence, Gilmour (Chapter 2) and Gilmour *et al.* (2013) demonstrated not only that *S. maximowiczii* was strongly supported as a member of a *Scirpus* and *Eriophorum* clade, thus warranting no change in its taxonomy, but that *Eriophorum crinigerum* was distantly related to these genera and best recognised as *Calliscirpus*, a new genus of two species (*C. criniger* and *C. brachythrix*) that was sister to Cariceae.

However, many intermediate species remain including species whose generic affinities are entirely unknown, but have traditionally been placed in Scirpeae. These taxa include *Eriophorum comosum* (Wall.) Nees, *Scirpus asper* J.Presl & C.Presl, and the genera *Karinia* Reznicek & McVaugh and *Cypringlea* M.T.Strong.

Eriophorum comosum has been treated as *Scirpus comosus* Wall., *Trichophorum comosum* (Wall.) A.Dietr., and even *Erioscirpus comosus* (Wall.) Palla (World Checklist 2010). Like a true *Eriophorum*, *E. comosum* displays numerous, long, white, silky bristles (Wu *et al.* 2010), and yet the number of its spikes in a compound anthela suggests a closer affinity to *Scirpus* as species of *Eriophorum* typically have very few spikes per inflorescence (Dai *et al.* 2010). However, *E. comosum* is unusual for *Eriophorum* and *Scirpus* because it does not possess a hybrid *Fimbristylis*-/*Schoenus*-type or *Schoenus*-type embryo; in fact, its embryo morphology suggests an affinity to species in the distantly related tribe Cypereae (Van der Veken 1965). Moreover, *E. comosum* thrives in dry crevice habitats in rocks or on cliffs, a strikingly different habitat from the cool, wet, temperate or arctic locations of most *Eriophorum* and *Scirpus* species (Ball & Wujek 2002; Wu *et al.* 2010). Both characteristics suggest not only that *E. comosum* may not be an *Eriophorum* or a

Scirpus, but it may even be distantly related to species of the Cariceae + Dulichieae + Scirpeae clade.

Scirpus asper has been called *S. glaucus* Nees & Meyen, *S. leptopus* Boeckeler, *S. asper* var. *polystachyus* C.B. Clarke, *S. subasper* Beetle, *Scirpus subasper* var. *diffusus* Beetle, *S. asper* var. *diffusus* (Beetle) Beetle and *S. subasper* var. *polystachyus* (C.B. Clarke) Beetle (World Checklist of Cyperaceae 2010). *Scirpus asper* has been noted to be similar to *Scirpus cubensis* (now *Oxycaryum cubense*) (Macbride 1936) and *Cypringlea analecta* (Beetle 1944). The leaves of *Oxycaryum* are more flaccid, the style bifid and bristles lacking and the genus is now known to be distantly placed in tribe Cypereae on the basis of molecular phylogenetic (Muasya *et al.* 2002) and developmental data (Muasya *et al.* 2009b; Larridon *et al.* 2011). *Scirpus asper* develops a *Schoenus*-type embryo (basal root cap and lateral first leaf), unlike that of a true *Scirpus*, which display a hybrid *Fimbristylis*-/*Schoenus*-type (semi-basal root cap and semi-lateral first leaf) embryo (Van der Veken 1965; Dhooge 2005). These discrepancies cast doubt on the placement of *S. asper* within *Scirpus* and possibly within the Cariceae + Dulichieae + Scirpeae clade itself.

Karinia is a Cyperaceae genus of which little is known. Originally described as *Scirpus mexicana*, the lone taxon *Karinia mexicana* is an endemic Mexican species (Reznicek & McVaugh 1993, 1994; World Checklist of Cyperaceae 2010). *Karinia mexicana* was described as a new genus of Scirpeae on the basis of morphology (Reznicek & McVaugh 1993), but it has subsequently been suggested that it may be best placed in Cypereae (González-Elizondo *et al.* 2007), possibly within the genus *Scirpoides* (Goetghebeur 1998). As *Karinia* has never been included in a molecular analysis and it was previously treated within *Scirpus*, it was deemed important to include it within analyses to

determine whether it might have affinities within the Cariceae + Dulichieae + Scirpeae clade.

An endemic Mexican genus, *Cypringlea* (*C. analecta* = *Scirpus analecta* and *C. coahuilensis* = *Scirpus coahuilensis*), was recently segregated from *Scirpus* based on the *Carex*-type embryo of the two species (Strong 2003). They were placed in their own genus based on habitat, leaf blade morphology and development, inflorescence morphology and rudimentary perianth bristles that were different from any other genus that develops *Carex*-type embryos (Strong 2003). A third species, *Cypringlea evadens* (C.D.Adams) Reznicek & S.González was later added to the genus (Reznicek & González 2008), however, this taxon has never been placed in a molecular analysis. This study is the first time that *Cypringlea* has been included in a molecular analysis to determine its evolutionary relationships within the Cariceae + Dulichieae + Scirpeae clade.

Despite the fact that Cariceae + Dulichieae + Scirpeae clade comprises about 40% of all Cyperaceae species and exhibits all of the ecological, biogeographical, and cytological diversity of sedges, relationships within the clade have never been examined in depth. No analysis has ever included more than 37 terminals outside of Cariceae, and support for relationships based on *rbcL* (Simpson *et al.* 2007) and later *rbcL* plus *trnL* and *rps16* (Muasya *et al.* 2009a) has always been poor. This study has not only thoroughly sampled the Cariceae + Dulichieae + Scirpeae clade using *matK* and *ndhF*, but it has also included taxa whose placement within the clade has never been analyzed molecularly before, such as the genera *Cypringlea* and *Karinia*, and unusual species such as *Eriophorum comosum* (recently published as *Erioscirpus comosus*; Yano *et al.* 2012) and *Scirpus asper*. Moreover, this analysis has also focused on determining the monophyly of *Scirpus*, which has not been

resolved, as well as investigating the sister group to Cariceae. Determining the sister group to this hyperdiverse tribe has significant implications for understanding its evolution, especially morphological adaptation and the role of chromosome fragmentation and fusion in the diversification of the group.

MATERIALS AND METHODS

MOLECULAR ANALYSIS

Outgroup Selection

Species from tribe Abildgaardieae were chosen as the outgroup for all analyses based on the analyses of Muasya *et al.* (1998) and Muasya *et al.* (2009a). These analyses have shown Abildgaardieae as part of the earliest diverging lineage within the sister group to the Cariceae + Dulichieae + Scirpeae clade.

DNA Extraction, Amplification, & Sequencing

Whole genomic DNA was extracted, amplified and sequenced for the cpDNA genes *matK* and *ndhF* as detailed in Chapter 2 (Materials and Methods) the specimens used are outlined in Appendix 1.

PHYLOGENETIC ANALYSIS

Initially, *matK* and *ndhF* were aligned using CLUSTAL X (Thompson *et al.* 1997) then minor adjustments were made manually using parsimony as a criterion for the acceptance or rejection of a proposed base movement (see Starr *et al.* 2004). When a more parsimonious alignment could not be found, both genes were assembled into a

single combined *matK* + *ndhF* matrix for analysis.

The data matrix was analyzed in PAUP* vers. 4.0b10 (Swofford 2002) using heuristic searches (HS) under the criterion of maximum parsimony (MP) with 1 000 000 RANDOM addition-sequence replicates, tree-bisection-reconnection (TBR) branch- swapping and multiple trees saved for each replicate (MULTREES=yes) to produce a strict consensus tree. Clade support was determined using bootstrap (BS; Felsenstein 1985) analyses with 10 000 replicates using a HS strategy, TBR branchswapping and a SIMPLE stepwise addition of taxa with the MULTREES command turned off. This strategy rapidly produces bootstrap support values that are almost identical to the values produced when multiple trees are saved for each replicate (DeBry & Olmstead 2000). The following conditions were used to describe the strength of the bootstrap support: strong 95-100% BS; very well 85-94% BS; well 75-84% BS; moderate 65-74% BS; weak 55-64% BS; and very weak <55% BS (Starr *et al.* 2004; Hillis & Bull 1993).

In order to determine whether the data could statistically reject the hypothesis of *Scirpus* being paraphyletic with respect to *Eriophorum* as well as the hypothesis that *Calliscirpus* may not be sister to Cariceae, constraint tree analyses were performed for the following conditions: 1) *Scirpus* was constrained to be monophyletic , 2) *Calliscirpus*, *Trichophorum*, *Oreobolopsis* and *Cypringlea* were constrained to form a monophyletic group and 3) *Eriophorum*, *Scirpus*, and *Calliscirpus* were constrained to form a clade 4) Cariceae, *Scirpus*, *Eriophorum*, *Zameioscirpus*, *Phylloscirpus*, and *Amphiscirpus* were constrained to form a monophyletic group 5) *Calliscirpus*, *Eriophorum*, *Scirpus*, *Zameioscirpus*, *Phylloscirpus*, and *Amphiscirpus* were constrained into a monophyletic

group and 6) All current taxa included in tribe Scirpeae (*Eriophorum*, *Scirpus*, *Zameioscirpus*, *Phylloscirpus*, *Amphiscirpus*, *Trichophorum*, *Oreobolopsis*, *Cypringlea*, and *Calliscirpus*) were constrained to form a monophyletic group. *Eriophorum comosum* was not constrained to be a part of a monophyletic Scirpeae tribe and *Scirpus asper* and *E. comosum* were not constrained to a part of a monophyletic *Scirpus* and *Eriophorum* clade as preliminary analyses determined that they were not members of this clade. Constraint analyses were performed under the same conditions as the maximum parsimony analyses described above and SH tests were conducted to determine whether differences between constrained and unconstrained analyses were statistically significant using the RELL test distribution by resampling estimated log-likelihood method (10000 replicates; Kishino *et al.* 1990; Shimodaira & Hasegawa 1999). Pairwise comparisons of absolute differences between taxa were conducted in PAUP (DSET DIST=ABS) (Swofford 2002), in order to gauge the number of mutations differentiating major clades within trees.

A Bayesian analysis was performed on the combined data set using MrBayes 3.0b4 (Ronquist 2003). Model selection was performed on each cpDNA locus separately using MrModeltest v.2 (Nylander 2004) and the Akaike Information Criterion (AIC; Akaike 1974) which provides an objective criterion for choosing the evolutionary model that best fits the data. Results suggested that a General Time Reversible + Gamma + Proportion Invariant (GTR + G + I) model of nucleotide substitution was the most appropriate model for both *matK* and *ndhF* (Zuur *et al.* 2009). Given that the same model was chosen for *matK* and *ndhF* and that both loci are linked and part of a genome that rarely undergoes recombination (Chiu & Sears 1985), a mixed model was not applied to the dataset in order to minimise the variance associated with estimating a large number of parameters. Four

Markov Monte Carlo Chains were run simultaneously in each Bayesian analysis for 5 000 000 generations with trees saved every 1000 generations. The first 100 000 trees were discarded because they were not sampled from the stationary phase (i.e., the posterior distribution). The remaining trees were summarized in a majority rule consensus tree with posterior probabilities (PP) used to evaluate clade support.

EMBRYOLOGICAL ANALYSIS

Embryographs of taxa throughout the Cariceae + Dulichieae + Scirpeae clade were extracted from previous studies (Chapter 2; Gilmour *et al.* 2013; Strong 2003; Van der Veken 1965; Dhooge 2005; Goetghebeur 1989). A character analysis was performed using the Bayesian tree (analysis performed as described above) and a matrix composed of the known embryo characters in MESQUITE 2.75 using the Mk1 (Markov k-state 1) model where the single parameter is the rate of change and any particular change is equally probable (Maddison & Maddison 2011) (Figure 16).

RESULTS

PHYLOGENETIC ANALYSIS

Phylogenetic reconstructions of the chloroplast matrix using parsimony (66 taxa, 2183 characters: 1120 *matK*, 1063 *ndhF*, 1483 characters constant, 455 parsimony-informative characters, tree length=1298, CI=0.58, RI=0.82) produced a fairly resolved strict consensus tree (Figure 14). Analyses placed a strongly supported Dulichieae sister to all remaining members of the Cariceae + Dulichieae + Scirpeae clade followed by the monotypic *Khaosokia* as sister to five distinct clades whose monophyly was 82% supported but whose relationships among themselves were only poorly resolved. Analyses recovered five distinct monophyletic groups in addition to the known monophyletic Dulichieae group: 1) the genus *Calliscirpus* (100% BS), 2) tribe Cariceae (100% BS), 3) *Trichophorum* + *Cypringlea* + *Oreobolopsis* (100% BS), 4) *Zameioscirpus* + *Phylloscirpus* + *Amphiscirpus* (69% BS) and 5) *Eriophorum* + *Scirpus* (99% BS). *Calliscirpus* and Cariceae formed a monophyletic group that was sister to a strongly supported *Trichophorum* + *Cypringlea* + *Oreobolopsis* clade. The *Zameioscirpus* + *Phylloscirpus* + *Amphiscirpus* clade was sister to the *Eriophorum* + *Scirpus* clade, although support for this relationships was weak (0.85 Bayesian; <50% Bootstrap). *Scirpus asper* was found to be strongly positioned within the *Zameioscirpus* + *Phylloscirpus* + *Amphiscirpus* clade whereas *Eriophorum comosum* appears to be distantly related to Cariceae + Dulichieae + Scirpeae species and is more closely related with *Karinia* to elements in tribe Cyperae such as the genus *Isolepis*.

The results of the constraint analyses are presented in Table 6. The constraint analysis that tested whether *Scirpus* was paraphyletic with respect to *Eriophorum* by forcing *Scirpus*

to be monophyletic (excluding *S. asper* and *E. comosum*) could not reject the possibility of a monophyletic *Scirpus* ($p > 0.05$). The constraint analysis for the monophyly for *Calliscirpus*, *Trichophorum*, *Oreobolopsis* and *Cypringlea* was also found to not be significant ($p > 0.05$). The constraint analysis that included *Eriophorum*, *Scirpus*, and *Calliscirpus* within a clade was found to be significant ($p\text{-value} = 0.001$), and could therefore be rejected. The constraint analysis that for the monophyly of Cariceae, *Eriophorum*, *Scirpus*, *Amphiscirpus*, *Phylloscirpus*, and *Zameioscirpus* was found to be significant ($p\text{-value} = 0.001$) and could be rejected. The constraint analysis for the monophyly of *Calliscirpus*, *Scirpus*, *Eriophorum*, *Amphiscirpus*, *Phylloscirpus*, and *Zameioscirpus* was found to be significant ($p\text{-value} = 0.001$) and could be rejected. The final constraint analysis constraining all taxa of Scirpeae (*Eriophorum*, *Scirpus*, *Amphiscirpus*, *Phylloscirpus*, *Zameioscirpus*, *Calliscirpus*, *Cypringlea*, *Oreobolopsis*, and *Trichophorum*) was found to be significant ($p\text{-value} = 0.001$) and could be rejected.

The absolute distances between the five major clades within the Cariceae + Dulichieae + Scirpeae clade ranged from 40 and 78 differences (Table 5). The smallest distance (40) was between *Calliscirpus* and *Trichophorum* + *Cypringlea* + *Oreobolopsis*, whereas the distance between *Calliscirpus* and Cariceae was 68. The largest distance (78) was between Cariceae and the *Zameioscirpus* + *Phylloscirpus* + *Amphiscirpus* clade.

The simplest model of sequence evolution that best fit *matK* and *ndhF* was GTR + G + I. The first 100 000 trees were discarded in the Bayesian analysis of the combined chloroplast matrix (2183 bp, 66 taxa) because they had not reached stationarity. The Bayesian majority rule consensus tree, derived from the combined analysis, is consistent with the strict consensus tree of MP analysis. The five distinct monophyletic groups

(*Calliscirpus*, *Carex* + *Kobresia*, *Trichophorum* + *Cypringlea* + *Oreobopsis*, *Zameioscirpus* + *Phylloscirpus* + *Amphiscirpus* and *Eriophorum* + *Scirpus*) recovered in parsimony analyses were also present in the Bayesian tree. The *Calliscirpus* group was found to be sister to the Cariceae clade, with a strong posterior probability of 0.83. *Scirpus asper* was found to group strongly (1.00 posterior probability) with the *Zameioscirpus* + *Phylloscirpus* + *Amphiscirpus* clade, whereas *Eriophorum comosum*, as well as *Karinia* were resolved outside of the Cariceae + Dulichieae + Scirpeae clade (Figure 15).

EMBRYOGRAPHY

The embryos of Cyperaceae species, representing the diversity found in the Cariceae + Dulichieae + Scirpeae clade along with the outgroup species from Abildgaardieae are displayed in Figure 16. Three embryo types are present within the clade: *Carex*-type, *Schoenus*-type and a hybrid *Fimbristylis*-/*Schoenus*-type hybrid (Van der Veken 1965; Goetghebeur 1986, Dhooge 2005). The character analysis suggests that the *Carex*-type embryo is most likely plesiomorphic state for the tribe with both the *Schoenus*- and hybrid *Fimbristylis*-/*Schoenus*-type embryos representing derived character states (Figure 16) that support the monophyly of the *Eriophorum* + *Scirpus* and the *Amphiscirpus* + *Phylloscirpus* + *Zameioscirpus* clades.

DISCUSSION

INTERMEDIATE SPECIES

The relationships of the intermediate species (*Eriophorum comosum*, *Scirpus asper*, and *Cypringlea* spp.) within the Cariceae + Dulichieae + Scirpeae clade have been somewhat resolved through the molecular analysis. Results indicate that *Eriophorum comosum* is not a member of the Cariceae + Dulichieae + Scirpeae clade, but appears to be most closely related to elements within tribe Cyperae, which is consistent with its *Cyperus*-type embryo morphology (Van der Veken 1965). Owing to poor taxonomic sampling outside of the Cariceae + Dulichieae + Scirpeae clade, the exact positioning of *E. comosum* within Cyperaceae is unclear in this analysis, but it can be stated that *E. comosum* is not a true *Eriophorum*, nor is it a member of the Cariceae + Dulichieae + Scirpeae clade. This corroborates the analysis of Yano *et al.* (2012) in suggesting that *E. comosum* is a member of tribe Cyperae and best treated in a separate genus as *Erioscirpus comosus* (Wall.) Palla.

According to my analysis, *Scirpus asper* is not a member of the genus *Scirpus*. Given that it resolved strongly (100%, 1.00) within the *Zameioscirpus* + *Phylloscirpus* + *Amphiscirpus* clade and it possesses a *Schoenus*-type embryo (Van der Veken 1965), a synapomorphy for this group, it would appear that *S. asper* is most closely related to these taxonomically difficult genera and may warrant recognition at the generic level. Morphologically, however, *Scirpus asper* does not fit well within this clade as unlike the other genera it is a large plant (20 – 40 cm) with an anthelate inflorescence of numerous spikelets (~10) whereas the other genera have very few spikelets and are typically highly

reduced (*Zameioscirpus* & *Phylloscirpus*: <12 cm; Dhooge *et al.* 2003; Dhooge & Goetghebeur 2004). *Amphiscirpus nevadensis* is an exception in the clade with a size between 10-70 cm and multiple spikelets, though its inflorescence is never anthelate in form (Ball & Wujek 2002). Although this might suggest that the placement of *S. asper* within this clade might be erroneous, the pattern of highly compound and reduced species being found in the same clade is in fact common throughout the Cariceae + Dulichieae + Scirpeae clade. For example, the genus *Cypringlea* may also have anthelate inflorescences and as many as 90 spikelets, whilst other members of the clade such as *Trichophorum cespitosum* can have a single spikelet and as few as three flowers (Ball & Wujek 2002; Strong 2003). Likewise, the most compound and reduced inflorescences in Cariceae are found in the same Caricoid clade (Starr & Ford 2009). Given that only a single sample of *S. asper* was examined in the current study, more samples and a more thorough study into the placement of *S. asper* is required to exclude the possibility of a laboratory error (e.g., contamination, mislabelling) and to determine whether it is best placed in an existing genus in this clade or may in fact be a new genus itself.

The constraint analysis testing the monophyly of Scirpeae was rejected. This means that the Scirpeae tribe (*Eriophorum*, *Scirpus*, *Amphiscirpus*, *Phylloscirpus*, *Zameioscirpus*, *Calliscirpus*, *Cypringlea*, *Oreobolopsis*, and *Trichophorum*) is probably paraphyletic.

Like many species previously treated in *Scirpus*, such as those from the recently named genus *Dracoscirpoides* (Muasya *et al.* 2012; tribe Cypereae), results suggest that *Karinia mexicana* is distantly related to *Scirpus* s.s. In both MP and Bayesian analyses, *Karinia mexicana* was completely removed from the Cariceae + Dulichieae + Scirpeae clade and strongly placed within a clade that includes *Eriophorum comosum* (= *Erioscirpus*

comosus) and *Isolepis aucklandica*, a genus firmly placed in tribe Cyperae in all previous molecular analyses (Muasya *et al.* 2001). As the focus of this research was on the Cariceae + Dulichieae + Scirpeae clade, taxonomic sampling outside the group was very limited and thus the sister group relationships of this unusual genus remain to be discovered. Although its position next to *Isolepis* and its *Cyperus*-type embryo would suggest a terminal placement somewhere in Cyperae, a detailed molecular analysis at the family level is necessary to determine its phylogenetic position within the Cyperaceae.

Molecular analyses confirm the original belief of Strong (2003) that *Cypringlea* is closely related to *Trichophorum* as their perfect flowers and *Carex*-type embryos would suggest. Nonetheless, relationships within the *Trichophorum* group are poorly supported and additional research will be needed to fully resolve the relationships and limits of the genera (*Trichophorum*, *Cypringlea*, and *Oreobolopsis*) in this clade. However, it is noteworthy that *Trichophorum* does not resolve as a monophyletic group, especially as the type for the genus, *T. alpinum*, is found as sister to *T. subcapitatum* and separate from all other species in the genus. These results suggest the possibility that *Trichophorum* may need to be divided into at least two separate genera.

SCIRPUS-ERIOPHORUM

Molecular analyses indicate that *Scirpus* and *Eriophorum* form a strongly supported clade with *Scirpus* paraphyletic with respect to a monophyletic *Eriophorum*. This close relationship is furthermore supported by embryo types: both *Scirpus* and *Eriophorum* display hybrid *Fimbristylis*-/*Schoenus*-type embryos which are not seen in any other genera in the

Cariceae + Dulichieae + Scirpeae clade (Dhooge 2005; Van der Veken 1965). This is consistent with the findings of Goetghebeur (1998) that closely related genera develop the same embryo type (Goetghebeur 1998) and it is consistent with the fact that the many closely (e.g., *Calliscirpus*; Chapter 2; Gilmour *et al.* 2013) and distantly (e.g., *Dracoscirpoides*; Muasya *et al.* 2012) related genera recently segregated from *Scirpus* do not possess these embryo types.

Although *Scirpus* was paraphyletic with respect to a monophyletic *Eriophorum* (93% BS) in this analysis, statistical support for this hypothesis was poor, and topological tests suggest that the data cannot reject the possibility that *Scirpus* and *Eriophorum* may yet prove to be natural sister genera. A considerable increase in taxonomic sampling combined with further molecular markers will be required to determine whether these two genera should be treated as separate or merged.

Within *Eriophorum*, the relationships of several of the strongly supported clades correspond to species groups distinguished in dichotomous keys for the Flora of North America (Ball & Wujek 2002). Taxa that usually develop two or more spikelets (*Eriophorum virginicum*, *E. tenellum*, and *E. gracile*) are found to resolve in one strongly supported clade (94% BS) whereas those that develop solitary spikelets are found in another (*E. scheuchzeri*, *E. chamissonis*, *E. russeolum*, *E. brachyantherum*, *E. callitrix* and *E. vaginatum*), but with two multispicate species *E. viridicarinatum* and *E. latifolium* in a basal grade. This topology suggests that the ancestor of *Eriophorum* was multispicate and it furthermore suggests that unispicate species evolved from multispicate species by reduction. As only 11 of the 17 species of *Eriophorum* were sampled in this study, a full understanding

of evolution with the genus awaits a study with a more complete taxonomic sampling of the genus.

CARICEAE SISTER GROUP

The chloroplast DNA analysis of *matK* and *ndhF* produced a fairly well resolved tree, but several branches with important taxonomic and evolutionary implications displayed very poor support. In particular, the genus *Calliscirpus* was resolved as sister to Cariceae, and part of a clade that includes a monophyletic group consisting of the genera *Trichophorum*, *Oreobolopsis* and *Cypringlea*. This monophyletic group was topologically tested using constraint analyses and the *Cariceae*, *Calliscirpus*, *Oreobolopsis*, *Trichophorum* and *Cypringlea* clade could not be rejected. However, the monophyly of an *Eriophorum*, *Scirpus*, *Amphiscirpus*, *Phylloscirpus*, *Zameioscirpus*, and Cariceae group and the *Calliscirpus*, *Scirpus*, *Eriophorum*, *Amphiscirpus*, *Phylloscirpus*, and *Zameioscirpus* group could both be rejected. Although this is the first time that the sister to Cariceae has resolved in an analysis comprising all the known genera in Cariceae + Dulichieae + Scirpeae clade support for this relationship was poor, and topological tests could not reject the possibility that *Calliscirpus* could also be a member of the *Trichophorum*, *Oreobolopsis* and *Cypringlea* clade. In fact, the absolute distances calculated during pairwise comparisons showed a smaller distance between *Calliscirpus* and the *Trichophorum* + *Oreobolopsis* + *Cypringlea* (40) clade than the distance between Cariceae and *Calliscirpus* (68). Moreover, embryo types are not useful for discerning relationships in this case as not only do they all share a *Carex*-type embryo, but this embryo type was determined to be plesiomorphic for the tribe in character analyses. However, constraint analyses reject the hypothesis that Cariceae could be sister to an

Eriophorum + *Scirpus* clade or to an *Eriophorum* + *Scirpus* + *Phylloscirpus* + *Amphiscirpus* + *Zameioscirpus* clade suggesting that the most likely sisters to Cariceae are either *Calliscirpus* or the *Trichophorum* + *Oreobolopsis* + *Cypringlea* (40) clade.

TABLES AND FIGURES

Table 5. Absolute distances between clades as calculated through pairwise comparison analysis in PAUP.

Clade 1	Clade 2	Absolute Distance Between Clades
<i>Calliscirpus</i>	Cariceae	68
<i>Calliscirpus</i>	<i>Trichophorum</i> + <i>Cypringlea</i> + <i>Oreobolopsis</i>	40
<i>Calliscirpus</i>	<i>Zameioscirpus</i> + <i>Phylloscirpus</i> + <i>Amphiscirpus</i>	43
Cariceae	<i>Scirpus</i> + <i>Eriophorum</i>	71
Cariceae	<i>Trichophorum</i> + <i>Cypringlea</i> + <i>Oreobolopsis</i>	69
Cariceae	<i>Zameioscirpus</i> + <i>Phylloscirpus</i> + <i>Amphiscirpus</i>	78
<i>Scirpus</i> + <i>Eriophorum</i>	<i>Calliscirpus</i>	42
<i>Scirpus</i> + <i>Eriophorum</i>	<i>Trichophorum</i> + <i>Cypringlea</i> + <i>Oreobolopsis</i>	60
<i>Scirpus</i> + <i>Eriophorum</i>	<i>Zameioscirpus</i> + <i>Phylloscirpus</i> + <i>Amphiscirpus</i>	50
<i>Trichophorum</i> + <i>Cypringlea</i> + <i>Oreobolopsis</i>	<i>Zameioscirpus</i> + <i>Phylloscirpus</i> + <i>Amphiscirpus</i>	60

Table 6. Constraint analyses calculated with constrained monophyletic trees. P-values were calculated during the analysis in PAUP.

Monophyletic Constraint	p-value
<i>Scirpus</i>	0.449
<i>Calliscirpus</i> , <i>Trichophorum</i> , <i>Oreobolopsis</i> , <i>Cypringlea</i>	0.382
<i>Calliscirpus</i> , <i>Scirpus</i> , <i>Eriophorum</i>	0.001
Cariceae, <i>Eriophorum</i> , <i>Scirpus</i> , <i>Zameioscirpus</i> , <i>Phylloscirpus</i> , <i>Amphiscirpus</i>	0.001
<i>Calliscirpus</i> , <i>Eriophorum</i> , <i>Scirpus</i> , <i>Zameioscirpus</i> , <i>Phylloscirpus</i> , <i>Amphiscirpus</i>	0.001
Scirpeae (<i>Calliscirpus</i> , <i>Trichophorum</i> , <i>Cypringlea</i> , <i>Zameioscirpus</i> , <i>Phylloscirpus</i> , <i>Amphiscirpus</i> , <i>Eriophorum</i> , <i>Scirpus</i>)	0.001

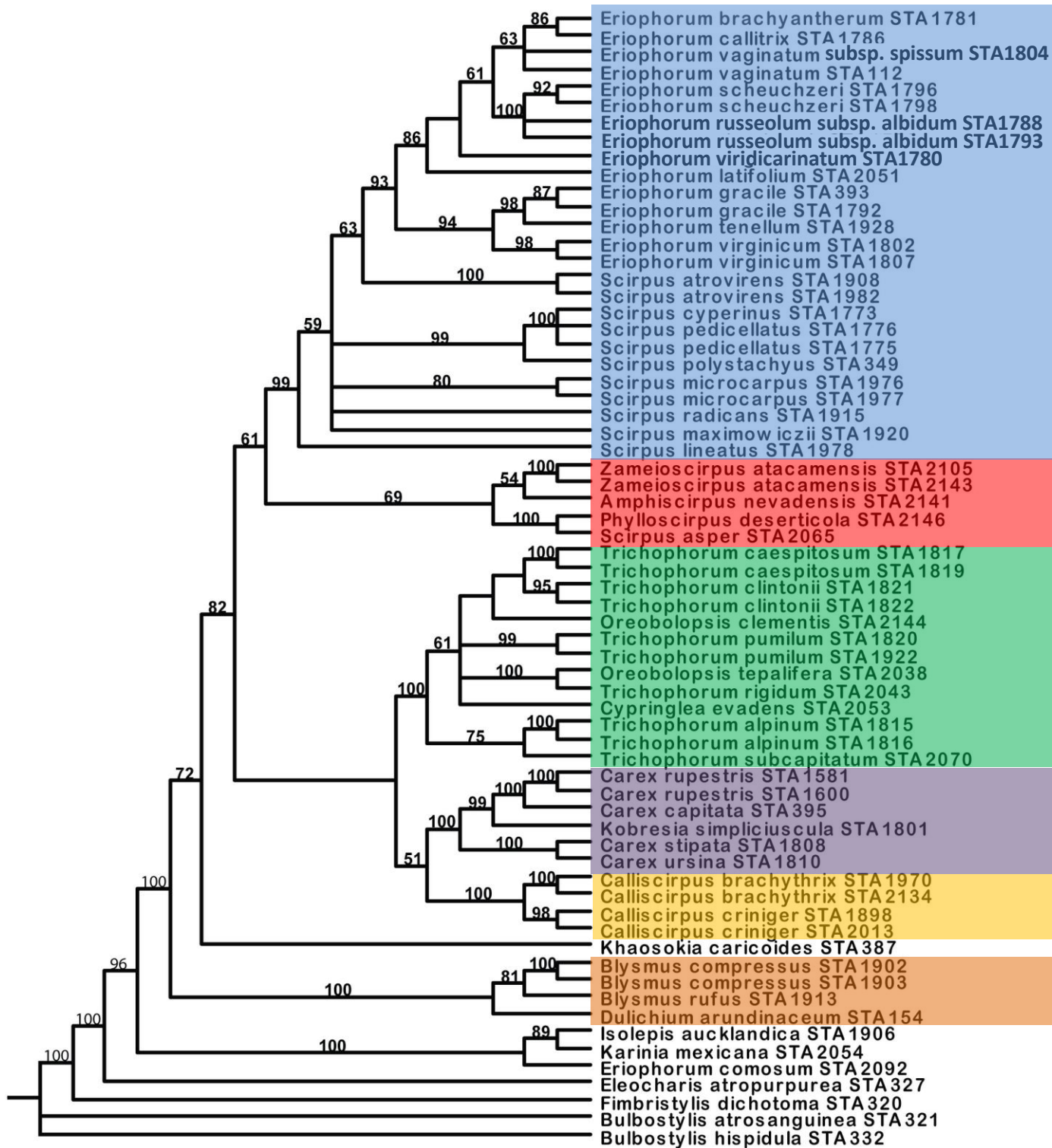


Figure 14. The strict consensus tree recovered during the combined cpDNA (*matK* + *ndhF*) parsimony analysis of the thoroughly sampled Cariceae + Dulichieae + Scirpeae clade. Bootstrap support values >50% are given above branches. Major clades are outlined in coloured boxes.

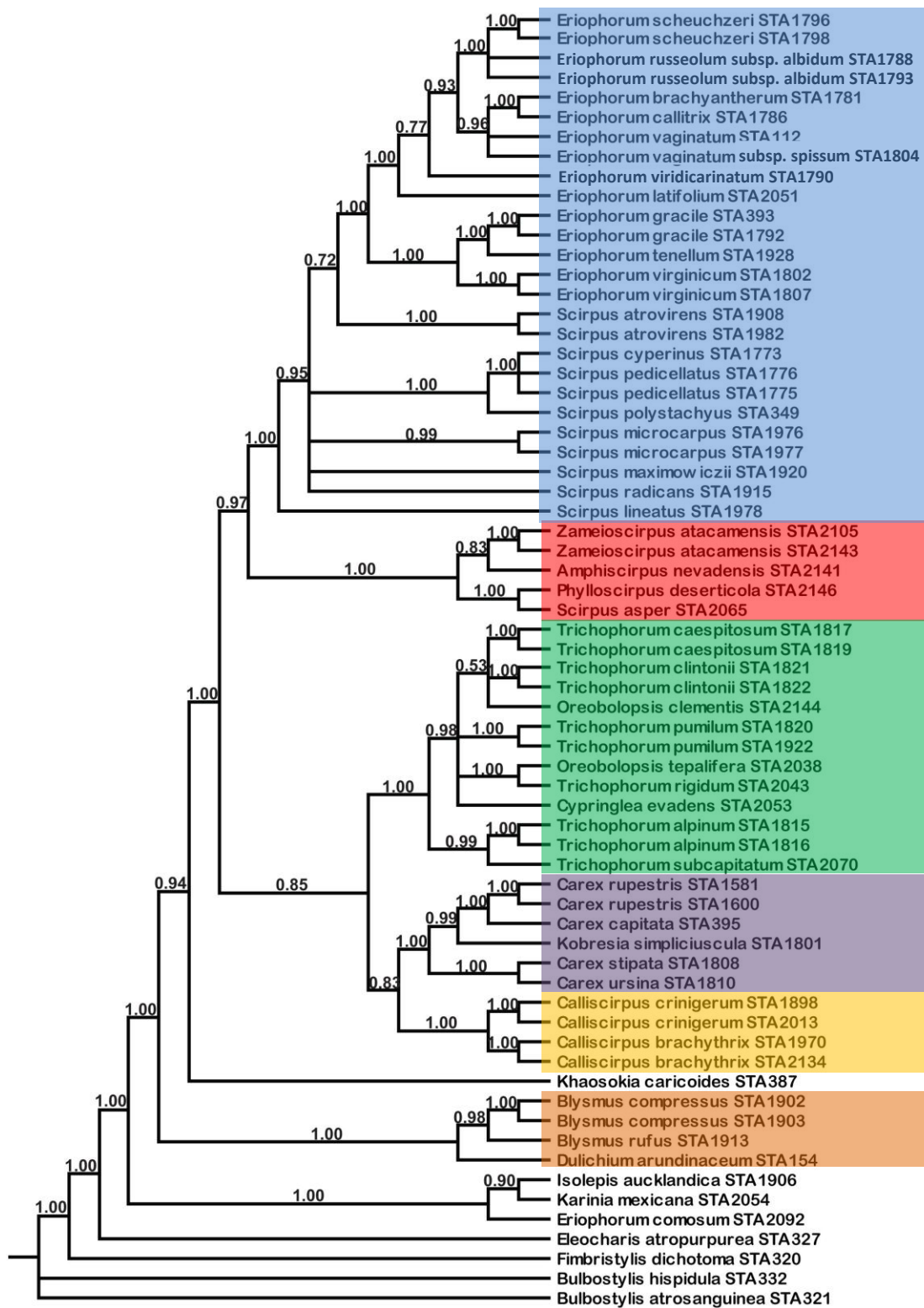


Figure 15. The strict consensus recovered during the combined cpDNA (*matK* + *ndhF*) Bayesian analysis of the Cariceae + Dulichieae + Scirpeae clade. Posterior probability values >0.50 are given above branches. Major clades are outlined in coloured boxes.

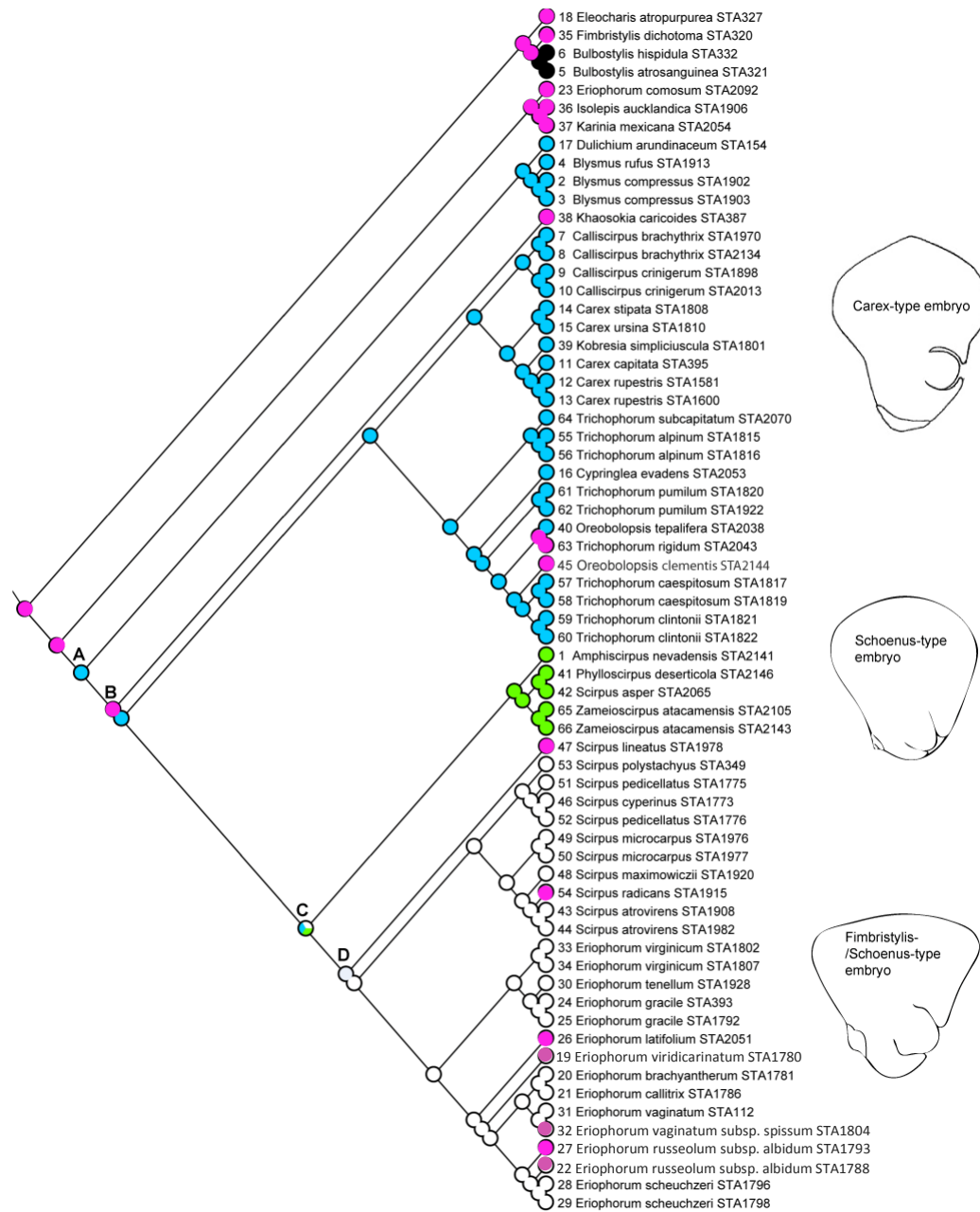


Figure 16. Embryo tree resolved through the character analysis based on the Bayesian tree using MESQUITE. Black: *Bulbostylis*-type embryo; Blue: *Carex*-type embryo; Green: *Schoenus*-type embryo; White: *Fimbristylis*-/*Schoenus*-type embryo; Pink: Embryo type unknown. Likelihood at node A: 0.975 *Carex*-type embryo; 0.016 *Bulbostylis*-type embryo; 0.0045 *Schoenus*-type embryo; 0.0045 *Fimbristylis*-/*Schoenus*-type embryo. Likelihood at node B: 0.973 *Carex*-type embryo; 0.007 *Bulbostylis*-type embryo; 0.01 *Schoenus*-type embryo; 0.01 *Fimbristylis*-/*Schoenus*-type embryo. Likelihood at node C: 0.326 *Carex*-type embryo; 0.006 *Bulbostylis*-type embryo; 0.334 *Schoenus*-type embryo; 0.334 *Fimbristylis*-/*Schoenus*-type embryo. Likelihood at node D: 0.004 *Carex*-type embryo; 0.004 *Schoenus*-type embryo; 0.992 *Fimbristylis*-/*Schoenus*-type embryo.

Chapter 4: Conclusions

Although the polyphyletic genus *Scirpus* L. s.l. (formerly >200 species) has been divided into more than 50 separate genera and now consists of only 64 species, its circumscription has been problematical. Three new genera have been segregated from *Scirpus* s.s. in the past decade, and the delimitation of *Scirpus* from its possible sister genus *Eriophorum* L. (c. 18 species) is still unresolved. The primary character used to delimit *Eriophorum* from *Scirpus*, both of which develop hybrid *Fimbristylis*-/ *Schoenus*-type embryos, is ≥ 10 smooth, elongate perianth bristles vs \leq six short, serrulate bristles or a lack of bristles, but some species display character combinations that make it difficult to place them in either genus. *Eriophorum crinigerum* (A. Gray) Beetle (= *Scirpus criniger* A. Gray), endemic to the California Floristic Province, USA, is one such species, possessing a unique combination of usually 6 barbed bristles and a *Carex*-type embryo (Chapter 2; Gilmour *et al.* 2013) that suggest affinities with both *Scirpus* and *Eriophorum*. In Chapter 2 I used molecular (*matK*, *ndhF*), morphological, and embryological data to demonstrate that *Eriophorum crinigerum* is not closely related to *Scirpus* or *Eriophorum*, but represents a new generic lineage, called *Calliscirpus* C. N. Gilmour, J. R. Starr, & Naczi (tribe Scirpeae). Within the genus, molecular (*matK*, ETS 1f) and morphological data strongly support the recognition of two species, each of which is restricted to a distinct region of high endemism. *Calliscirpus criniger* (A. Gray) C. N. Gilmour, J. R. Starr, & Naczi comb. nov. is common to the Klamath-Siskiyou and North Coast mountain ranges of Oregon and California and develops wider floral scales and longer ligule hairs, than the new species, *Calliscirpus brachythrix* C. N. Gilmour, J. R. Starr, & Naczi, which is unique to the Sierra Nevada

mountain range of California and develops narrower floral scales and shorter ligule hairs.

Scirpus maximowiczii, another transitional species that has blurred the limits of *Scirpus* and *Eriophorum*, and has been cited as morphologically similar to *Calliscirpus* species in the past, is strongly placed within a *Scirpus* s.s. + *Eriophorum* s.s clade (100% bootstrap) on the basis of molecular and embryological data. Any similarity between *Calliscirpus* and *S. maximowiczii* is therefore not due to recent common ancestry. Tree support and taxonomic sampling in Chapter 2 were not sufficient to resolve the delimitation of *Eriophorum* s.s. from *Scirpus* s.s.

Given the recent descriptions of many new taxa within the Cariceae + Dulichieae + Scirpeae clade (*Calliscirpus*, *Zameioscirpus*, *Cypringlea*), a re-evaluation of relationships within the clade with increased taxonomic sampling was undertaken in Chapter 3 using molecular and embryological data. Results suggested that the Cariceae + Dulichieae + Scirpeae clade can be divided into five major lineages, an *Eriophorum* and *Scirpus* clade (Scirpeae), a *Zameioscirpus*, *Amphiscirpus*, *Phylloscirpus* and *Scirpus asper* clade, a *Trichophorum*, *Oreobolopsis*, and *Cypringlea* clade, a *Carex*, *Kobresia*, and *Calliscirpus* clade, and a *Blysmus* and *Dulichium* clade. *Khaosokia caricoides* is currently recognized as its own lineage, however, its evolutionary relationships have yet to be thoroughly studied. *Calliscirpus* was found to be distantly related to *Eriophorum* and *Scirpus*, but sister to Cariceae. Although there is poor molecular support along the backbone of the tree, these relationships are supported by embryological data. *Calliscirpus* develops the *Carex*-type embryo along with *Carex* and *Kobresia*. However, *Scirpus* and *Eriophorum* develop the hybrid *Fimbristylis*-/*Schoenus*-type embryo, supporting the evolutionary distance between these two genera and *Calliscirpus*.

In particular, the relationships regarding *Calliscirpus* to Cariceae as well as to *Eriophorum* and *Scirpus* were of interest for not only placing *Calliscirpus* within the clade, but also of the possibility that it could be sister to Cariceae. In the study I used molecular and embryological data to determine and support evolutionary relationships within the clade. Molecular DNA markers (*matK*, *ndhF*) and embryological data strongly suggested a distant relationship between *Calliscirpus* and *Eriophorum* and *Scirpus*. Shimodaira-Hasegawa tests using constraint trees suggested the molecular data could not reject the hypothesis of a monophyletic *Scirpus* clade (p-value = 0.449) or a monophyletic *Calliscirpus*, *Trichophorum*, *Oreobolopsis*, and *Cypringlea* clade (p-value = 0.382). Constraint analyses constructed using the molecular data supported the hypotheses of a monophyletic *Scirpus* clade, however the monophyly vs. paraphyly of *Scirpus* could not be resolved. Additional research will be required to determine the true evolutionary relationships between *Scirpus* and *Eriophorum* as well as the sister group to Cariceae given that the Cariceae + Dulicheae + Scirpeae clade was not fully resolved and the backbone of the clade is very weakly supported (< 50% bootstrap). A character analysis using the maximum likelihood analysis of the embryo types strongly suggested (0.975 likelihood) the *Carex*-type embryo as ancestral for the tribe with the hybrid *Fimbristylis*-/*Schoenus*-type and *Schoenus*-type embryos representing derived types. The embryological data supports an *Eriophorum* and *Scirpus* clade (*Fimbristylis*-/*Schoenus*-type embryo) and a *Zameioscirpus*, *Phylloscirpus*, and *Amphiscirpus* clade (*Schoenus*-type embryo). Both *Eriophorum comosum* and *Karinia*, two poorly studied taxa that were possibly members of the Cariceae + Dulicheae + Scirpeae clade were removed from the clade, requiring additional research to determine their relationships within the Cyperaceae family.

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Appendix 1: Specimens used in Chapter 3 for DNA Analysis

Taxon	Count.	Prov/State	Locality	Herb.	Accession #	Collector & #	Collection Date	DNA ID
<i>Amphiscirpus nevadensis</i>	Canada	Saskatchewan		CAN	574006	Hudson 5177	Sep. 9 1994	STA2141
<i>Blysmus compressus</i>	-	-	-	CAN	376441	Kotowicz 871	1968	STA1902
<i>Blysmus compressus</i>	-	-	-	CAN	329258	-	1962	STA1903
<i>Blysmus rufus</i>	-	-	-	CAN	363006	Jokela	Aug. 9 1958	STA1913
<i>Bulbostylis atrosanguinea</i>	Kenya	-	-	K	-	Muasya 1037	-	STA321
<i>Bulbostylis hispidula</i>	Kenya	-	-	K	-	Muasya 1025	-	STA332
<i>Calliscirpus brachythrix</i>	U.S.A.	California	Alpine County	CAN	-	Starr & Thibeault	2007	STA1970
<i>Calliscirpus brachythrix</i>	U.S.A.	California	Sierra County	CHSC	58530	Ahart & Oswald 5099	Jul. 12 1992	STA2134
<i>Calliscirpus criniger</i>	U.S.A.	California	Del Norte County	DAO	249788	Tracy 9380	May 10 1931	STA1898
<i>Calliscirpus criniger</i>	U.S.A.	Oregon	Josephine County	CAN	-	Starr 10S-056	2010	STA2013
<i>Carex capitata</i>	-	-	-	CAN	-	Starr 06-016	-	STA395
<i>Carex rupestris</i>	U.S.A.	Colorado	-	CAN	-	Starr 10S-029	Jul. 24 2010	STA1581

Taxon	Count.	Prov/State	Locality	Herb.	Accession #	Collector & #	Collection Date	DNA ID
<i>Carex rupestris</i>	Montenegro	-	Durmitor National Park	-	-	Mejias 139PJM10	Jul. 16 2010	STA1600
<i>Carex stipata</i>	Canada	Ontario	-	CAN	581060	Dugal & Camfield 3728	May 23 1991	STA1808
<i>Carex ursina</i>	Denmark	Greenland	-	CAN	18341	Porsild 8828	Aug. 8 1943	STA1810
<i>Cypringlea evadens</i>	Mexico	Guerrero	-	MICH	1138001	Rawlins & Sholes 2830	Jun. 30 1982	STA2053
<i>Dulichium arundinaceum</i>	Canada	Manitoba	-	FHO	-	Ford, Punter, & Stewart 94233	-	STA154
<i>Eleocharis atropurpurea</i>	Kenya	-	-	EA	-	Muasya et al. 725	-	STA327
<i>Eriophorum brachyantherum</i>	Canada	British Columbia	-	CAN	411873	Gillett & Boudreau 17512	Jul. 27 1977	STA1781
<i>Eriophorum callitrix</i>	Canada	Northwest Territories	-	CAN	28108	Porsild & Porsild 4753	Jun. 7 1928	STA1786
<i>Eriophorum comosum</i>	China	Yunnan	-	A	-	Hing et al. 22413	Nov. 11 2004	STA2092
<i>Eriophorum gracile</i>	Canada	Northwest Territories	-	CAN	479515	Talbot 6237-4	Aug. 10 1976	STA1792
<i>Eriophorum gracile</i>	-	-	-	CAN	-	Starr 06014	2006	STA393

Taxon	Count.	Prov/State	Locality	Herb.	Accession #	Collector & #	Collection Date	DNA ID
<i>Eriophorum latifolium</i>	Finland	Fennia		OSC	122824	Jokela s.n.	Jul. 20 1965	STA2051
<i>Eriophorum russeolum</i> subsp. <i>albidum</i>	Canada	Quebec	-	CAN	584970	Gauthier 75-208	Jul. 6 1975	STA1793
<i>Eriophorum russeolum</i> subsp. <i>albidum</i>	Canada	Alberta	-	CAN	250516	Pegg s.n.	Jun. 19 1957	STA1788
<i>Eriophorum scheuchzeri</i>	Denmark	Greenland	-	CAN	311395	Jorgensen & Larsson 66-1555	Aug. 6 1966	STA1796
<i>Eriophorum scheuchzeri</i>	U.S.A.	Alaska	-	CAN	374814	Argus & Chunys 5813	Aug. 5 1966	STA1798
<i>Eriophorum tenellum</i>	Canada	Ontario	-	CAN	521628	Dugal & Shchepanek 6354	Aug. 23 1984	STA1928
<i>Eriophorum vaginatum</i>	United Kingdom	England	-	-	-	Starr & Scott 98007	-	STA112
<i>Eriophorum vaginatum</i> subsp. <i>spissum</i>	Canada	Newfoundland & Labrador		CAN	28406	Porsild 12	Jul. 12 1937	STA1804
<i>Eriophorum virginicum</i>	Canada	Quebec	-	CAN	395985	Shchepanek 1415	Sep. 9 1975	STA1802
<i>Eriophorum virginicum</i>	Canada	Newfoundland & Labrador		CAN	457555	Dickson & Brunton 3214	Sep. 25 1979	STA1807

Taxon	Count.	Prov/State	Locality	Herb.	Accession #	Collector & #	Collection Date	DNA ID
<i>Eriophorum viridicarinatum</i>	Canada	Ontario	-	CAN	435381	Shea 11351	Jul. 11 1976	STA1780
<i>Fimbristylis dichotoma</i>	Kenya	-	-	EA	-	Muasya 1006	-	STA320
<i>Isolepis aucklandica</i>	New Zealand	Cantebury District	-	CAN	416674	McIntosh s.n.	Feb. 12 1977	STA1906
<i>Karinia mexicana</i>	Mexico	Arandas	-	MICH	1136756	McVaugh 26621	Aug. 30 1990	STA2054
<i>Khaosokia caricoides</i>	-	-	-	-	-	Middleton et al. 4071	-	STA387
<i>Kobresia simpliciuscula</i>	Canada	Yukon Territory		CAN	318729	Porsild 1825	Aug. 11 1968	STA1801
<i>Oreobolopsis clementis</i>								STA2144
<i>Oreobolopsis tepalifera</i>	Bolivia	Cochabamba	-	NY	-	Wood 10463	Jan. 27 1996	STA2038
<i>Phylloscirpus deserticola</i>	Argentina	-	-	US	3363415	Ru 9797	Mar. 4 1997	STA2146
<i>Scirpus asper</i>	Chile	Valaparaíso	-	MICH	-	Landrum 3834	Nov. 11 1981	STA2065
<i>Scirpus atrovirens</i>	U.S.A.	Ohio	Paulding County	CAN	320674	Stuckey 6020	Sep. 13 1967	STA1908
<i>Scirpus atrovirens</i>	Canada	New Brunswick	Charlotte County	CAN	521238	Shchepanek & Dugal 5974	Aug. 6 1984	STA1982

Taxon	Count.	Prov/State	Locality	Herb.	Accession #	Collector & #	Collection Date	DNA ID
<i>Scirpus cyperinus</i>	Canada	Ontario	-	CAN	406410	<i>Shchepanek & Dugal</i> 6269	Aug. 21 1986	STA1773
<i>Scirpus lineatus</i>	Canada	Ontario	Norfolk County	CAN	370570	<i>Cruise</i> 1388	Aug. 17 1050	STA1978
<i>Scirpus maximowiczii</i>	-	-	-	CAN	344882	-	1967	STA1920
<i>Scirpus microcarpus</i>	Canada	Ontario	Ottawa-Carleton County	CAN	581102	<i>Dugal & Camfield</i> 6354	Aug. 23 1984	STA1976
<i>Scirpus microcarpus</i>	Canada	Saskatchewan	Meadow Lake	CAN	429634	<i>Baldwin & MacPherson</i> 10638	Aug. 13 1966	STA1977
<i>Scirpus pedicellatus</i>	Canada	Quebec	-	CAN	445079	<i>Houle</i> 76-1185	Aug. 24 1976	STA1775
<i>Scirpus pedicellatus</i>	Canada	New Brunswick	-	CAN	540736	<i>Haber</i>		STA1776
<i>Scirpus polystachyus</i>	Australia	-	-	K	-	<i>Pullen</i> 4091	-	STA349
<i>Scirpus radicans</i>	-	-	-	CAN	136190	<i>Samuelsson</i> 296	Jul. 3 1934	STA1915
<i>Trichophorum alpinum</i>	Canada	Quebec	-	CAN	231240	<i>Baldwin</i> 5678	Jun. 9 1954	STA1815
<i>Trichophorum alpinum</i>	U.S.A.	Alaska	-	CAN	299445	<i>Spetzman</i> 4941	Aug. 18 1963	STA1816

Taxon	Count.	Prov/State	Locality	Herb.	Accession #	Collector & #	Collection Date	DNA ID
<i>Trichophorum caespitosum</i>	Canada	British Columbia	-	CAN	589883	Saarela 1219	Jul. 24 2007	STA1817
<i>Trichophorum caespitosum</i>	Canada	Nunavut	-	CAN	585981	Aiken & Iles 02- 048	Jul. 6 2002	STA1819
<i>Trichophorum clintonii</i>	Canada	Quebec	-	CAN	395366	Cayouette 74- 52	Jun. 10 1974	STA1821
<i>Trichophorum clintonii</i>	Canada	Ontario	-	CAN	440584	Pratt 128	Jun. 6 1978	STA1822
<i>Trichophorum pumilum</i>	Canada	Yukon	-	CAN	589169	Bennett et al. 06-097	Jul. 20 2006	STA1820
<i>Trichophorum pumilum</i>	-	-	-	CAN	381789	Mejland	May 7 1963	STA1922
<i>Trichophorum rigidum</i>	Bolivia	La Paz	-	NY	-	Harling & Anderson 21139	Jan. 26 1985	STA2043
<i>Trichophorum subcapitatum</i>	China	Hunan	-	CAS	1021971	Zhang-chun 1903	May 26 1998	STA2070
<i>Zameioscirpus atacamensis</i>	Argentin a	-	-	US	3363409	Ru 9885	Mar. 13 1997	STA2105
<i>Zameioscirpus atacamensis</i>	Argentin a	-	-	US	3363408	Ru 9884	Mar. 13 1997	STA2143

Appendix 2: Published article in *Kew Bulletin*

Gilmour, C. N., Starr, J. R., & Naczi, R. F. C. (2013). *Calliscirpus*, a new genus for two narrow endemics to the California Floristic Province, *C. criniger* and *C. brachythrix* sp. nov. (Cyperaceae). *Kew Bull.* 68: doi: 10.1007/s12225-012-9420-2.

Appendix 3: Errata in Appendix 2

The following errata were discovered within the published paper presented in Appendix 2 before the completion of this thesis. Luther Pass, Eldorado Co., California, 30 July 1971, *Mott* 42071 (CAS) originally identified as *Calliscirpus brachythrix* C.N. Gilmour, J.R. Starr, & Naczi was reidentified as *Eriophorum gracile* Koch in A.W. Roth. Warren Creek, Mono Co., California, 10 Sept. 1984, *W. Knight & I. Knight* 5431 (CAS) originally identified as *Calliscirpus brachythrix* C.N. Gilmour, J.R. Starr, & Naczi was reidentified as *Carex microptera* Mack. There is another specimen collection by *W. Knight & I. Knight* with the same collection number as above, 5431, as well as the same collection date, 10 Sept, 1984, collected on the trail between Tioga Lake and Bennetville, Mono Co., California (CAS). However, this sheet has collections of both *Calliscirpus brachythrix* C.N. Gilmour, J.R. Starr & Naczi and *Carex microptera* Mack. Ontario, Canada, 11 July 1976, *Shea* 11351 (CAN 435381) originally identified as *Eriophorum crinigerum* Honck. was reidentified as *Eriophorum viridicarinatum* (Engelm.) Fernald.

Also note the removal of the localities for the misidentified specimens does not change the range given for *C. brachythrix* in Chapter 2 or Appendix 2 as *C. brachythrix* is commonly found in both Eldorado and Mono Counties, California.