Systematics and ecology of the moss genus *Scleropodium* (Brachytheciaceae)

By

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Abstract

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Scleropodium is a genus of six species in the Brachytheciaceae. Although they are common in north temperate zones, they have not received monographic treatment in over a century. The aims of this study were to test species circumscriptions within the genus with molecular data, complete a thorough global taxonomic treatment of the genus, and to quantitatively investigate the ecological preferences of the species.

A molecular phylogenetic study was conducted using 104 individuals spanning the range of morphological variation and the geographic extent of the genus. Maximum Parsimony and Bayesian phylogenetic analyses and a statistical parsimony network analysis of ITS and the chloroplast rps4, bsbA2 and trnG regions were performed. Although slight differences were found among analyses, there were six clear molecular groups. Five of these corresponded directly to the species *Scleropodium californicum*, *S. cespitans*, *S. julaceum*, *S. obtusifolium* and *S. touretii*. The sixth species, *S. occidentale*, is new to science and is described here. It is similar in ecology and morphology to S. obtusifolium, but has several diagnostic features in both molecular markers and morphological characters. Molecular data also revealed that S. australe, previously recognized as an endemic species in Tasmania, is nested in phylogenetic analyses within European S. touretii.

For the taxonomic treatment, specimens from across the global distribution of the genus were obtained and examined. Earlier taxonomic problems are resolved and new determinations of many herbarium specimens resulted in substantial changes in the known geographic distributions of the species. Nomenclatural problems are addressed, including lectotypification of *S. cespitans*, neotypification of S. touretii, and snynonymization of *S. colpophyllum* with *S. cespitans* and *S. australe* with *S. touretii*. Two Korean species, *S. brachyphyllum* and *S. coreense*, are excluded from *Scleropodium*.

A study of niche differences among five of the six species was conducted in the coast ranges of California. The study employed twenty-eight100m2 plots to investigate mesosite and microsite differences among species. Differences could be found in all species pairs except for *S. obtusifolium* and *S. occidentale*, both of which are restricted to seasonal drainages. Environmental factors that were important at broad spatial scales included slope and tree canopy cover. Finer scale differences at the scale of individual moss patches were found in distance of the moss patches from drainages, substrate type and shade. Although niche differences were observed and quantified, niche overlap was extensive and mixed moss patches with two species growing intertwined were common. Niche differences among sexes within species was also

investigated, but results were mostly not significant. In the more common species, there were trends toward female sex ratio bias and restriction of male individuals to more mesic habitats, but these results were mostly confounded by numbers non-expressing individuals.

For Mom, who inspired my passion for living things,
For Dad, who taught me to contemplate,

And for Tracy, who tolerates a house full of moss specimens.

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Preface

This dissertation presents the results of an investigation into the evolution, ecology and taxonomy of *Scleropodium*, a small genus of six mosses. The inspiration for the project came from my interest in the flora and vegetation of California and desire to work on a local problem. California is the global center of diversity of the genus, with all six species occurring in sympatry in the Coast Ranges of the state. The species are all very common, so they are well represented in herbaria and logistically easy to study in the field. The problem, which I hope I have made progress toward fixing here, is that the species circumscriptions have been poorly understood. Over the past few decades, this has been a vexing problem for bryologists in California in that specimens from one of the most common local genera could not be consistently identified to species with much confidence, even by seasoned local bryologists.

My goal in undertaking this project was fairly simple. Following the path of botanists over the last few centuries, I just wanted to take a group of plants that were poorly understood and bring some order to the chaos. As a bryologist in California, I often spend time making collections in areas where *Scleropodium* species are dominant members of the cryptogam community. In a way, working on the systematics of this genus would directly serve some of my other interests in studying the ecology and floristics of bryophytes in California.

In addition to the taxonomic problems, *Scleropodium* poses some interesting questions that any plant ecologist enjoys wrestling with. How can several very closely related species live in sympatry? Are they engaging in niche partitioning? If so, along which environmental axes? If not, how do they coexist and how did they diverge? These are old questions that ecologists have been chipping away at for decades. As one who dabbles in ecology, these questions are very interesting to me, especially in a group of organisms, the mosses, in which the rules are slightly different than for the more commonly studied angiosperms. Despite the intrigue, studying the ecology of *Scleropodium* species wasn't really feasible when I started this project for the simple reason that I (along with everyone else) couldn't confidently identify the species. In fact, some wondered even if the species were good natural groups or whether there was some serious taxonomic work that needed to be done.

My approach in engaging these problems was to start with molecular phylogenetic analyses to determine how many genetic groups occur within the genus. I didn't pay much attention to the names on specimen labels, but focused on sequencing across the geographic extent and range of morphological variation within the group. Results of those analyses showed fairly convincingly that there are six species in the genus worthy of recognition. Historical morphological species circumscriptions were actually nicely congruent with results from the molecular analyses, and so most of the taxonomic work didn't involve too much tinkering with the existing circumscriptions. Instead there was a new species to describe that earlier workers hadn't noticed yet and a few names for ecological variants that needed to be synonymized. This culminated in a healthy amount of museum work. Because the genus is so common, there were many hundreds of specimens to examine and a good proportion of these were misidentified. An interesting outcome of this was a fairly drastic realignment of geographic distributions for several species.

The new distribution maps rekindled my interest in the ecological relationships among the species. Armed with the ability to identify the species, I went into the field to get insight into whether or not these species are really sympatric at a small spatial scale, and whether they

overlap ecologically. It turns out that several of them are quite distinct from others ecologically. People just weren't aware of it because we couldn't tell them apart.

To me, this captures the essence of why basic systematic research is important. This project began very simply by asking the questions "how many species?" and "how do you tell them apart?" In the course of answering these questions, I discovered and named a new species now known to be common in western North America. In Tasmania, I provided evidence that a species being considered for conservation priority was actually an introduced population from Europe (i.e. it is an exotic, not a rare species). Reinterpretation of type specimens of two species described from Korea revealed that they are not *Scleropodium* and that there are currently no species in the genus known from the entire Asian continent. The distributions of five existing species were clarified, resulting in several new state records, major range extensions and in one case a substantial range size reduction. Patterns in the molecular data strongly suggest that, although the genus is probably old, diversification of the extant taxa is recent, perhaps coinciding with the development of a Mediterranean climate in California. While documentation of diversification of angiosperm lineages in California is now commonplace, this study is among the first to document recent diversification of a bryophyte lineage in California (the other being the closely related genus *Homalothecium*). In studying niche differentiation, I found that the species tend toward different niches at small spatial scales, but that niche overlap is extensive even to the extent of mixed patches with individuals of multiple species growing intertwined. All these insights from one of the most common genera along the west coast of North America.

In today's academic climate, where scientists are evaluated by their grant money and the impact factors of their papers, we should remember that there is no substitute for investing deeply in a comprehensive understanding of the natural history, ecology and evolution of organisms. My experience with a few species over a few years has been sufficient to convince me that there is a great need for baseline information in organismal biology. My experience as a graduate student in a prestigious ecology and evolution program has convinced me that the majority of students are responding to pressure of funding agencies and impact factors by investing time in methodological and theoretical advances at the expense of understanding the organisms they study. I hope this dissertation can be viewed as an example of the intellectual fulfillment that comes from science deeply rooted in natural history.

The dissertation is divided into five chapters. The first chapter provides the results for my molecular phylogenetic study investigating species circumscriptions within the genus *Scleropodium*. The second chapter is a global monograph of the genus based on the reciprocal illumination of the molecular work and extensive herbarium work and field observations. In the third chapter, results are provided from an ecological study documenting niche differences, or lack thereof, among five of the six species in the genus. The fourth chapter is the description of new species to science based on the morphological and molecular work in the previous chapters. The fifth and final chapter is the documentation, based on molecular and morphological data, of an introduced population of *Scleropodium* in Tasmania that had once been thought to be an endemic species, along with the necessary taxonomic changes.

CHAPTER 1. Species delimitation and cryptic diversity in the moss genus *Scleropodium* (Brachytheciaceae)

Abstract

Cryptic lineage diversification is an important component of global biodiversity, but it presents challenges to our ability to catalog and understand that diversity. Because of their relative morphological simplicity and broad geographic distributions, bryophytes are an ideal study group for investigating this phenomenon. This study generated molecular data from 109 ingroup individuals to test morphological species circumscriptions and examine patterns of cryptic lineage diversification within the small north temperate moss genus *Scleropodium* (Brachytheciaceae). Maximum Parsimony and Bayesian phylogenetic analyses and statistical parsimony network analyses of ITS and chloroplast rps4, psbA2 and trnG regions indicate that the genus comprises six distinct molecular groups. Five of these molecular groups correspond to previously recognized species: *S. californicum* (Lesq.) Kindb., *S. cespitans* (Müll.) Koch, *S. julaceum* Lawton, *S. obtusifolium* (Mitt.) Kindb. in Macoun and *S. touretii* Brid. (Koch). However, the sixth group does not correspond to any existing species. Maximum parsimony and Bayesian posterior probability support for the monophyly of species varied widely and depended on both the dataset (ITS, chloroplast, combined) and the analysis method (Parsimony/Bayesian). Low phylogenetic resolution of species is attributable to the lack of informative DNA sequence vaiation and incongruent placements of three accessions in the chloroplast and ITS gene trees, both suggesting recent divergence within the genus. Re-examination of the herbarium vouchers for the sixth molecular group reveals that they form a group nested within the morphological circumscription of *S. obtusifolium*. One subtle morphological character (relative frequency of a costa spine) was identified that has utility in discriminating these two genetically distinct but morphologically very similar species.

Introduction

Cryptic species are an important component of global diversity (Bickford et al., 2007). While the careful cataloging of these species is urgent as a stand-alone goal, documentation of cryptic diversity also underpins the understanding of the ecological and evolutionary mechanisms driving lineage diversification (Hebert et al., 2004; Condon et al., 2008; Vieites et al., 2009). Recognition of cryptic species is straightforward when well-defined reciprocally monophyletic lineages are discovered within morphologically defined species, or where accessions attributed to a species are robustly supported as polyphyletic on gene trees (see Funk & Omland, 2003; De Queiroz, 2007). However, cases in which cryptic diversity is nested such that potential species are paraphyletic on gene trees can be more problematic to interpret, especially where resolution is poor. Despite the difficutly of detection, this is an expected pattern for cryptic lineages in which divergence is not complete or in which reticulation is present (e.g. Lu et al., 2010).

In most studies, cryptic species, almost by definition, have been discovered and delimited using molecular data. However, cryptic diversification is not fundamentally different from diversification with associated morphological evolution. Therefore discovery of cryptic species in lineages undergoing rapid divergence, reticulation and other processes which reduce or eliminate the expectation of monophyletic species are expected to be common (as in De Queiroz, 2007). This poses a clear challenge to the documentation of cryptic diversity that only reinforces

the need for systematic studies incorporating complete taxon sampling and dense sampling of multiple accessions within species.

Bryophytes (mosses, liverworts and hornworts) provide interesting systems for the study of cryptic diversification. As traditionally recognized, bryophyte species are generally broadly defined, with each species encompassing significant morphological variation and occupying broad geographical distributions often spanning several continents (Schofield, 1988). In recent molecular studies of bryophytes incorporating population level sampling, evidence for cryptic diversity is frequently revealed (Shaw, 2000; 2001; Feldberg et al., 2004; Hentschel et al., 2007; Wachowiak et al., 2007; Hedenäs & Eldenas, 2007; Fuselier et al., 2009; Heinrichs et al., 2009; 2011). As in many other taxa, molecular studies also reveal some species to be non monophyletic, reinforcing the need for more studies that include dense population-level sampling in order to uncover possible cryptic diversity (Stech & Wagner, 2005; Draper et al., 2007; Vanderpoorten & Shaw 2010).

The moss genus *Scleropodium* provides an ideal system within which to study cryptic diversity. The genus is placed within a large family, Brachytheciaceae (ca. 350 spp), that has been the subject of extensive recent study using molecular methods. Given the limitations of morphology to circumscribe species and genera (Robinson, 1962; Huttunen et al., 2007; Ignatov & Huttunen, 2002), molecular data have been needed to elucidate the backbone phylogeny (Huttunen et al., 2007; Huttunen & Ignatov, 2004) as well as to ascertain phylogenetic relationships within and among species to provide solid foundations for species delimitation (Blockeel et al., 2005; Vanderpoorten et al., 2005; Huttunen et al., 2008; Wynns et al., 2009; Aigoin et al., 2009; Hedenäs et al., 2009; Draper & Hedenäs, 2009a; Draper & Hedenäs, 2009b; Hutsemekers et al., 2010; Carter 2010). Although sampling has previously been incomplete, *Scleropodium* is thought to be monophyletic and is isolated on a long branch within a Homalothecioideae + Brachythecioideae clade (Huttunen et al., 2004; Huttunen et al., 2007), with the most recent analyses placing the genus in a trichotomy with Homalothecioideae and Brachythecioideae (Huttunen et al., 2007).

Scleropodium is a small genus, with between five and ten species depending on interpretation. The native distribution includes western North America, western Europe, the Mediterranean Basin, and Macaronesia, with the highest diversity along the Pacific coast of North America. Two species have been described from Korea, but are only doubtfully placed in the genus (Takaki, 1955). Another species was described from Tasmania, but that species has been shown to be an introduced population of *S. touretii* from Europe (Hedenäs, 1996; Hedenäs, 2002; Carter 2010). New collections of *Scleropodium* from the Pacific coast of North America since the last taxonomic treatments (Grout, 1899; Lawton, 1967) have raised questions about the circumscriptions of species in that region. The most recent floristic treatment of the region (Norris and Shevock 2004a; 2004b) highlighted these difficulties and informally proposed the recognition of a new species, *Scleropodium* species A, to account for morphological variation within *S. obtusifolium*. The current study employs molecular data to examine patterns of molecular variation within the genus *Scleropodium*. The specific goals were to 1) use molecular data to test morphological species circumscriptions in the genus and 2) to investigate patterns of morphologically cryptic molecular variation within the genus.

Methods

Sampling & DNA isolation

Specimens were chosen to span the geographic distribution and range of morphological variation within *Scleropodium* (Table 1.1). Recently collected material for the two Korean taxa, *S. coreense* Card. and *S. brachyphyllum* Card. could not be obtained, so these two putative species were not included in the study. Specimens of several doubtfully distinct taxa, namely, *S. touretii* var. *teneriffae* Card. et. Wint., *S. touretii* var. *colpophyllum* (Sull.) Grout, and *S.* species A, a putative segregate species from *S. obtusifolium* suggested by Norris & Shevock (2004b), were included to assess the genetic and evolutionary distinctiveness of these entities using molecular data. After pilot studies assessing variation in several molecular markers, the following markers were chosen: the 5.8S subunit and flanking internal transcribed spacers ITS1 and ITS2 of nrDNA(hereafter ITS), part of the photosystem II protein psbA (psbA2), the chloroplast ribosomal small protein 4 (rps4) and transfer RNA^{GLY} (UCC) (trnG). Isolation of DNA was conducted using the DNEasy Plant Mini-prep kit (Qiagen, Germantown, Maryland, USA) following the manufacturer's protocol. After extraction, Genomic DNA was diluted to 1:100 for use in PCR reactions. PCR amplifications were performed using AccuPower PCR premix tubes (Bioneer Co., South Korea). Primers and PCR protocols followed Shaw et al. (2003b). Cleaning of PCR amplicons was conducted with Exonuclease I and Antarctic Phosphotase (New England Biolabs Inc., Ipswich MA, USA). Clean PCR amplicons were cycle sequenced at the UC Berkeley DNA sequencing facility (http://mcb.berkeley.edu/barker/dnaseq/index.html).

ITS polymorphism

In a previous morphological and molecular analysis of intercontinental disjunctions of three mosses including *S. touretii*, Shaw et al. (2003a) found two divergent ITS copy types within several individuals of *S. touretii*. Through cloning, they determined that the two copy types varied at a single nucleotide position. To check for the presence of this polymorphism in the current dataset, Genbank sequences from Shaw et al. (2003a) were aligned with the ITS sequences generated for this study. From this alignment, the polymorphic site could be identified in each of the new sequences. Chromatograms were rechecked and all could be unambiguously scored for one of the two copy types, or for both by the presence of a double peak at that position. Accessions with both copy types were scored with an ambiguity code (Y) at the site of the double peak to indicate the presence of both the cytosine and thymine copy types.

Phylogenetic analyses

Sequences were aligned using MUSCLE (Edgar, 2004) and then checked by eye in Mesquite version 2.5 (Maddison & Maddison, 2008). Parsimony informative indels in both ITS and the chloroplast regions were scored following the simple coding method (Simmons and Ochoterena, 2000) to generate a separate indel matrix. Maximum parsimony analyses were implemented in PAUP* 4.10b10 (Swofford, 2002) using ITS, chloroplast and combined datasets that excluded non-informative characters and identical accessions. Of the 109 individuals in the full dataset, 39 were identical across all four regions (including indels) to at least one other individual in the dataset. An additional 33 individuals only differed by autapomorphies and were also excluded. Analyses used a heuristic tree search strategy using TBR branch swapping with 100 random addition sequence replications. A bootstrap analysis with 1000 pseudoreplicates and each replicate limited to 100,000,000 rearrangements was used to assess clade support. Bayesian

analyses for each of the three datasets (chloroplast, ITS, combined) were conducted using MrBayes (Huelsenbeck & Ronquist, 2001). Substitution models for each of the four regions were obtained using the AIC criterion in MrModeltest2.3 (Nylander, 2004). A separate model with identical forward and reverse transition rates was used for the indel matrix (Lewis, 2001). Analyses were run for fifteen million generations, with sampling every 1000 generations. The first 25% of sampled trees were discarded for the burn-in. Each run employed two simultaneous runs each with three hot chains and one cold chain. Stationarity was checked by ensuring that the average standard deviation of split frequencies was below 0.01 and by graphing the log likelihood scores for each run.

After visualizing the tree topologies and haplotype networks (see methods below), it was apparent that three accessions, *S. cespitans* 12, *S. touretii* 20, and *S. californicum* 19, were responsible for a large proportion of the incongruence among analyses. To further investigate this, a parsimony analysis using the combined dataset but excluding the three accessions was run using the same parameters of the previous parsimony analysis. Three additional Bayesian analyses were conducted (ITS, chloroplast and combined datasets) using the same methods as the previous Bayesian analyses, but excluding the three accessions in each run. To examine the effects of removing these three individuals from the analyses, constraint trees were generated to filter the post-burnin trees to assess the posterior probability of monophyly of each species. The constraint trees were generated in Mesquite and the filtering was conducted in PAUP*.

Network analyses

Because of generally low support in phylogenetic analyses, a statistical parsimony network was generated for the combined dataset using TCS version 2.1 (Clement et al., 2000) to further explore the relationships among sequences in the dataset. Outgroups and the indel matrix were excluded from the analysis.

Results

Molecular data

Sequence lengths for the four regions used were: ITS 694-729bp, rps4 558-571bp, psbA2 638-651bp and trnG 526-541bp. Aligned lengths were: ITS 745, rps4 588, psbA2 668, trnG 543. The number of variable positions in the alignments (and percent parsimony informative relative to the total sequence length) were: ITS 99(7.4%), rps4 22(2.3%), psbA2 35(3.7%) and trnG 43 (4.2%). A total of 56 indels were discovered and the 24 that were parsimony informative were coded in a separate matrix.

3.2 ITS polymorphism

The polymorphic ITS position found by in Shaw et al. (2003a) is at position 483 in the alignment used in this analysis. Shaw et al. found a copy type with a cytosine at position 483 in accessions from Macaronesia and North America, while copy types with either a thymine at position 483 or with both copy types were found in individuals from Europe and Macaronesia (data from Genbank). In the current study, individuals with the cytosine copy only were found throughout the distribution of the species, whereas individuals with a thymine copy only or possessing both copies were restricted to the Old World (Table 1.2). Both datasets demonstrate the presence of the cytosine copy throughout the range but no evidence for the thymine copy in the new world. Dense sampling of individuals of other species in the genus confirmed that the thymine copy is

present only in Old World *S. touretii*, with all other species possessing a cytosine fixed at the same position.

3.3 Phylogenetic analyses

Results of Bayesian analyses of the chloroplast and ITS datasets are shown in Figure 1.1. Both trees indicate strong support for the monophyly of the genus but display short branch lengths, generally low posterior probabilities for monophyletic species, and poor resolution of relationships among species. Both analyses revealed a group of accessions (*Scleropodium* species B, to avoid confusion with Norris & Shevock's [2004b] *S.* species A) which did not form a monophyletic group, but also could not readily be assigned to any existing species. The chloroplast dataset provided strong support for the monophyly of *S. julaceum*, *S. obtusifolium* and *S. californicum*. *Scleropodium touretii* and the accessions forming the group *S.* species B received no support, and *S. cespitans* was paraphyletic. Backbone support for the relationships among species was moderate, with *S. touretii* accessions unresolved and the remaining species forming a clade. Within the clade, *S. cespitans* was paraphyletic below a polytomy including *S. californicum*, *S.* species B, *S. julaceum* and *S. obtusifolium*. The ITS dataset provided somewhat different results. Only *S. julaceum* was recovered as a monophyletic species. *Scleropodium obtusifolium* and *S.* species B received no support, while *S. californicum* and *S. touretii* were polyphyletic. *Scleropodium cespitans* was rendered non-monophyletic by the inclusion of a single accession of *S. touretii* (*S. touretii* 20) within an otherwise monophyletic clade with very low support. Unlike the chloroplast dataset, the ITS dataset yielded almost no information about the relationships among species.

Parsimony analyses of the ITS and chloroplast datasets yielded topologies similar to those produced by the Bayesian analyses (trees not shown). The chloroplast analysis resulted in 21,228 equally parsimonious trees with a length of 164, Consistency Index 0.8232, and Retention Index 0.9388. The ITS dataset produced 270,350 equally parsimonious trees with length 159, Consistency Index 0.8365, and Retention Index 0.8865. Bootstrap support for each of the six species is provided in Figure 1.1. The only substantive difference between the Bayesian analysis and the parsimony analysis was the recovery of a monophyletic *S. touretii* (Bootstrap support < 70%) in the parsimony analysis of the chloroplast dataset that was not recovered in the Bayesian analysis of the same dataset.

Because most of the incongruence between the dataset involved either single or small groups of individuals, rather than major differences among species relationships, the two datasets were combined and subjected to Bayesian and Maximum Parsimony analyses (Figure 1.2). The parsimony analysis resulted in 201,105 equally parsimonious trees with a tree length of 333, Consistency Index 0.8078, and Retention index 0.9146. In the parsimony analysis, three of the six species were monophyletic with strong to moderate support, *S.* species B was monophyletic with weak support, *S. obtusifolium* and *S. cespitans* received no support, and no species had supported branches indicating non-monophyly.

In the Bayesian analysis of the combined dataset, *S. californicum*, *S. obtusifolium* and *S. julaceum* were monophyletic with strong support, accessions of *S.* species B were unresolved and hence this species received no support, and *S. cespitans* and *S. touretii* were both paraphyletic.

Relationships among species were mostly poorly resolved in both analyses, and incongruent where supported branches were recovered. In the parsimony analysis, *S. touretii*, *S. julaceum* and *S. cespitans* made up a basal polytomy with a clade comprising *S. obtusifolium*, *S.*

occidentale and *S. californicum*. In contrast, the Bayesian analysis recovered *S. touretii* and then *S. cespitans* forming paraphyletic grades, with the remaining species nested within the grade. *Scleropodium julaceum* and *S. obtusifolium* formed a clade sister to *S.* species B, with that clade sister to *S. californicum*.

Because three accessions (*S. cespitans* 12, *S. californicum* 19, *S. touretii* 20; see Figure 1.1) appeared to have a strong affect on the monophyly of species, separate analyses were conducted after excluding the three accessions from the dataset (Figure 1.3). The parsimony analysis resulted in 6231 equally parsimonious trees with a tree length of 213, Consistency Index 0.7230, and Retention index 0.9424. Clades corresponding to all six species were recovered in this analysis, with all but *S.* species B receiving bootstrap support of greater than 70%. The Bayesian analysis recovered strongly supported clades corresponding to four of the six species, *S. obtusifolium*, *S. californicum*, *S. julaceum* and *S. touretii*. *Scleropodium* species B was unresolved and *S. cespitans* was paraphyletic with Old World accessions (*S. cespitans* 104, *S. cespitans* 105 and *S. cespitans* 110) sister to a clade including *S. julaceum*, *S. californicum*, *S. species* B and *S. obtusifolium*. In both the parsimony and Bayesian analyses, the relationships among species were better resolved than in analyses including *S. cespitans* 12, *S. californicum* 19, and *S. touretii* 20. In these analyses, *S. touretii* was sister to the rest of the genus and *S. julaceum* was sister to a clade comprising the remaining four species.

The monophyly of each of the species was also tested using constraint trees on the Bayesian analyes of the ITS, chloroplast and combined datasets excluding *S. cespitans* 12, *S. californicum* 19, and *S. touretii* 20 (Table 1.3). In the analysis of the ITS dataset, the posterior probability of monophyly of *S. californicum* increased from 0.00 to 0.98 and the support for *S. cespitans* increased from 0.32 to 0.96. In the chloroplast analysis, the posterior probability for a monophyletic *S. touretii* increased from 0.12 to 0.99. In the combined analysis, *S. californicum*, *S. julaceum, S. obtusifolium* and *S. touretii* received a posterior probability of at least 0.99, with support for *S. cespitans* and *S.* species B remaining well below 0.50.

Accessions representing the four previously recognized taxa, *S. touretii* var. *colpophyllum*, *S. touretii* var. *teneriffae*, *S.* species A (a segregate from *S. obtusifolium*) of Norris and Shevock (2004b), and *S. australe* received no support in any of the phylogenetic analyses. Very little genetic structure was found within *S. obtusifolium*, and none of the structure corresponded to vouchers (*S. obtusifolium* 10, *S. obtusifolium* 24) representing *S.* species A. More genetic structure was found within *S. touretii*, however this was best explained by geography (see Figure 1.4) and did not correspond to specimens representing the morphology associated with *S. touretii* var. *colpophyllum* and *S. touretii* var. *teneriffae*. *Scleropodium australe*, represented by *S. touretii* 70, was found to be conspecific with *S. touretii* (Carter, 2010) using a subset of the current molecular dataset, and the results of the current study confirm this.

Network analysis

A statistical parsimony network analysis was conducted using the combined dataset excluding indels, and including all individuals in the ingroup. Results of this analysis (Figure 1.4) support the recognition of six genetically distinct groups within the dataset corresponding to the five previously known species plus *S.* species B. *Scleropodium cespitans* and *S. touretii*, the two species with intercontinental disjunctions, display clear differences among geographically separated populations. Accessions *S. cespitans* 12 and *S. touretii* 20, two specimens which strongly influenced the monophyly of *S. cespitans* and *S. touretii* in the Bayesian phylogenetic

analyses, were connected by a branch in this analysis, further implicating the similarity of these two accessions in the poor resolution of the phylogenetic analyses.

Discussion

Molecular variation and global distribution

The problems associated with the occurrence of multiple ITS copy types within individual plants are well understood (Alvarez and Wendell, 2003). While paralogy is problematic for resolution of relationships among species, the presence of multiple copy types within a species can potentially provide insights into genetic population structure and/or the parentage of putative hybrids. In *S. touretii*, the presence of two ITS copy types in the Old World (Macaronesia, Europe and the Mediterranean Basin) with only one copy type in North America provides evidence for some level of genetic isolation between these two populations. The results of the statistical parsimony network also reveal that there is some degree of genetic isolation between populations on either side of the Atlantic Ocean, but suggest that isolation is not complete. The molecular markers used in this study do not provide sufficient resolution to test hypotheses for vicariance versus dispersal in explaining the current distribution of *S. touretii*. However, other studies of trans-Atlantic disjunctions in moss species have found compelling evidence in support of recent dispersal (Shaw et al., 2003; Huttunen et al., 2008). Based on the results presented here, the population structure within *S. touretii* is also consistent with either ongoing or recent (although highly restricted) dispersal rather than ancient dispersal or vicariance.

Poor resolution and low support for morphologically circumscribed species

When analyzed in a statistical parsimony network, the molecular data presented here cluster all ingroup accessions into six unambiguous groups, with five of the groups corresponding directly to morphologically circumscribed species and the sixth group forming a cryptic cluster falling within the morphological circumscription of *S. obtusifolium*. Phylogenetic analyses, however, did not consistently recover well-supported monophyletic clades corresponding to all six species. Across analyses, branch lengths were short and the placement of accessions into monophyletic clades corresponding to the morphologically defined species, as well as the support for those clades, depended on both the dataset used (ITS, chloroplast, combined) and the analysis employed (Maximum Parsimony, Bayesian). In general the chloroplast data provided greater resolution and support for clades that were consistent with morphologically circumscribed species. A second general trend was that the Maximum Parsimony analyses tended to more consistently recover the morphologically circumscribed species as monophyletic, whereas the Bayesian analysis tended to recover paraphyletic species, especially *S. touretii* and *S. cespitans*. The third feature of the results is that accessions of some morphologically defined species are unresolved (i.e. no support for monophyly or for non-monophyly). This is most clearly illustrated in Figure 1.1 by *S. touretii* in the chloroplast tree and *S. obtusifolium* in the ITS tree.

Non-monophyly and poor resolution of morphologically defined species is a common finding in phylogenetic studies of disparate taxonomic groups with dense sampling within species (Funk & Omland, 2003; Vanderpoorten & Shaw, 2010). Three possible explanations for this pattern (reviewed in Funk & Omland, 2003) are: 1) Morphological circumscriptions do not accurately reflect phylogeny (inaccurate taxonomy), 2) species are well defined, but are subject to introgression or have experienced hybridization, 3) The species in question have diverged recently and/or rapidly. Each of these three are discussed in turn below.

The presence of a sixth molecular group, *Scleropodium* species B, that is clearly genetically distinct from *S. obtusifolium*, indicates that the current taxonomy of the group does not adequately reflect the evolutionary divergence history of the group (see section 4.3 below). However, this does not explain the general lack of resolution across the remaining species. In cases of taxonomic lumping, species are expected to form multiple, supported, non-sister clades (i.e. a polyphyletic species). In cases of taxonomic splitting, the expectation is accessions of multiple species form a single clade or are intermixed with each other in one or more clades. With the exception of *S.* species B, the results of this study do not conform to either of these expectations, and so discrepancy of morphological circumscriptions and the molecular data is not a likely explanation for the systematic low support and resolution throughout the genus.

Another possible explanation for poor resolution and non-monophyly is gene flow among otherwise well supported species or clades, either in the form of introgression or the presence of hybrids. Initially this is an appealing explanation, especially because the removal of only three out of 105 ingroup individuals substantially increases posterior probability support for three species (Table 1.3). If introgression were occurring, the prediction would be that the nuclear dataset (ITS) would be concordant with the morphological circumscriptions, while individual incongruent accessions would be found in the chloroplast dataset. In fact this is true only for accession *S. cespitans* 12, with the opposite being true for *S. touretii* 20 and *S. californicum* 19 (Fig. 1.1). The fact that multiple copies of ITS are known in one species of the genus (Shaw et al., 2003a; Table 1.2) further suggests that ITS may be subject to problems with paralogy and may be less trustworthy than the chloroplast dataset in this study.

If a hybrid origin for some accessions or species was causing the poor resolution, expectations would include incongruence between gene trees, a monophyletic hybrid species rendering one of its parents paraphyletic, or two parental species and the hybrid species polyphyletic with each other (reviewed in Funk & Omland, 2003). In *Scleropodium*, none of these patterns are strongly evident. If hybrid speciation has occurred, the most likely candidates are *S.* species B and *S. cespitans*, both of which lack strong support in most of the phylogenetic analyses. In gametophyte morphology, sporophyte morphology and ecology, *S.* species B is intermediate between *S. obtusifolium* and *S. cespitans* (much closer to *S. obtusifolium* in all three categories). With the data currently available, however, any argument for a hybrid origin of *S.* species B or other species in the genus is speculative. In addition, hybrid speciation could only explain poor resolution throughout the tree and does not provide insight into the incongruent placement of the three accessions mentioned previously.

The third possible explanation is recent divergence of species within *Scleropodium*. The expected molecular pattern for a recently derived group could include incomplete lineage sorting as well as species that have differentiated but have not been separated for long enough to form reciprocally monophyletic species clades. The results presented here are consistent with both of these expectations. The different results of Bayesian and Maximum Parsimony analyses, as well as the short branch lengths throughout the tree suggest a pattern of recent divergence (see Figure 1.4). In addition the presence of accessions (e.g. *S. cespitans* 12, *S. californicum* 19, *S. touretii* 20) that proved problematic in the phylogenetic analyses is consistent with the expected pattern of ancestral polymorphism associated with recently diverged lineages (Funk & Omland, 2003).

With the data currently available, recent divergence appears to be the most likely explanation for poor phylogenetic resolution within *Scleropodium*. Although moss species are sometimes thought of as "ancient" or slow to diverge, studies of species diversification including divergence time estimates in the moss genera *Homalothecium* (Huttunen et al., 2008) and

Mitthyrhidium (Wall, 2005) have demonstrated net species diversification rates of 0.5 (0.28-1.0) and 0.560 (0.556-0.564) species per lineage per million years, respectively. While not as fast as the most rapid angiosperm rates recorded, these are quite fast compared to many angiosperm lineages (Baldwin & Sanderson, 1998; Hughes & Eastwood, 2006). A conclusion of recent divergence is also consistent with the concentration of *Scleropodium* within the Mediterranean climatic region of California (Carter, unpublished data). The onset of the Mediterranean climate in California is estimated at less than seven million years ago (Millar, 2011) and was responsible for the substantial diversification of a wide array of vascular plant lineages (Raven & Axelrod, 1978; Ackerly, 2009). It seems reasonable that the origin of the Mediterranean climate would have had a similar effect on bryophyte lineages, and the few studies to employ population level sampling of Californian bryophytes suggest that this may indeed be the case (Fernandez et al., 2006; Huttunen et al., 2008; Hedenäs et al., 2009).

Evidence for an undescribed species of *Scleropodium*

Recognition of cryptic diversity is typically based on the discovery of non-monphyly of a single morphologically circumscribed species (Bickford et al., 2007). This is particularly convincing when the branches supporting the morphologically cryptic clades are long and well supported. The results of this study present an interesting alternative case. The group of twelve accessions labeled "Species B" in Figures 1.1-1.4 were recovered as a monophyletic clade only in the Maximum Parsimony analyses of the combined dataset, and the bootstrap support for the clade was low (<70) in both cases. In all other analyses, there was no support for the monophyly of the group, but also no support for any branches that would render the group non-monophyletic. The accessions are not assignable to any of the existing five species based on the phylogenetic analyses, and the statistical parsimony network suggests that this group is as genetically different from the other five species as they are from each other.

All twelve voucher specimens for the accessions of *S.* species B were originally identified as *S. obtusifolium*. The accessions of *S.* species B were collected from localities throughout California, as were the accessions of *S. obtusifolium*, indicating that the two species are potentially sympatric. *Scleropodium obtusifolium* is a rheophyte (growing seasonally submerged in flowing streams) which occurs throughout western North America. As with most rheophytic mosses, phenotypic plasticity is high in *S. obtusifolium* and is likely associated with environmental factors including stream flow rates, as well as seasonality of the stream and elevation. This habitat affinity was thought to be unique in the genus with the other species occurring on rocks, soil and tree bases in terrestrial environments (Grout, 1899). Because of the ecological distinctness of *S. obtusifolium* relative to its congeners, detailed morphological analyses of the species relative to its congeners were never undertaken. Re-examination of the vouchers of *S.* species B and *S. obtusifolium* have revealed that at least one subtle morphological character, the relative frequency of a spine at the terminus of the costa (leaf midrib) may have utility in discriminating the two species. A full morphological reanalysis of these two species will accompany a forthcoming formal taxonomic treatment elsewhere.

Conclusions

The genus *Scleropodium* contains six species including one cryptic species not previously recognized. Phylogenetic analyses do not consistently support the monophyly of all species with the markers used in this study, however the six species form distinct molecular groups in a statistical parsimony network. Poor resolution of species and of relationships among species is

most consistent with recent diversification, although alternative explanations cannot be rejected. Patterns of molecular variation in New World and Old World populations of S. touretii and S. cespitans are not consistent with hypotheses of vicariance or ancient dispersal.

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Figures and Tables

Accession ID	Taxon	Voucher (Herbarium)	Locality	Genbank Accession #: ITS, rps4, psbA2, trnG
Homnut*	*Homalothecium nuttallii*	Carter 336 (UC)	Lopez Cyn., S.L. Obispo Co., CA, USA	GU306137, GU329714, GU329724, GU329734
Kinprae*	*Kindbergia praelonga*	Carter 1506 (UC)	Cambria, S.L. Obispo Co., CA, USA	GU306138, GU329715, GU329725, GU329735
Brachfrig	*Brachythecium frigidum*	Lenz 927 (UC)	Tennant, Siskiyou Co., CA, USA	HM771719, HM771813, HM771907, HM772001
Brachasp	*Brachythecium asperrimum*	Shevock 13119 (UC)	Sequoia Natl. For., Tulare Co., CA, USA	HM771743, HM771837, HM771931, HM772025
CASFr19	*Scleropodium californicum*	Shevock 18897 (UC)	San Francisco, San Francisco Co., CA, USA	HM771731, HM771825, HM771919, HM772013
CASlo35	*Scleropodium californicum*	Carter 1097 (UC)	CA Polytechnic St. Univ., S. L. Obispo Co., CA, USA	HM771743, HM771837, HM771931, HM772025
CASFr54	*Scleropodium californicum*	Shevock 19120 (UC)	Yerba Buena Is., San Francsico Co., CA, USA	HM771755, HM771849, HM771943, HM772037
CASlo03*	*Scleropodium cespitans*	Carter 667 (UC)	Price Canyon, S.L. Obispo Co., CA, USA	HM367713, HM367723, HM367728, HM367718
CASlo06	*Scleropodium cespitans*	Carter 603 (UC)	Bob Jones Trail, S.L. Obispo Co., CA, USA	HM771723, HM771817, HM771911, HM772005
CASlo12	*Scleropodium cespitans*	Carter 008 (UC)	Cerro Alto, S.L. Obispo Co., CA, USA	HM771728, HM771822, HM771916, HM772010
CASlo33*	*Scleropodium cespitans*	Carter 1839 (UC)	Cerro Alto, S.L. Obispo Co., CA, USA	HM367712, HM367722, HM367727, HM367717
CASlo36	*Scleropodium cespitans*	Carter 902 (UC)	Pennington Crk., S.L. Obispo Co., CA, USA	HM771744, HM771838, HM771932, HM772026
CAMen45	*Scleropodium cespitans*	Carter 2226 (UC)	Mackerricher St. Pk., Mendocino Co., CA, USA	HM771751, HM771845, HM771939, HM772033
WACla72	*Scleropodium cespitans*	Norris 90299 (UC)	Joyce, Clallam Co., WA, USA	HM771768, HM771862, HM771956, HM772050
CASCz82	*Scleropodium cespitans*	Kellman 721 (UC)	Corallitos, Santa Cruz Co., CA, USA	HM771773, HM771867, HM771961, HM772055
CASlo88	*Scleropodium cespitans*	Carter 1014 (UC)	Chorro Valley, S.L. Obispo Co., CA, USA	HM771778, HM771872, HM771966, HM772060
Turk104*	*Scleropodium cespitans*	Nyholm 876b/71 (S)	Izmir Province, Turkey	GU306130, GU329707, GU329717, GU329727
Italy105*	*Scleropodium cespitans*	Bisang 930106 (S)	Sardegna, Italy	GU306131, GU329708, GU329718, GU329728
Spain110*	*Scleropodium cespitans*	Een sn (14 III 1998) (S)	Andalucia, Spain	GU306132, GU329709, GU329719, GU329729
CASlo07	*Scleropodium julaceum*	Carter 1080 (UC)	CA Polytechnic St. Univ., S.L. Obispo Co., CA, USA	HM771724, HM771818, HM771912, HM772006
CAFre09	*Scleropodium julaceum*	Shevock 17115 (UC)	Pinehurst, Fresno Co., CA, USA	HM771726, HM771820, HM771914, HM772008
CASlo13	*Scleropodium julaceum*	Carter 982 (UC)	Las Pilitas Rd., S.L. Obispo Co., CA, USA	HM771729, HM771823, HM771917, HM772011
CASlo31	*Scleropodium julaceum*	Carter 1599 (UC)	Rock Ranch, S.L. Obispo Co., CA, USA	HM771740, HM771834, HM771928, HM772022
CASlo32	*Scleropodium julaceum*	Carter 1280 (UC)	Paso Robles, S.L. Obispo Co., CA, USA	HM771741, HM771835, HM771929, HM772023
CASlo34	*Scleropodium julaceum*	Carter 1140 (UC)	Chimeneas Ranch, S.L. Obispo Co., CA, USA	HM771742, HM771836, HM771930, HM772024
CAKer38	*Scleropodium julaceum*	Shevock 12879 (UC)	Kern River, Kern Co., CA, USA	HM771745, HM771839, HM771933, HM772027
CAKer39	*Scleropodium julaceum*	Shevock 16923 (UC)	Jawbone Cyn., Kern Co., CA, USA	HM771746, HM771840, HM771934, HM772028
CACon40	*Scleropodium julaceum*	Norris 109491 (UC)	Berkeley, Contra Costa Co., CA, USA	HM771747, HM771841, HM771935, HM772029
CATul52	*Scleropodium julaceum*	Laeger 1242 (UC)	Greenhorn Mts., Tulare Co., CA, USA	HM771754, HM771848, HM771942, HM772036
ORBen63	*Scleropodium julaceum*	Carter 2487 (UC)	McDonald Exp. For., Benton Co., OR, USA	HM771762, HM771856, HM771950, HM772044
CALak08	*Scleropodium obtusifolium*	Carter 1939 (UC)	Cobb, Lake Co., CA, USA	HM771725, HM771819, HM771913, HM772007
CATou10	*Scleropodium obtusifolium*	Shevock 29993 (UC)	Yosemite,Mariposa Co., USA	HM771727, HM771821, HM771915, HM772009
WAJef17	*Scleropodium obtusifolium*	Norris 98543 (UC)	Olympic Natl. Pk., Jefferson Co., WA, USA	HM771730, HM771824, HM771918, HM772012
CAFre22	*Scleropodium obtusifolium*	Shevock 14957 (UC)	Kings River, Fresno Co., CA, USA	HM771733, HM771827, HM771921, HM772015
CAFre23*	*Scleropodium obtusifolium*	Shevock 17188 (UC)	Indian Creek, Fresno Co., CA, USA	GU306133, GU329710, GU329720, GU329730
CAFre24	*Scleropodium obtusifolium*	Shevock 14516 (UC)	Monarch Wilderness, Fresno Co., CA, USA	HM771734, HM771828, HM771922, HM772016
CATeh26	*Scleropodium obtusifolium*	Shevock 19376 (UC)	Yolla Bolly Mts, Tehema Co., CA, USA	HM771735, HM771829, HM771923, HM772017
CAFre27	*Scleropodium obtusifolium*	Shecock 15607 (UC)	Sequoia Natl For., Fresno Co., CA USA	HM771736, HM771830, HM771924, HM772018
CAKer28	*Scleropodium obtusifolium*	Shevock 15545 (UC)	Piute Mts, Kern Co., CA, USA	HM771737, HM771831, HM771925, HM772019
CAMrp41	*Scleropodium obtusifolium*	Shevock 31752 (UC)	Yosemite Natl. Pk., Mariposa Co., CA, USA	HM771748, HM771842, HM771936, HM772030
CAMen43	*Scleropodium obtusifolium*	Carter 2800a (UC)	Hopland, Mendocino Co., CA, USA	HM771750, HM771844, HM771938, HM772032
CAMrp60	*Scleropodium obtusifolium*	Shevock 15239 (UC)	Mariposa, Mariposa Co., CA, USA	HM771760, HM771854, HM771948, HM772042
CAVen69	*Scleropodium obtusifolium*	Sagar 066 (UC)	Santa Monica Mts., Ventura Co., CA	HM771766, HM771860, HM771954, HM772048
CASCz80	*Scleropodium obtusifolium*	Kellman 728 (UC)	Diablo Gulch, Santa Cruz Co., CA, USA	HM771771, HM771865, HM771959, HM772053
CARiv81	*Scleropodium obtusifolium*	Shevock 32560 (UC)	San Bernardino Mts., Riverside Co., CA, USA	HM771772, HM771866, HM771960, HM772054
NVHm86	*Scleropodium obtusifolium*	Shevock 32484 (UC)	Santa Rosa Range, Humboldt Co., NV, USA	HM771776, HM771870, HM771964, HM772058
CARiv87	*Scleropodium obtusifolium*	Shevock 32550 (UC)	San Bernardino Mts., Riverside Co., CA, USA	HM771777, HM771871, HM771965, HM772059
Portugal49*	*Scleropodium touretii*	Een sn (2 IV 1994) (UC)	Sancta Clara-a-Velha, Portugal	GU306134, GU329711, GU329721, GU329731
CASlo02*	*Scleropodium touretii*	Carter 1825 (UC)	Cerro Alto, S.L. Obispo Co., CA, USA	HM367715, HM367725, HM367730, HM367720
CASlo04	*Scleropodium touretii*	Carter 1288 (UC)	Klau Mine Rd., S.L. Obispo Co., CA, USA	HM771722, HM771816, HM771910, HM772004
CASlo20	*Scleropodium touretii*	Carter 1386 (UC)	Cambria, S.L. Obispo Co., CA, USA	HM771732, HM771826, HM771920, HM772014
CABut30	*Scleropodium touretii*	Carter 2032 (UC)	La Porte, Butte Co., CA, USA	HM771739, HM771833, HM771927, HM772021
CABut47	*Scleropodium touretii*	Carter 2060 (UC)	Sly Creek Dam, Butte Co., CA, USA	HM771752, HM771846, HM771940, HM772034

Table 1.1 Voucher specimens and Genbank accession numbers. Vouchers with an asterisk (*) were previously used by Carter (2010). Page 1 of 2.

CASlo51	*Scleropodium tourettii*	Carter 1533 (UC)	Los Berros Cyn., S.L. Obispo Co., CA, USA	HM771753, HM771847, HM771941, HM772035
ORVien66	*Scleropodium tourettii*	Carter 2582 (UC)	Viento St. Pk., Hood River Co., OR, USA	HM771763, HM771857, HM771951, HM772045
CATul67	*Scleropodium tourettii*	Shevock 12994 (UC)	Sequoia Natl. For., Tulare Co., CA, USA	HM771764, HM771858, HM771952, HM772046
CATuo68	*Scleropodium tourettii*	Colwell 04-40 (UC)	Yosemite Natl. Pk., Tuolomne Co., CA, USA	HM771765, HM771859, HM771953, HM772047
Tasmania70*	*Scleropodium tourettii*	Seppelt 27568 (UC)	Hobart, Tasmania	GU306129, GU329706, GU329716, GU329726
CABut71	*Scleropodium tourettii*	Dillingham 1812 (UC)	Lassen Natl. For., Butte Co., CA, USA	HM771767, HM771861, HM771955, HM772049
CAMen73	*Scleropodium tourettii*	Carter 3009 (UC)	Angelo Reserve, Mendocino Co., CA, USA	HM771769, HM771863, HM771957, HM772051
CAMen74	*Scleropodium tourettii*	Carter 2285 (UC)	Mendocino, Mendocino Co., CA, USA	HM771770, HM771864, HM771958, HM772052
CASCz75*	*Scleropodium tourettii*	Carter 2867 (UC)	Nicene Marks St. Pk., Santa Cruz Co., CA, USA	HM367714, HM367724, HM367729, HM367719
CAMon84	*Scleropodium tourettii*	Kellman 2966 (UC)	Palo Colorado Rd., Monterey Co., CA, USA	HM771774, HM771868, HM771962, HM772056
CASCz85	*Scleropodium tourettii*	Kellman 1891 (UC)	Little Creek, Santa Cruz Co., CA, USA	HM771775, HM771869, HM771963, HM772057
CASBa90	*Scleropodium tourettii*	Shevock 32573 (UC)	Santa Ynez Mts., Santa Barbara Co., CA, USA	HM771780, HM771874, HM771968, HM772062
Portugal94	*Scleropodium tourettii*	Sales 058 (E)	Esremadura: Nazare, Portugal	HM771782, HM771876, HM771970, HM772064
Italy95*	*Scleropodium tourettii*	Long 38021 (E)	Umbria, Italy	GU306135, GU329712, GU329722, GU329732
France97	*Scleropodium tourettii*	Long 35401 (E)	Dept. Pyrenees Orientalis, France	HM771783, HM771877, HM771971, HM772065
France100	*Scleropodium tourettii*	Long 23474 (S)	Brittany, France	HM771784, HM771878, HM771972, HM772066
Madeira102	*Scleropodium tourettii*	Hedenas sn (7 IV 1990) (S)	Madeira, Portugal	HM771785, HM771879, HM771973, HM772067
Grce106*	*Scleropodium tourettii*	Een G027 (S)	Crete, Greece	GU306136, GU329713, GU329723, GU329733
Madeira109	*Scleropodium tourettii*	Hedenas sn (12 VI 1998) (S)	Madeira, Portugal	HM771786, HM771880, HM771974, HM772068
England111	*Scleropodium tourettii*	Paton sn(15 Aug 1961) (S)	East Cornwall, England	HM771787, HM771881, HM771975, HM772069
CITen112	*Scleropodium tourettii*	Heinrichs 6169 (S)	Tenerifae, Canary Islands, Spain	HM771788, HM771882, HM771976, HM772070
France125	*Scleropodium tourettii*	Vanderpoorten 439 (LG)	Corsica, France	HM771789, HM771883, HM771977, HM772071
Italy126	*Scleropodium tourettii*	Vanderpoorten 559 (LG)	Province de Toscane, Italy	HM771790, HM771884, HM771978, HM772072
France128	*Scleropodium tourettii*	Vanderpoorten M5 (LG)	department du Gard, France	HM771791, HM771885, HM771979, HM772073
CIGrCa129	*Scleropodium tourettii*	Vanderpoorten GCA25 (LG)	Gran Canaria, Canary Islands, Spain	HM771792, HM771886, HM771980, HM772074
Madeira130	*Scleropodium tourettii*	Vanderpoorten M32 (LG)	Madeira, Portugal	HM771793, HM771887, HM771981, HM772075
CILaPm131	*Scleropodium tourettii*	Vanderpoorten PALM1462 (LG)	La Palma, Canary Islands, Spain	HM771794, HM771888, HM771982, HM772076
Corsica132	*Scleropodium tourettii*	Vanderpoorten COR2007/245(LG)	Corsica, France	HM771795, HM771889, HM771983, HM772077
France133	*Scleropodium tourettii*	Vanderpoorten Merc62 (LG)	Alpes Maritimes, France	HM771796, HM771890, HM771984, HM772078
Morocco134	*Scleropodium tourettii*	Cano sn (17 III 1997) (S)	Ibel Bouhalla, Morocco	HM771797, HM771891, HM771985, HM772079
CAVen136	*Scleropodium tourettii*	Sagar 440 (UC)	Santa Monica Mts., Ventura Co., CA, USA	HM771799, HM771893, HM771987, HM772081
ORBen137*	*Scleropodium tourettii*	Carter 2459 (UC)	McDondald Exp For., Benton Co., OR, USA	HM367716, HM367726, HM367731, HM367721
CASRI138	*Scleropodium tourettii*	Norris 102066 (UC)	Santa Rosa Is., Santa Barbara Co, CA, USA	HM771800, HM771894, HM771988, HM772082
CASRI139	*Scleropodium tourettii*	Norris 102261 (UC)	Santa Rosa Is., Santa Barbara Co, CA, USA	HM771801, HM771895, HM771989, HM772083
CASRI140	*Scleropodium tourettii*	Norris 102125 (UC)	Santa Rosa Is., Santa Barbara Co, CA, USA	HM771802, HM771896, HM771990, HM772084
CIHier142	*Scleropodium tourettii*	Gonzalez-Mancelo 18770 (UC)	Hierro, Canary Islands, Spain	HM771803, HM771897, HM771991, HM772085
CIHier143	*Scleropodium tourettii*	Gonzalez-Mancelo 18757 (UC)	Hierro, Canary Islands, Spain	HM771804, HM771898, HM771992, HM772086
CIHier144	*Scleropodium tourettii*	Gonzalez-Mancelo 18755 (UC)	Hierro, Canary Islands, Spain	HM771805, HM771899, HM771993, HM772087
CILaPm146	*Scleropodium tourettii*	Gonzalez-Mancelo 18759 (UC)	La Palma, Canary Islands, Spain	HM771806, HM771900, HM771994, HM772088
CILaPm147	*Scleropodium tourettii*	Gonzalez-Mancelo 18767 (UC)	La Palma, Canary Islands, Spain	HM771807, HM771901, HM771995, HM772089
CITen148	*Scleropodium tourettii*	Gonzalez-Mancelo 18763 (UC)	Tenerifae, Canary Islands, Spain	HM771808, HM771902, HM771996, HM772090
CILaPm149	*Scleropodium tourettii*	Gonzalez-Mancelo 18751 (UC)	La Palma, Canary Islands, Spain	HM771809, HM771903, HM771997, HM772091
CASDg155	*Scleropodium tourettii*	Laeger 797 (UC)	Interstate 15, San Diego Co., CA, USA	HM771810, HM771904, HM771998, HM772092
ORMar159	*Scleropodium tourettii*	Norris 83279 (UC)	Willamette Natl. For., Marion Co., OR, USA	HM771812, HM771906, HM772000, HM772094
CASlo01	*Scleropodium* species B	Carter 1838 (UC)	Cerro Alto, S.L. Obispo Co., CA, USA	HM771721, HM771815, HM771909, HM772003
CASlo29	*Scleropodium* species B	Carter 1659 (UC)	Black Mtn., S.L. Obispo Co., CA, USA	HM771738, HM771832, HM771926, HM772020
CATul42	*Scleropodium* species B	Shevock 17645 (UC)	Sequoia Natl. For., Tulare Co., CA, USA	HM771749, HM771843, HM771937, HM772031
CATul56	*Scleropodium* species B	Shevock 12952 (UC)	Sequoia Natl. For., Tulare Co., CA, USA	HM771756, HM771850, HM771944, HM772038
CAFre57	*Scleropodium* species B	Shevock 12697 (UC)	Sequoia Natl. For., Fresno Co., CA, USA	HM771757, HM771851, HM771945, HM772039
CAMad58	*Scleropodium* species B	Shevock 17095 (UC)	O'Neals, Madera Co., CA, USA	HM771758, HM771852, HM771946, HM772040
CATul59	*Scleropodium* species B	Shevock 14602 (UC)	Mineral King Rd., Tulare Co., CA, USA	HM771759, HM771853, HM771947, HM772041
CATul61	*Scleropodium* species B	Shevock 15211 (UC)	Sequoia Natl. For., Tulare Co., CA, USA	HM771761, HM771855, HM771949, HM772043
CASlo89	*Scleropodium* species B	Carter 1402 (UC)	Cuesta Ridge, S.L. Obispo Co., CA, USA	HM771779, HM771873, HM771967, HM772061
CASlo91	*Scleropodium* species B	Carter 1414 (UC)	Cuesta Ridge, S.L. Obispo Co., CA, USA	HM771781, HM771875, HM771969, HM772063
CAVen135	*Scleropodium* species B	Sagar 580 (UC)	Santa Monica Mts., Ventura Co., CA, USA	HM771798, HM771892, HM771986, HM772080
CALAn156	*Scleropodium* species B	Shevock 21667 (UC)	San Gabriel Mts., Los Angeles Co., CA, USA	HM771811, HM771905, HM771999, HM772093

Table 1.1. Voucher specimens and Genbank accession numbers. Vouchers with an asterisk (*) were previously used by Carter (2010). Page 2 of 2.

A

Representative accession	Identity of position 483 in ITS alignment
S. touretii 04 (N. America)	AGAGCGTCA**C**AGGGTCG
S. touretii 100 (Europe)	AGAGCGTCA**T**AGGGTCG
S. touretii 126 (Europe)	AGAGCGTCA**C**AGGGTCG
S. touretii 129 (Macaronesia)	AGAGCGTCA**C**AGGGTCG
S. touretii 142 (Macaronesia)	AGAGCGTCA**Y**AGGGTCG
S. californicum 54	AGAGCGTCA**C**AGGGTCG
S. cespitans 03	AGAGCGTCA**C**AGGGTCG
S. julaceum 09	AGAGCGTCA**C**AGGGTCG
S. obtusifolium 28	AGAGCGTCA**C**AGGGTCG
S. species B 156	AGAGCGTCA**C**AGGGTCG

B

Geographic region	Number of individuals with each copy		
Present study	C	T	C&T
Europe/Medit.	6	9	0
Macaronesia	9	1	3
North America	23	0	0
Shaw et al. 2003			
Europe/Medit.	0	3	2
Macaronesia	1	0	0
North America	7	0	0

Table 1.2. Multiple ITS copies in *Scleropodium touretii*. A) Alignment of representative individuals of S. touretii and other species demonstrating the two ITS copy types (C and T) and an individual with both copy types (Y). All other species in the genus have a C at the same position. B) Geographical distribution of individuals with C, T or both copy types from the present study and from Shaw et al. (2003a).

	ITS		**Chloroplast**		**Combined**	
	All	Without 12,19,20	All	Without 12,19,20	All	Without 12,19,20
S. californicum	0.00	0.98	1.00	1.00	1.00	1.00
S. cespitans	0.32	0.96	0.00	0.11	0.00	0.32
S. julaceum	1.00	0.99	0.99	1.00	1.00	1.00
S. obtusifolium	0.09	0.09	0.93	0.98	0.99	0.99
S. touretii	0.00	0.00	0.12	0.99	0.05	1.00
S. species B	0.42	0.42	0.05	0.04	0.10	0.11

Table 1.3. Posterior probabilities supporting the monophyly of each species for six Bayesian phylogenetic analyses. Each dataset (ITS, chloroplast, combined) was analyzed using the full set of 109 individuals and a set of 106 individuals (without S. californicum 19, S. cespitans 12, S. touretii 20). The number of trees generating the post-burnin distribution in each analysis was 22,501.

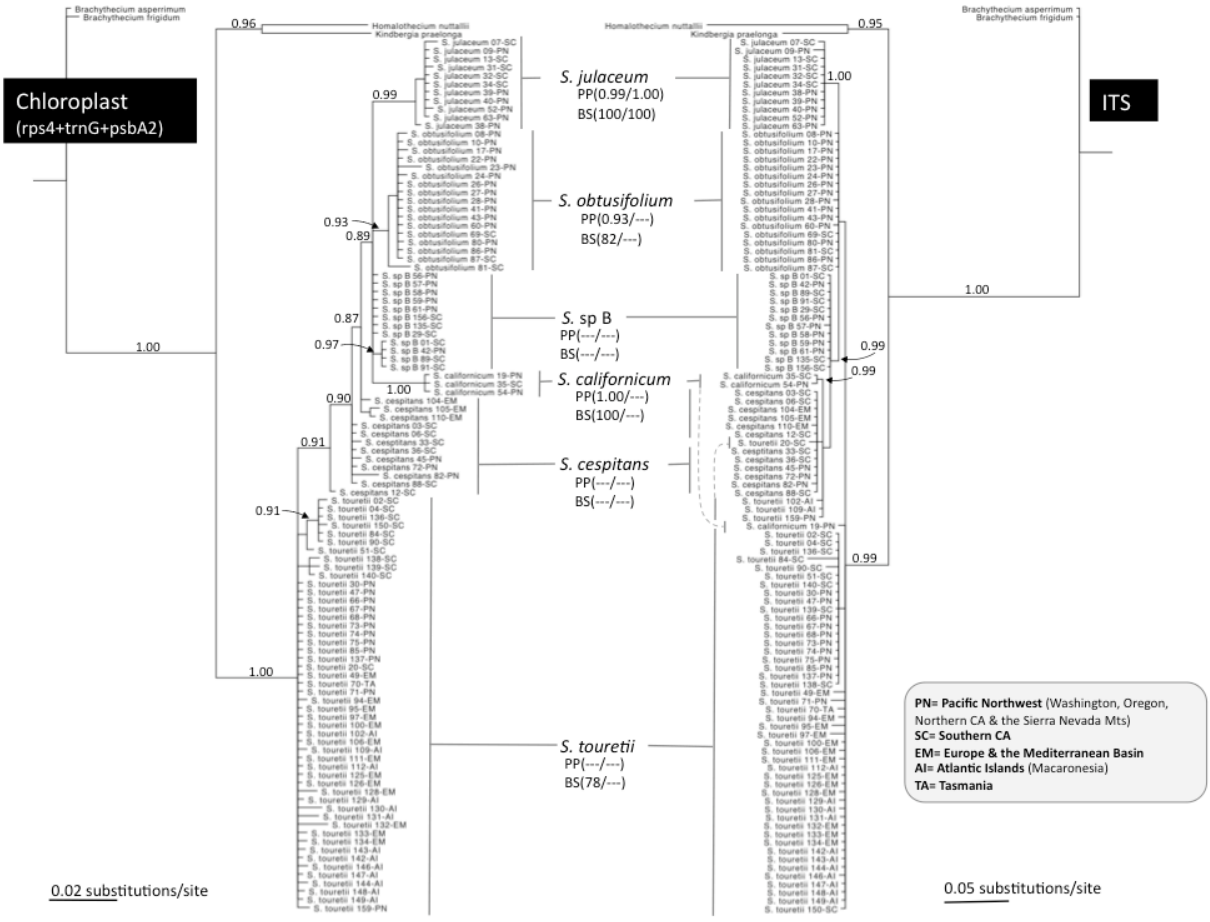

Figure 1.1. Fifty percent majority rule consensus trees from Bayesian analyses of ITS and chloroplast datasets. Support values are posterior probabilities. Support for the monophyly of morphologically circumscribed species (PP=Posterior probability, BS= Parsimony bootstrap) is indicated below the species names. Values below 0.85 and 70%, respectively, are not reported.

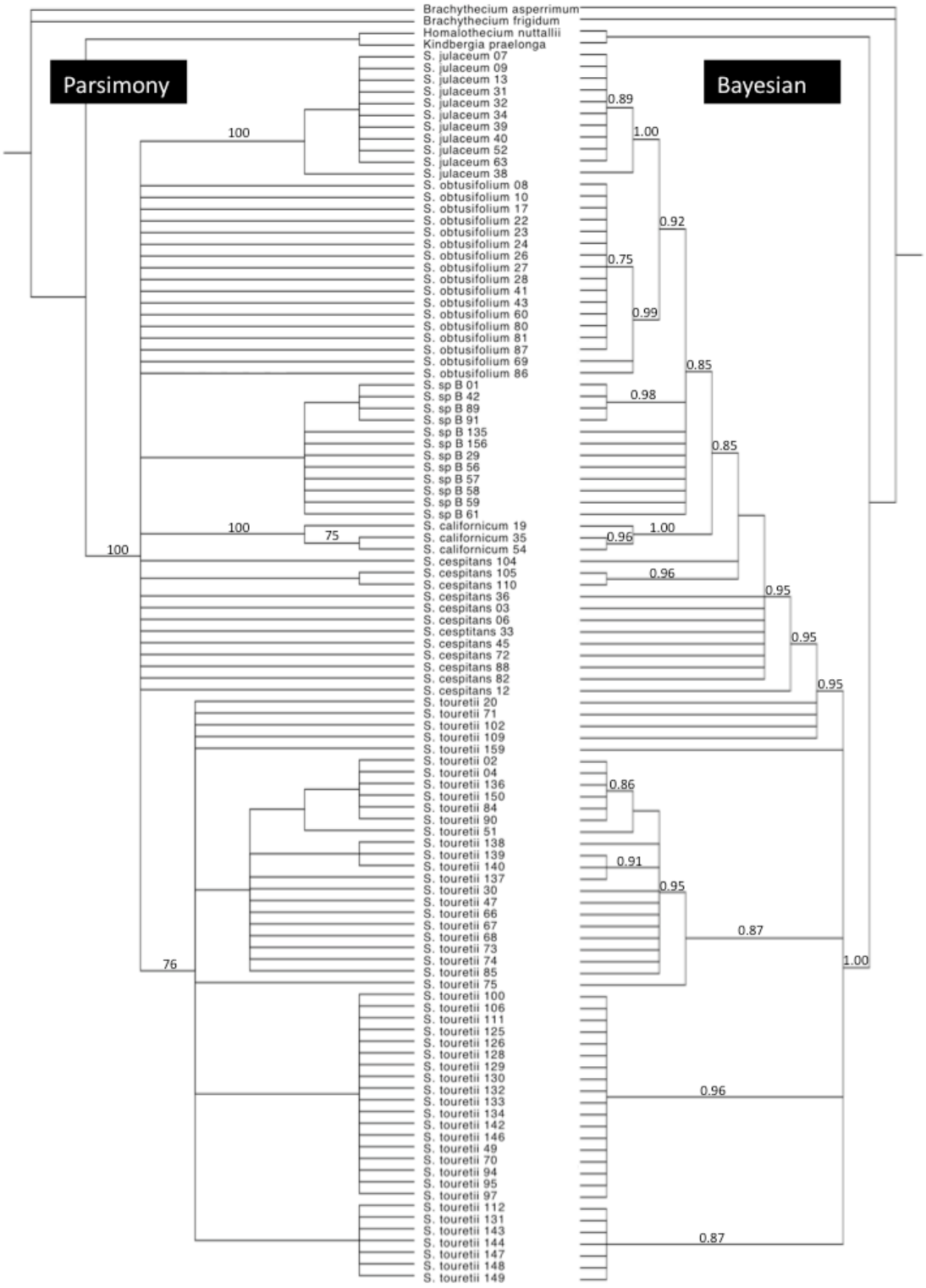

Figure 1.2. Strict consensus maximum parsimony tree (left) and fifty percent majority rule consensus tree from the Bayesian analysis (right) using a combined dataset of ITS, rps4, trnG and psbA2 sequences. Support values are bootstrap support (> 70%) and posterior probabilities (> 0.85), respectively.

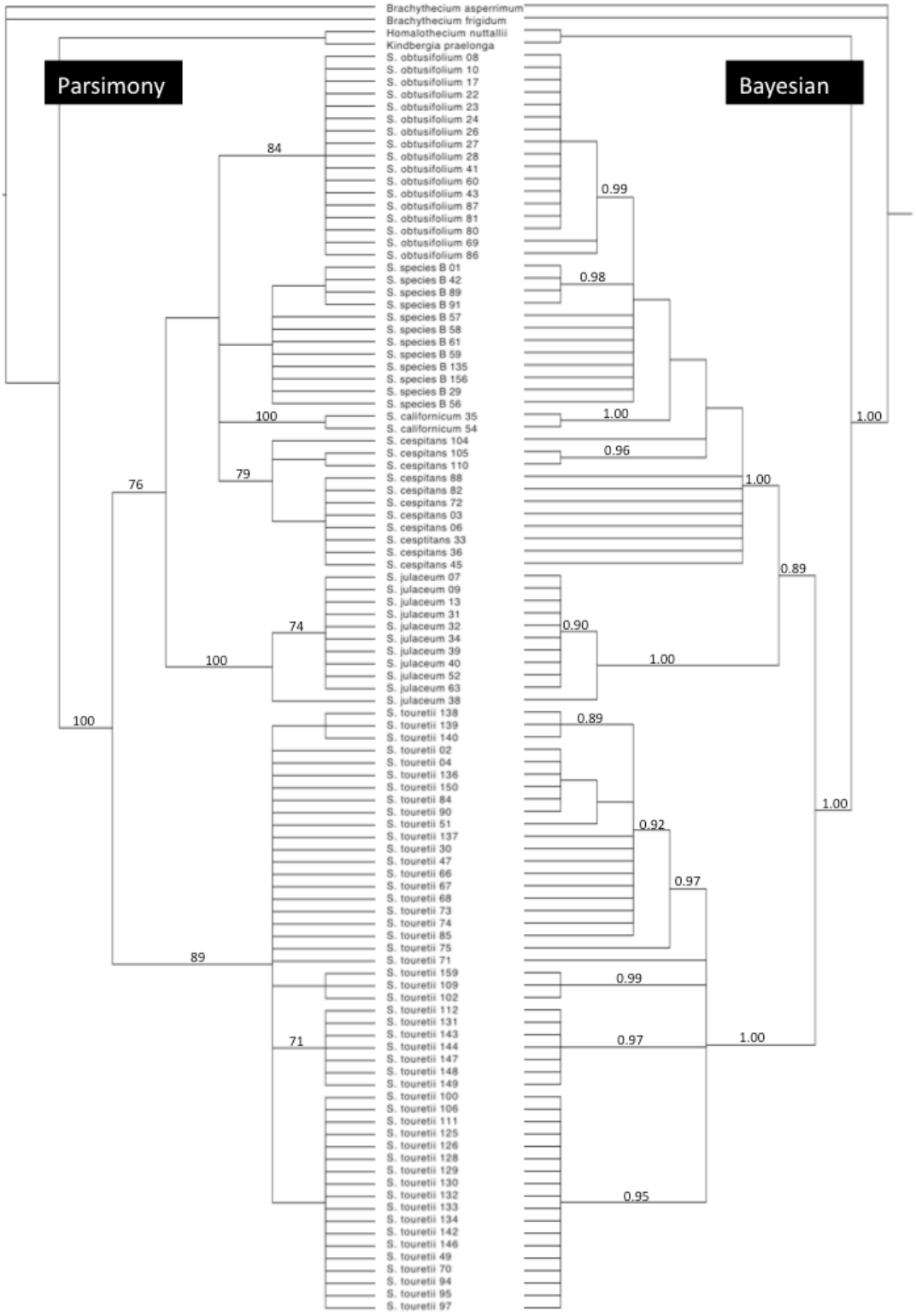

Figure 1.3. Strict consensus maximum parsimony tree (left) and fifty percent majority rule consensus tree from the Bayesian analysis (right) using a combined dataset of ITS, rps4, trnG and psbA2 sequences. Accessions *S. cespitans* 12, *S. californicum* 19 and *S. touretii* 20 were excluded from the analyses. Support values are bootstrap support (> 70%) and posterior probabilities (> 0.85), respectively.

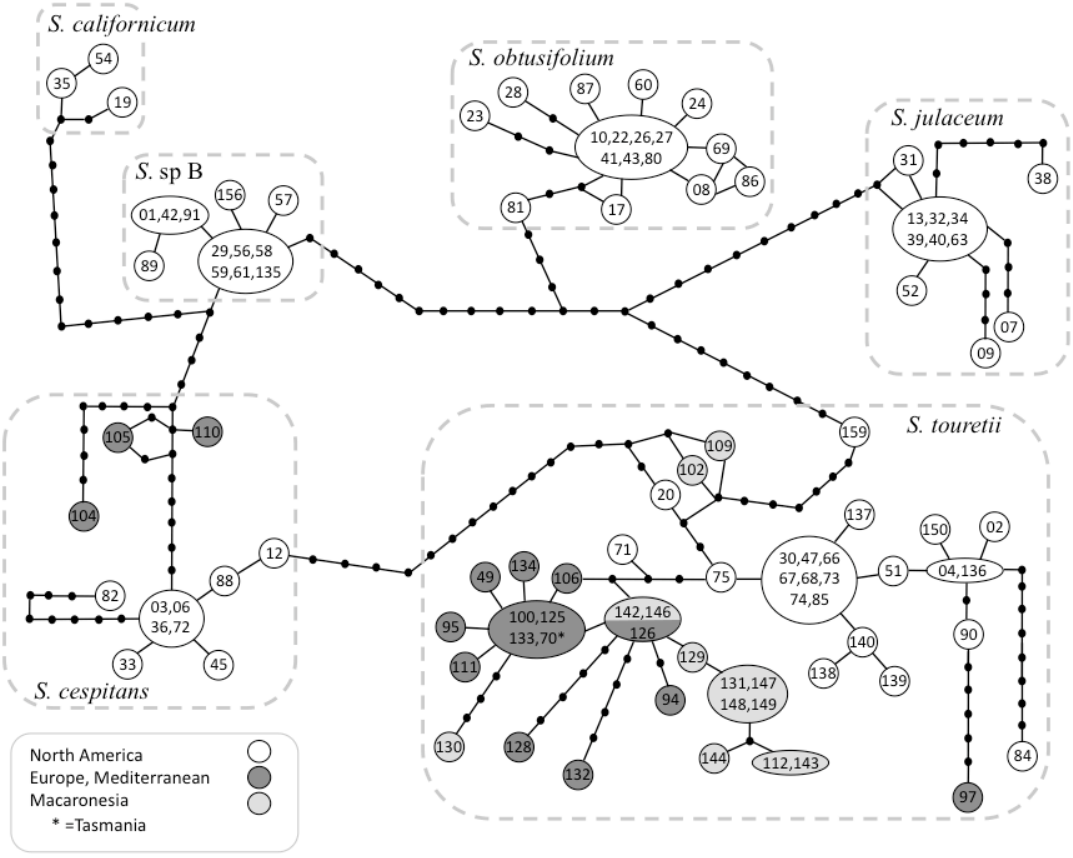

Figure 1.4. Statistical parsimony network for the combined dataset used in the phylogenetic analyses. Solid points indicate missing haplotypes. Dashed lines circumscribe morphologically defined species. Grayscale indicates geographic origin of the voucher specimens.

CHAPTER 2. A monograph of the moss genus *Scleropodium* (Brachytheciaceae)

Abstract

Scleropodium is a genus of six species with a native range including western North America, western Europe, the Mediterranean Basin, and Macaronesia. Informed by a recent molecular phylogeny of the genus, new descriptions, illustrations, range maps, and a key are presented. In addition, notes on morphological characters, ecology, and distribution, including several new state and country records, for all six species are provided based on field observations and examination of herbarium specimens from across the entire ranges of all species. Excluded taxa are discussed, including those earlier reported from Korea (*S. brachyphyllum* Cardot & *S. coreense* Cardot), Tasmania (*S. australe* Hedenäs), North America (*S. colpophyllum* (Sulliv.) Grout[= *S. touretii* var. *colpophyllum* (Sulliv.) Lawton]) and the Canary Islands (*S. touretii* var. *teneriffae* Card. et Wint.). In addition, a lectotype is designated for *S. cespitans* (Müller) Koch and a neotype for *S. touretii* (Bridel) Koch, and earlier nomenclatural confusion with both *S. cespitans* and *S. touretii* is clarified.

Introduction

The genus *Scleropodium* (Brachytheciaceae) is a small group of six to nine traditionally recognized species occurring in the north-temperate zone and Tasmania. Monophyly of the genus has been supported by large family-level phylogenetic analyses (Vanderpoorten et al 2005, Huttunen et al 2007). However, morphological species circumscriptions within the genus have been challenged recently as the number of available collections has increased from California, where all species in the genus are sympatric and appear to intergrade morphologically. This study employs the results of a recent molecular phylogeny (Carter, ms accepted) to clarify morphological species circumscriptions, clarify several nomenclatural problems, and discuss distributions and ecological preferences of the six currently recognized *Scleropodium* species.

The placement of *Scleropodium* relative to other genera within Brachytheciaceae remains a difficult problem. In a revision of the family based on morphology, Ignatov & Huttunen (2002) placed the genus tentatively in the Brachythecioideae, with *Kindbergia*, *Eurhynchiadelphus*, *Brynhia*, *Brachythecium*, *Sciurohypnum*, and *Unclejackia*. However, they noted that it was unclear to them whether the genus belongs in the Brachythecioideae or the closely related Homalothecioideae, which includes *Homalothecium*, *Brachytheciastrum*, and *Eurhynchiastrum*. Phylogenetic analysis of a combined dataset using ITS2, the chloroplast *psbT-H* and *trnL-F* regions, and 63 morphological characters underscored this ambiguous placement (Huttunen & Ignatov 2004). Phylogenetic analyses employing gaps in the chloroplast and ITS2 datasets recovered *Scleropodium* as the first to diverge within the Homalothecioideae, but an analysis with gaps treated as missing data resolved *Scleropodium* as sister to the Homalothecioideae + Brachythecioideae clade.

The instability at the base of the Homalothecioideae + Brachytheciodeae clade was substantiated by Huttunen et al. (2004) using a dataset overlapping with the Huttunen & Ignatov (2004) analysis. They found a *Scleropodium* + *Kindbergia* clade falling within the Homalothecioideae clade rather than within the Brachythecioideae clade. This result was substantiated by Vanderpoorten et al. (2005), who used an entirely different dataset (ITS and the chloroplast *rps4* & *atpB-rbcL*) in a recircumscription of *Brachytheciastrum* (Homalothecioideae). Their study recovered the *Scleropodium* + *Kindbergia* clade as sister to

Homalothecioideae, rendering Brachthyecioideae paraphyletic below Homalothecioideae. Based on this result they informally suggested the inclusion of Homalothecioideae within Brachythecioideae.

In the most recent study, Huttunen et al. (2007), using a dataset of *trnL*, *psbT-H*, and ITS2, found Brachythecioideae and Homalothecioideae to be reciprocally monophyletic, but without strong support. In this analysis, *Scleropodium* falls in a trichotomy with Brachythecioideae and Homalothecioideae. Given the instability of phylogenetic placement of *Scleropodium*, subfamilial placement of the genus is currently unclear, although at least two concrete conclusions can be drawn about the evolution of *Scleropodium* in the context of its close relatives. First, the Homalothecioideae + Brachytheciodeae + *Scleropodium* group is consistently resolved as a well supported group. Second, regardless of its exact placement, it is clear that *Scleropodium* diverged early in the evolution of this large Homalothecioideae + Brachythecioideae clade.

Like many genera in Brachytheciaceae, *Scleropodium* lacks well defined morphological synapomorphies. This problem has been recognized throughout the family (Robinson 1962, Ignatov & Huttunen 2002), and in *Scleropodium* specifically (Blockeel et al. 2005), with some of the problems due to taxonomic misinterpretations, but with many others caused by morphological convergence and phenotypic plasticity throughout the family. Most genera can be successfully described by combinations of characteristics, however. For *Scleropodium*, the most useful combination of characteristics is: lamina cells smooth, elongate and flexuose, leaf margins entire (less commonly minutely serrulate) in the proximal half and entire to serrate in the distal half, leaf plications absent, costa ending above mid-leaf in a spine (except in the aquatic *S. obtusifolium*), plants dioicous, seta typically papillose. As in other genera, variation among individual specimens is high, and specimens can be found that are not perfectly circumscribed by this combination of characteristics.

The genus has never been subjected to a global taxonomic treatment, but the species have been discussed in several regional taxonomic treatments and floras (North America: Grout 1899, 1928, Lawton 1967, 1971, Norris & Shevock 2004a,b, Malcolm et al 2009, Ignatov in prep; Europe: Smith 1978, 2004, Australia: Hedenas 2002, East Asia: Takaki 1955). The distribution of the genus includes the western half of the United States, extending into southwestern Canada and northwestern Mexico, Europe, from Great Britain to Spain and throughout the Mediterranean Basin from Turkey to Morocco, and Macaronesia (Canary Is., Azores, Cape Verde Is., Madeira). Two species, excluded in this treatment, have been described from the Korean peninsula in East Asia (see excluded species), and another species was described from Tasmania, although that species is now considered an anthropogenic introduction of European *S. touretii* (Carter 2010).

A recent molecular phylogenetic analysis found *Scleropodium* to contain six species (Carter ms accepted). That study sampled 104 individuals across the geographic distribution and morphological variation throughout the genus and resulted in a phylogeny employing ITS and the chloroplast *rps4*, *trnG*, and *psbA2* regions (Fig. 2.1). The analysis supported the monophyly of five traditionally recognized species, *S. californicum, S. cespitans, S. julaceum, S. obtusifolium*, and *S. touretii*, and also indicated the presence of a previously overlooked species, *S. occidentale* (Carter, in review).

The broad goal of the present study is to provide the first global taxonomic treatment of *Scleropodium*. Specific goals were to: 1) update the taxonomy of *Scleropodium* using information obtained from a molecular phylogenetic study of the genus 2) clarify the morphological circumscriptions of the recognized entities based on the molecular data and a

much larger set of specimens than was available for earlier treatments, 3) provide a key, illustrations, distribution maps, and ecological notes to aid in identification of specimens, and 4) clarify several nomenclatural problems.

Treatments

Scleropodium Bruch. & Schimp., Bryol. Eur. Fasc. 55-56. 1853.

Plants yellow to dark green, in thin to robust dense mats. Branching sympodial and irregular, branches often indistinguishable from young stems. Stems to 6cm, branches shorter, to 3cm. Stems and branches often curved distinctly either distally or proximally in most species. Shoots weakly to very strongly julaceous, attenuate or cylindrical, < 2mm in diameter (dry). Leaves strongly to not concave, deltate, oval, elliptic, ovate, or lanceolate; apex rounded to acuminate or cuspidate; margins plane, entire to distally serrulate or serrate. Costa ½ to 9/10 leaf length, occasionally forked distally, ending gradually or abruptly, ending in one or more spines (spine absent in *S. obtusifolium*). Decurrencies rare (except in *S. californicum*). Plications absent. Lamina leaf cells elongate hexagonal to long vermicular, 4-20:1, with rounded ends, shorter in leaf apex, 2-8:1; Cells in alar region differentiated, slightly inflated and thin walled or thick walled with regular or irregular thickenings, 0.5-3:1, irregular to rounded or rectangular, shorter and wider than lamina cells. Cells in leaf apex shorter than lamina cells.

Dioicous. Perichaetial leaves sheathing in proximal half, with distal half spreading to squarrose, lanceolate, ecostate. Seta red-brown to brown, usually papillose (often more strongly proximally than distally), sinistrose or not twisted, 5-22mm in length. Capsule <2.5mm, 1.5-3.5:1, erect to pendant, symmetric or slightly asymmetric, typically constricted just below mouth when dry (except *S. obtusifolium*). Stomata few, near base of capsule, pores broadly elliptic, 2:1. Exothecial cells irregularly rounded to rectangular with rounded ends, 10–25 μm × 25–75 μm. Annulus of 2–3 rows of cells. Peristome double. Exostome dark orange-red, striate below, papillose above. Cilia appendiculate (except *S. cespitans*). Operculum short conic to short rostrate. Calyptra cucullate, naked. Spores 11-19μm, smooth to very minutely papillose.

Growing on rocks, soil, and tree bases in moderate shade to full sun, less commonly in deep shade. Two species growing on rocks submerged or in splash zone of seasonal or year-round drainages or creeks. Several species common in urban areas.

Scleropodium californicum (Lesq.) Kindberg, Bryinearum exoticarum 1888, p. 35.

HOLOTYPE: *Bolander s.n., 20 Aug. 1865*. (NY). California, San Francisco.

Hypnum californicum Lesq., Trans. Amer. Philos. Soc. 13:13. 1865.

Plants light-green to yellow, in loose, thin mats. Stems mostly <5cm, branches mostly < 2cm. Stems and branches not consistently curved, diameter consistent throughout (not attenuate), 0.3-0.5(-0.7)mm in diameter, julaceous or not. Leaves not concave, 0.75-1.45mm × 0.35-0.70mm, ovate, less commonly lanceolate; apex long acuminate, acuminations appressed or slightly spreading; margins entire or occasionally minutely serrulate in distal 2/3. Costa ending gradually near base of acumen, spine-tipped in at least some leaves. Cells of the lamina vermicular with rounded ends, 7-15:1, 4-4.5μm × 40-70μm, slightly shortened in the apex, 4-10:1; juxtacostal

cells irregular with rounded ends, wider than lamina cells, in 2-5 rows from leaf base; alar cells more or less isodiametric and rounded, rectangular or slightly irregular, much wider than lamina cells, walls often thickened, forming 7-14 rows at leaf margin, in triangular patch abruptly differentiated from lamina cells.

Seta 9-14(-22)mm, red to reddish brown, generally twisted, roughened by prorate cells or smooth. Capsule more or less horizontal, <2mm, slightly to strongly curved and asymmetric. Spores 11-18μm, minutely papillose.

MORPHOLOGICAL NOTES (Fig. 2.2). This species is the least julaceous in the genus. In the field it can often be identified by its small size, relatively long internodes (the branch/stem often visible without removing leaves), and long-acuminate apices. When growing in its preferred habitat of soil among grasses and small forbs, it also exhibits a distinctively open, loose habit. In these situations it is more likely to be confused with *Homalothecium arenareum* or *Brachythecium albicans* than other *Scleropodium* species, but it is usually much smaller, and often less julaceous, than either of these two species. When growing on bare soil, it can be confused with *S. julaceum*, but that species typically lacks a long apiculus (but see discussion under *S. julaceum*).

Under the microscope, the species is usually easily identified by the presence of leaf decurrencies composed of typically 2-6 elongate rectangular cells. This feature is unique in the genus. Other useful characteristics include the long acumination of the leaves, the combination of many (usually >10) rows of quadrate cells in the alar region with relatively long laminal cells, and a costa that tapers gradually. The species also generally lacks any distal serration or serrulation except in very rare instances.

ECOLOGY & DISTRIBUTION (Fig. 2.3). This species appears to be restricted to fog influenced areas along the immediate coast from southern Oregon to northern Baja California, Mexico. Although previously reported from the Sierra Nevada (Norris & Shevock 2004), all voucher specimens from that region have been assigned to other taxa. It is common in central California in grassy areas, coastal scrub, coastal dune swales, and open oak woodlands, and exhibits a noticeable preference for well lit areas over heavily shaded areas. It commonly grows intermingled with grasses and forbs, a characteristic shared with the much larger *S. touretii*.

NOMENCLATURAL AND TAXONOMIC NOTES: The name most often applied to this taxon in the literature is *S. californicum* (Lesq.) Ren. & Card. 1893, Revue Bryologique 20:20, but Kindberg, in 1888, was the first to use the combination *S. californicum,* therefore the appropriate name is *S. californicum* (Lesq.) Kindberg. Sullivant & Lesquereux's Musci Boreali Americani no. 511 is not the type, although that collection is labeled as an isotype in PC and possibly other herbaria.

Scleropodium cespitans (Müller) Koch, Leafl. of Western Bot. 6:31. 1950.

LECTOTYPE: *Wilson, W. 1849. English Bot. Suppl. plate 2878* (NY!, HU, G) See nomenclatural notes section below for discussion.
Type locality: Near Warrington, England

Hypnum caespitosum Wilson, English Bot. Suppl. pl. 2878. 1849.
Hypnum cespitans Müller, Synopsis muscorum frondosorum omnium hucusque cognitorum vol. 2, fasc 7-10. 1851. non Pal.-Beauv., 1805
Hypnum lentum Mitt., Journ. Linn. Soc. 8:36. 1865.
Eurhynchium colpophyllum Sullivant. Icon. Musc. Suppl. 95. *pl. 71*. 1874
Eurhnchium macounii Kindb. Rev. Bryol. 22:85. 1895.
Eurhynchium colpophyllum var. *flagelliforme* Barnes, Bot. Gaz. 16:207. 1891.
Brachythecium appleyardiae McAdam & Smith, J. Bryol. 11:591-598. 1981

Plants olive to yellow green, forming dense mats. Stems mostly <3cm long, branches < 2cm long. Stems and branches often curved strongly in the proximal half, typically strongly attenuate, 0.5-0.9mm in diameter at broadest point, strongly julaceous. Leaves slightly to strongly concave, 1.1-1.6mm × 0.4-0.8mm, ovate to narrowly lanceolate or oblong; apex acuminate in mature stem leaves to broadly cuspidate or obtuse in branch leaves; margins entire or distally serrate. Costa narrowing gradually in mature leaves to ending abruptly in one or more spines in branch leaves, 70-95% leaf length. Cells of lamina vermicular with rounded ends, 7-12:1, 5-6μm ×45-65μm, shortened in apex; juxtacostal cells wider than lamina cells, in 2-3 rows; alar cells thin-walled and inflated or smaller, isodiametric and thick walled in 5-8 rows near the margin.

Seta 7-12mm, orange to brown, twisted, roughened by prorate cells, especially below. Capsule cylindrical to slightly asymmetrical, 2-4:1, 1-2mm × 0.3-0.7mm, erect to inclined. Spores 15-19μm, smooth to minutely papillose.

MORPHOLOGICAL NOTES (Fig. 2.4). This is a variable species that can be quite difficult to identify. Smaller specimens are easily confused with *S. julaceum* and *S. californicum*, while robust specimens can be confused with *S. touretii*. In the field, well developed *S. cespitans* branches show a combination of strong proximal curvature (often more than 90 degrees) and attenuation. Each branch is both strongly curved and widest in diameter near the point of attachment to the stem and then straightens and narrows gradually toward the shoot apex. Individuals with short branches or with less julaceous branches may not exhibit this combination of characteristics, but when present, it is highly diagnostic. This species also produces sporophytes more readily than the other species in the genus, and it is the only species with consistently +/- erect capsules (this character state is present but uncommon in *S. julaceum*).

Under the microscope, the species can usually be differentiated from *S. julaceum* and *S. californicum* by the fewer rows of quadrate cells in the alar region. In comparison with *S. touretii*, the leaves of *S. cespitans* are generally smaller and the lamina cells are shorter.

ECOLOGY & DISTRIBUTION. In the Old World, this species is widespread from northwestern Europe through the Mediterranean Basin, but is apparently relatively uncommon throughout most of that range. It has been reported from England, Ireland, Scotland, Wales, Belgium, Corsica, France, Italy, the Netherlands, Portugal, Sardinia, Spain, Turkey, Tenerife (Smith 2004).

In North America, it is common along the Pacific coast, from southern California to British Columbia. The species also occurs sporadically in the Sierra Nevada, but is much less common there than in the coastal regions of California (Fig. 2.5).

This is the only species in the genus that has a decided preference for a woody substrate. Approximately half of the specimens examined were collected from tree bases and tree trunks (mostly *Quercus* and *Salix*). It is common in live oak woodlands, coastal scrub, and *Salix* thickets. Inland, it is associated with riparian corridors. This is the only species in the genus that commonly is collected with sporophytes in North America (about 40% of specimens examined compared to 15-20% for the other species). In Britain and Ireland, sporophytes are reported as being "extremely rare" (Hill et al. 1994). Sporophyte capsules are always erect to slightly inclined, which is unique in the genus (except for very uncommonly in *S. julaceum*). The species is common as an urban weed on concrete walls and in irrigated horticultural plots (especially in coastal areas) in both its Old World and New World distribution.

NOMENCLATURAL AND TAXONOMIC NOTES: Several misunderstandings regarding the nomenclature and the type of *S. cespitans* have been propagated through the literature. The species was first recognized as *Hypnum caespitosum* by W. Wilson in 1841 (Wilson in Hooker 1841. Journal of Botany v. III p. 385): "This yet unpublished species, nearly allied to *H. blandum* [=*Scleropodium touretii*], but with an erect capsule, secund foliage, though rather abundant near Warrington, has not been elsewhere observed." Shortly thereafter, the name was formally published (Wilson, 1849. English Botanical Supplement, pl. 2878). The name *H. caespitosum* had already been used (*H. caespitosum* (Hedw.) Schrad. 1803, J. Bot. (Schrader) [Basionym: *Leskea caespitosa* Hedw., Spec. Musc. 1801, pl. 49: f. 1—5]), so *H. caespitosum* Wilson is an invalid later homonym. The name *Hypnum caespitosum* had also been invalidly published for a separate entity by Pallisot-Beauvois in 1805.

Carolo Müller apparently recognized that *H. caespitosum* Wilson was invalid, and in 1851 he published the name *H. cespitans* with direct reference to Wilson's *H. caespitosum*:

> ***251. H. cespitans*** C. Müll.; *dioicum*; cespites latissimi decumbentes, viridissimi vel atro-virides; caulis longe repens, ramis brevibus teretibus strictis confertis subtenuibus assurgentibus vix ramulosis pinnatim vage divisus; folia caulina conferta appressa, *haud secunda, humore erectopatentia*, subrotundate ovalia, breviter acuminata, concave, estriata, margine basi parum revoluto, apice vix conspicue denticulato, nervo ultra medium evanido viridi, cellulis brevibus *angustissimis* plerumque sordide chlorophyllose virentibus mollibus, alaribus *vix conspicuis* quadratis; perich. Pauca appressa, e basi vaginante *minute tenerrime* reticulata pellucida enervi breviter stricte et acute acuminata, integerrima; theca in ped. Breviusculo rubente *ubique papilloso erecta cylindraceo-oblonga angusta brevis*, late annulata, oper. *conico brevi obtuso apiculato*; persit. d. ext. rufescentes, superne subserrulati, int. angusti perforati flavi leaves, ciliis ***1-2*** *brevissimus laevibus*.
>
> *H. caespitosum* Wils. Engl. Bot. Suppl. t. 2878.
>
> *Patria*. Anglia, in muris prope Warrington Lancashire, ubi Wilson detexit, quocum Martio 1847 legit W.P. Schimper, qui nobis benevole communicavit. Ad arborum radices in pratis irriguis arenaque suffuses prope Aquas Tarbellicas Pyrenaeorum occidentalium, ubi in planitie legit Spruce, cujus specimina nostro Montagne debemus. *H. murale* habitu peraffine, sed notis laudatis primo adspectu distinguendum et pulchra species.
>
> (Müller, Carolo. 1851. Synopsis Muscorum Frondosum omnium hucusque cognitorum. vol.2. Berlin. Sumptibus Alb. Foerstner. pp 354-355.)

In the above protologue no type specimen is designated for the name *H. cespitans* Müller, but the unambiguous reference to *H. caespitosum* Wilson indicates that Müller regarded the two names as applying to a taxon of identical circumscription. The type specimen of *H. cespitans* Müller therefore should be the type for the invalid *H. caespitosum* Wilson. However, Wilson (1849) also failed to designate a type in the protologue for *H. caespitosum*:

2878. HYPNUM caespitosum
Tufted Feather Moss

CRYPTOGAMIA Musci

Gen. Char. *Fruit-stalks* lateral. *Peristome* double: outer one of 16 teeth; inner, a membrane cut into 16 equal segments, and usually with intermediate filiform processes. *Calyptra* dimidiate.
Spec. Char. Stems creeping, with short, simple, incurved branches. Leaves ovate, concave, spreading, second, serrulate, with plane margins, nerved above half way. Fruit-stalks rough. Capsule erect, oblong. Lid conical, subrostrate.

This new species was gathered in Nov. 1836 at Longford, near Warrington, where both previously and since it has been observed, growing abundantly in fruit, and in extensive patches upon walls built of sandstone, in places where it is exposed to inundation, in company with *H. rutabulum*. It occurs also about the roots of trees in the same neighbourhood, and in a few similar situations elsewhere about Warrington, but mostly in a barren state. More recently this moss has been observed near Frodsham in Cheshire, in places not exposed to inundation. It has been carefully observed for several years, and found to be constant in the above characters. It is therefore now, with Dr. Taylor's concurrence, proposed as a new species. It differs from *Hypnum rutabulum* in its second, glossy, patent and more rigid foliage, which is by no means acuminated, but rather obtuse, and when dry exhibits no appearance of striae. When growing it much resembles *H. blandum*, (*H. illicebrum*, *Schwaegr. Suppl. v. i. P.2.225*,) and, as in that species, the branches are incurved. It differs from both in the much more erect capsules, which are of a more oblong shape. In the Frodsham specimens the capsule is more elongated and slightly curved, and the lid has a short inclined beak. – W. W.

(Wilson, W. 1849. English Botanical Supplement. vol. 4. plate 2878)

An attempt was made in this study to find specimens unambiguously associated with the protologue of *Hypnum caespitosum*, but was unsuccessful. However, Wilson's plate 2878 clearly shows the erect-inclined capsule, short lamina cells, and distal leaf serration that differentiate that taxon from *Scleropodium touretii*, the only other *Scleropodium* in Europe.
Under article 7.3 of the ICBN, an appropriate course of action is the designation of Wilson's plate 2878 as the lectotype for the name *H. caespitosum* Wilson. Because Müller's *H. cespitans* is based directly on *H. caespitosum* Wilson, Wilson's plate 2878 can be considered the lectotype for *H. cespitans* Müller.

Müller's protologue for *H. cespitans* includes mention of two specimens that are still in existence in the Kew collections on permanent loan to BNHM. These are the Spruce collection from the Pyrenees Mts. (no date or collector number) and the Schimper collection from March of 1847. Both specimens are identifiable as *Scleropodium cespitans* and the Schimper collection has sporophytes in good condition. These two specimens appear adjacent to one another on the same herbarium sheet.

Hypnum cespitans Müller was transferred to *Scleropodium* by L. Koch (Koch, 1950. Mosses of California: an annotated list of species. Leaflets of Western Botany 6:1-40.[p. 31])

Scleropodium colpophyllum has been interpreted differently as either a subspecies of *S. touretii* (Lawton 1967, 1971, Sharp 1995, Ignatov (in prep), as a unique species (Grout 1899, 1928, Norris & Shevock 2004a,b), or as a synonym of *S. cespitans* (Robinson 1962). Reinterpretation of the morphological variation in *S. cespitans* and *S. touretii* has been possible using vouchers from an earlier molecular analysis that clearly differentiated these two species (Carter in review). Based on this new evidence, it is clear that there is a considerable amount of overlap in the morphological variation of *S. cespitans* and *S. touretii*. Based on examination of keys, species descriptions, specimens identified as *S. colpophyllum* by various authors, and my own experience in the field, it is clear that the name *S. colpophyllum* has been used (and used differently by different authors) to accommodate the overlap in variation between *S. cespitans* and *S. touretii*. The result is that some workers (e.g. Grout 1899) have believed that *S. colpophyllum* is related to *S. cespitans* (because their concept of *S. colpophyllum* included mostly characteristics of *S. cespitans*), while others (e.g. Lawton 1967) have argued that *S. colpophyllum* is closer to *S. touretii* (because their concept included mostly characteristics of *S. touretii*). The molecular data are clear, however, in that specimens fall out into two groups corresponding to *S. cespitans* and *S. touretii*. A clearer picture of the morphological variation expressed by these two species, as is presented in this paper, eliminates the need for this confusing name. The type of the basionym *Eurhynchium colpophyllum* Sullivant, at FH, was not examined in this study; however, Sullivant's plate 71 in the protologue is unambiguously *S. cespitans* based on the short leaf cells and narrow, erect capsules.

Scleropodium julaceum Lawton, Bull. Torrey Bot. Club 1967

> HOLOTYPE: *MacFadden 18595* (DUKE). California, Los Angeles Co., Sepulveda Canyon, on rock. March 1939.
> Isotype at CAN

Paratypes: California, *Los Angeles Co.*: Sepulveda Canyon, *MacFadden 18593* (MACF); Mint Canyon, *MacFadden 8035* (MACF, DUKE); Pasadena, *Palmer s.n.* (DUKE); Wolfskill Canyon, *R.R. Inglis 30 Apr. 1944* (MACF); Arroyo Seco, *N. Sweet 346* (MACF), Kingman 6 May 1911(FH); San Gabriel Mts, Eaton's Canyon, Kingman 11 Feb 1910 (FH, DUKE); Millards's Canyon, Kingman 18 Dec 1909 (FH); Rubis Canyon, Kingman 18 Feb 1911 (FH). *Tulare Co.*: near Sequoia Park, *MacFadden 21863* (MACF, CAN). *San Diego Co.*: 82 miles n.e. of Lakeside, *Wiggins, 9 Apr. 1954* (CAN). *Alameda Co.*: Univ. of CA, Berkeley Campus, *Hermann 17303* (CAN). *Mendocino Co.*: Little Lake Valley, *Branscomb 159* (MACF).

Plants olive green to dark green, in dense mats. Stems mostly <3cm, branches mostly < 2cm. Stems and branches not consistently curved, diameter consistent throughout (not attenuate), diameter <0.5(.75)mm, typically strongly julaceous, slightly less so in poorly developed specimens. Leaves not concave, 0.3-0.8mm × 0.5-1.2mm, ovate to broadly-ovate; apex typically acute, ranging from acuminate to obtuse; margins serrulate to serrate distally or entire throughout. Costa strong, ending abruptly in one or more spines, 50-85% leaf length; cells of lamina elongate hexagonal to short-vermicular, 1:4-8, 5-8μm × 30-45μm, cells in apex shorter, rhomboidal to elongate hexagonal with rounded corners, 1-3:1; juxtacostal cells wider than lamina cells, irregularly rectangular to hexagonal in 2-5 rows from leaf base; alar cells 1-2:1,

rounded to rectangular, wider than lamina cells, thick or thin walled, in 9-17 rows at the leaf margin.

Seta 5-8mm, twisted or not, brown, roughened by prorate cells or smooth. Capsule slightly asymmetric to cylindrical, <1.5mm, horizontal to inclined. Spores 12-19μm, smooth to minutely papillose.

MORPHOLOGICAL NOTES (Fig. 2.6). This species is often recognizable in the field by the combination of its small size, tightly julaceous branches, and lack of long acuminate apices. On typical specimens, branches have tightly imbricate leaves and the diameter of the (dry) leafy shoot does not vary much over most of the length of the branch, in contrast to *S. cespitans*, which tends to have leafy shoots that are much larger in diameter at the proximal end with a gradual tapering toward the shoot apex.

Under the microscope, *S. julaceum* has several good diagnostic features. The short, often almost hexagonal lamina cells are unique in the genus. The strong, abruptly ending costa, usually with one or more distal spines, is useful in separating the species from *S. californicum*, with which it shares several rows of quadrate cells across the leaf base. Throughout most of its range, the species also has a characteristic ovate leaf shape with a blunt apex. In very wet areas, especially along the immediate coast and in urban settings, the species develops an apiculus and strongly resembles *S. californicum*. In these cases, the lamina cell shape is usually sufficient to diagnose the species.

ECOLOGY & DISTRIBUTION (Fig. 2.7). Although only described fairly recently (Lawton 1967), this is a common species throughout drier regions of cismontane California. Previously, the species was recorded only from central and southern coastal California. An improved understanding of the morphological differences between this species and *S. cespitans* has resulted in the extension of the known distribution northward to Vancouver Island and inland to the foothills of the Sierra Nevada. Although present in Oregon, Washington, and British Colombia, all collections from outside of California, with the exception of several from the southern coast of Oregon, are from well populated areas. The species is also a common weed of garden beds in northern and central California, so it is possible that the northern end of the range is the result of human introduction. In the south, the species is not yet known from Mexico, but is expected in the Mediterranean climatic region of northwestern Baja California below San Diego.

This species has a wide ecological niche breadth and can be found on rocks, soil, or the base of hardwoods in full sun or, less commonly, deep shade. It is most common in mesic to very dry oak woodland and chaparral communities and is rare in the coniferous communities of northwestern California and the Sierra Nevada. There is apparently only modest ecological overlap between *S. julaceum* and *S. californicum*, with *S. julaceum* common in dry woodlands away from the immediate coast and S. *californicum* primarily in more mesic woodlands and coastal scrub communities that receive more fog. *Scleropodium julaceum* and *S. touretii* can often be found growing together in mixed patches, often with *Claopodium whippleanum*, but the size difference between *S. julaceum* and *S. touretii*, even in the field, easily separates the two species.

NOMENCLATURAL AND TAXONOMIC NOTES. This taxon was described relatively recently (Lawton 1967), but many older collections of it exist, often under the names *S. cespitans* var. *sublaeve* or *S. apocladum*. The relationship of these two names to the current

circumscription of *S. julaceum* is discussed thoroughly by Lawton (1967). The holotype of this species is at DUKE, but duplicates were distributed widely in 1942 as Grout's North American Musci Pleurocarpi Supplement no. 70 (as *S. apocladum*). In the protologue of *S. julaceum*, no mention is made of Grout's exsiccati, but Lawton indicated that she examined isotypes of *MacFadden 18595* at CAN and MACF. The MACF collections were integrated into LAM, which was subsequently integrated into UC. The MACF isotype examined by Lawton has been lost; however, one of Grout's exsiccati no. 70 originally sent to UC remains in that herbarium, as do all of the MACF paratypes cited by Lawton (1967), including *MacFadden 18593*, which was collected at the same place and on the same date as the type, *MacFadden 18595*. A specimen from Mint Canyon, Los Angeles County, cited by Lawton in her protologue, was distributed by Grout in his N. Amer. Musc. Pleuro. Suppl. as no. 35. The original MacFadden specimen examined by Lawton is at UC, as is one of Grout's no. 35.

Scleropodium obtusifolium (Mitt.) Kindb. in Macoun, Cat. Canad. Pl. 6: 202. 1892

HOLOTYPE Drummond's Musci Americani no. 193. (BM!)
Type Locality: Canadian Rocky Mountains, Jasper National Park, junction of Snake Indian and Athabasca Rivers (see Bird 1967)
Isotypes at BM(!), FH(!), G

Hypnum obtusifolium Hook., Drumm. Musc. Am. no. 193.
Stereodon obtusifolius Mitt., Journ. Linn. Soc. 8: 42. 1865.
Rhynchostegium obtusifolium (Mitt.) Jaeg., Ber. St. Gall. Naturw. Ges. 1876-77:377. 1878.

Plants yellow green to light green, forming dense mats. Stems to 10cm, often anchored to substrate with leaves degraded by erosion, branches typically <2cm. Stems and branches often hooked or curled distally, diameter consistent throughout (not attenuate), to 2mm in diameter, strongly julaceous, with leaves appressed. Leaves concave (0.6-)0.8-1.6mm × (0.6-)1.3-2.6mm, broadly oval to ovate, occasionally orbicular; apex rounded to obtuse, occasionally cuspidate; margins entire, rarely serrulate at extreme apex, often distally broadly inrolled. Costa gradually tapering, 70-85% leaf length. Cells of leaf lamina vermicular with rounded ends, 7-18:1, 5-7μm × 45-80μm, shorter in leaf apex, >2:1; juxtacostal cells enlarged, irregular, thin or thick walled, alar cells generally enlarged, occasionally smaller, slightly to much-inflated and thin-walled, if thick-walled then walls unevenly thickened, in <8 rows near margin.

Seta 9-16mm, orange to brown, twisted or not, roughened by prorate cells or not. Capsule short-cylindrical, symmetrical, 1.2-2.0 x 0.8-1.1mm, horizontal to pendant. Spores 15-19μm, minutely papillose.

MORPHOLOGICAL NOTES (Fig. 2.8). This species has, until recently, not been much discussed because its very different ecological preferences are reliable for diagnosis. A notable exception is Grout's (1899) comment: " There can be no doubt that this [*S. obtusifolium*] should be regarded as a subspecies of *S. illecebrum* [=*S. touretii*]. The plants nearest the type are always submerged and nearly always sterile. A complete series can be traced from the typical form described above to typical *S. illecebrum*." The comment was repeated in Grout's 1928 treatment of the genus, but was not addressed in later treatments (Lawton 1967, Lawton 1971, Norris & Shevock 2004b). The recently described *S. occidentale* is in some ways intermediate between *S.*

touretii and *S. obtusifolium*, and it may be that Grout was examining specimens attributable to that taxon.
Because of its large size, *S. obtusifolium* is only likely to be confused with *S. touretii* and *S. occidentale*. It can be differentiated from *S. touretii* based on the habitat, an entire distal leaf margin, and usually the lack of a leaf acumination, although *S. obtusifolium* can have cuspidate leaves. From *S. occidentale*, it differs in usually lacking a spine at the tip of the costa and generally having slightly larger leaves that are slightly more oval-ovate compared to the lanceolate-ovate leaves of *S. occidentale*.

Norris and Shevock (2004b) identified a variant of *S. obtusifolium* as *S.* "species A". That entity has leaves wider than long, and has both leaves and cells of the lamina much shorter than those of typical *S. obtusifolium*. In addition, *S.* "species A" has reduced internodes, stiffened branches, and strongly concave leaves, giving leafy shoots a distinctly rigid and tightly julaceous appearance. The entity is known from several collections, primarily in the central and southern Sierra Nevada.

Comparison of DNA sequences (ITS and the chloroplast *rps4*, *trnG*, and *psbA2* regions) of four specimens representing the *S.* "species A" morphology to typical *S. obtusifolium* revealed that there were no differences between *S.* "species A" and several typical *S. obtusifolium* accessions over the 2544 base pair dataset (Carter, ms accepted). Close morphological examination of several hundred specimens from across the range of *S. obtusifolium* suggest to me that *S.* "species A" represents the extreme end of the broad spectrum of morphological variation typical of many aquatic bryophytes. Based on these results, *S.* "species A" is not recognized in this treatment. Voucher specimens conforming to this morphology are listed below under *S.* "species A" and Genbank numbers for these vouchers were listed by Carter (in press).

Vouchers for the *Scleropodium* "species A" morphotype (sensu Norris & Shevock 2004b)
Specimens below are housed at UC.
CALIFORNIA. Fresno Co., *Shevock 14505*, *Shevock 12831*; Mariposa Co., *Shevock 29993*; Calaveras Co., *Norris 99429*; Mendocino Co., *Carter 2964*.

ECOLOGY & DISTRIBUTION (Fig. 2.9). *Scleropodium obtusifolium* has a broader North American distribution than the other species in the genus. Common from Vancouver, British Columbia to the mountains of southern California, it also occurs throughout the Rocky Mountains, although it is apparently less common in the Rockies than it is in coastal states. The species is represented by at least several records in all the Rocky Mountain states except for Colorado, where a recent floristic treatment (Weber & Whitmann 2007) found no specimens to document the presence of the species in that state. Ecologically the species is restricted to aquatic and semi-aquatic sites with fast moving water. It reaches its greatest abundance in small to medium sized creeks in which it grows on rocks that are seasonally submerged or in the splash zone. Occasionally the species can be found in steep drainages through relatively arid habitats including chaparral and *Quercus douglasii* woodland, where individuals are dry and dormant for at least June-September each year.

NOMENCLATURAL AND TAXONOMIC NOTES. The first name published for what is currently recognized as *Scleropodium obtusifolium* (Mitten) Kindberg in Macoun was *Hypnum obtusifolium* Hooker. A protologue was not published in a volume, but distributed with T.

Drummond's Musci Americani (Rocky Mountains) in 1828. Drummond's number 193 is labeled:

> 193. Hypnum obtusifolium, *nov. sp.* caulibus laxe ramosis, foliis undique imbricates valde concavis obtusissimis integerrimis, nervo ante apicem evanescente.
> Hab.—In a rivulet on the Rocky Mountains

The name *Hypnum obtusifolium* Hooker (1828) is an invalid later homonym of *H. obtusifolium* (Turner) Bridel, published in 1812. The next available name for *H. obtusifolium* Hooker is *Stereodon obtusifolius* Mitten 1864. That name was published without a protologue and was published by Mitten as *Stereodon obtusifolius* Hook. et Wils., apparently as a replacement name for Hooker's *Hypnum obtusifolium*. In writing up the bryophytes in Macoun's 1892 Catalog of plants of the 49th parallel, Kindberg made the new combination *Scleropodium obtusifolium* (Mitten) Kindberg. For the current study, Drummond's number 193 was examined at BNHM (on permanent loan from K).

Scleropodium occidentale Carter, The Bryologist *in press*

> HOLOTYPE: *Carter 1838* (UC) California, San Luis Obispo Co., Cerro Alto Campground. 4 May 2007. Isotype at NY.

Plants yellow green to dark green, forming dense mats. Stems typically <2.5cm, occasionally to 5cm. Branches typically <l.5cm. Stems and branches slightly to strongly curved, with strongest curvature near middle or proximal (not distal), typically attenuate, 0.8-1.2mm in diameter at widest point, julaceous. Leaves slightly concave, 1.0-1.7mm × .50-1.15mm, ovate, occasionally lanceolate to elliptic; apex acute to rounded or cuspidate, occasionally acuminate; margins entire or with serrulations restricted to cusp/acumination, often widely inrolled distally. Costa ending gradually or abruptly in a spine, (50-)70-95% leaf length. Cells of lamina vermicular with rounded ends, 8-12:1, 4-7μm × 45-60μm, cells in apex shorter, 2-5:1; juxtacostal cells irregular, enlarged, in 2-3 rows from leaf base; alar cells irregular or rectangular 1-5:1, wider than lamina cells, with walls thickened or cells slightly inflated, in 2-5 rows near margin.

Seta 11-15mm, orange to brown, twisted or not, rough throughout by prorate cells or becoming smooth distally. Capsule symmetric, strongly to slightly strangulate, 1.8-2.5mm × 0.5-0.9mm, horizontal to pendant. Spores 15-19μm, minutely papillose.

MORPHOLOGICAL NOTES (Fig. 2.10). This species can only be confused with *S. obtusifolium*, based on both habitat and leaf morphology. The combination of large leaves with entire distal margins sets these two apart from the other members of the genus. The most reliable single character differentiating the two species is presence or absence of a spine at the tip of the costa. The spine is formed from the most distal cell of the costa being unattached to the lamina for about half its length. Visualization requires a leaf whole mount with the abaxial side up and focusing up and down through the leaf under high magnification. This characteristic is present on at least some leaves (usually not all leaves) of roughly 90% of specimens of *S. occidentale* examined. In *S. obtusifolium*, this characteristic is very uncommon. The leaf apex in *S. occidentale* is typically acute, but occasionally becomes acuminate or apiculate. Leaf apices in *S. obtusifolium* tend to be broader, obtuse to truncate or less commonly apiculate to cuspidate. The

leaf shape in *S. occidentale* also tends to be ovate in comparison with the ovate to oval or broadly elliptic leaves of *S. obtusifolium*.

ECOLOGY & DISTRIBUTION (Fig. 2.11). This species is documented primarily from within cismontane California, with several additional records extending to southwestern British Columbia and Nevada. It appears to prefer slightly drier habitats than does *S. obtusifolium*. Most specimens of *S. occidentale* are from seasonal drainages or creeks in oak woodlands or chaparral and occupy the driest extreme of the habitats occupied by *S. obtusifolium*. It may be that *S. occidentale* even replaces *S. obtusifolium* ecologically, but more detailed work needs to be done to confirm this. In the southern Sierra Nevada, which has been well collected by J. Shevock, it is clear that there are differences in the elevational distributions of the two species. *Scleropodium obtusifolium* is common along the entire gradient from the oak-dominated foothills up through the white fir (*Abies concolor*) belt and decreases in abundance in the red fir (*A. magnifica*) belt. *Scleropodium occidentale* is also common through the oak-pine associations of the foothills but appears to drop out below the coniferous forests. In the foothill woodlands where they overlap, it appears that *S. occidentale* prefers the smaller tributaries, with *S. obtusifolium* preferring the larger drainages; however, this observation is anecdotal and requires quantitative verification. In the Peninsular and Transverse ranges of southern California, *S. occidentale* appears to extend to somewhat higher elevations and has been documented from the white fir belt.

Scleropodium touretii (Brid.) Koch, Rev. Bryol. & Lichenol. 1949

> NEOTYPE: *F. Camus s.n. 15 April 1904* (PC0024100)(PC!). France, Seine-et-Oise, St-Rémy-lès-Chevreuses, talus de bois. See nomenclatural notes section below for discussion.

Muscus terrestris surculis kali geniculati aut illecebrae, aemulis etc. Vaillant Botan. Paris 137 *pl 25.f.7*. 1727.
Hypnum illecebrum L., Sp. Pl. 1129. 1753. non Hedw., 1801
Hypnum touretii Brid., Sp. Musc. 2:185. 1812
Hypnum illecebrum Schwaegr., Suppl. I, part 2: 225. 1816
Hypnum blandum Lyell in Hook. & Tayl., Musc. Brit., (2nd ed)176. 1827. Suppl. *pl.5*. 1827.
Scleropodium illecebrum Schimper, Bryologia Europea 6 (55-56) M:3, pl. 557. 1853.
Scleropodium touretii (Brid.) Koch var. *teneriffae* Winter. Hedwigia 55:121. 1914.
Scleropodium australe Hedenäs 1996. Nova Hedwigia 62:451-465.

Plants light-green to yellow, forming robust mats. Stems to 6cm, branches and young stems gen <1.5cm. Stems and branches often curved strongly (especially distally), diameter consistent throughout (not attenuate), 0.8-1.6mm in diameter, strongly julaceous to spreading and nearly complanate. Leaves concave, 1.0-2.4mm × 0.8-1.6mm, mature stem leaves ovate to deltate, young stem leaves and branch leaves ovate-lanceolate to oblong; apex in well developed leaves acuminate, in younger leaves acuminate, acute, obtuse or cuspidate; margins entire in well-developed leaves, entire to serrate in distal 1/3 in younger leaves. Costa narrowing gradually toward apex in well developed leaves, narrowing gradually or ending abruptly without narrowing in younger leaves, often spine-tipped in all leaves, 55-85% leaf length. Cells of the lamina vermicular with rounded ends, 8-20:1, 35-90µm × 3.5-6.0µm, cells in the leaf apex shorter and

broader, 4-7:1; juxtacostal cells irregular, with rounded ends, 1-3:1, wider than lamina cells; alar cells irregular, 0.5-3:1, walls thicker or thinner than lamina cells, in <8(-10) rows from leaf base, transitioning rather abruptly into lamina.

Seta 9-16mm, brown to reddish-brown, twisted or not, densely roughened by prorate cells. Capsule cylindrical, 2-3:1,<2mm, horizontal. Spores 11-14μm, smooth to minutely papillose.

MORPHOLOGICAL NOTES (Fig. 2.12). This species is highly variable. In the field, it is a large moss that ranges from tumid and tightly julaceous to nearly complanate with spreading leaves, depending on the microenvironment. Most specimens have short acuminations that are reflexed when dry. This characteristic is only shared with *S. californicum*, which is a much smaller species. Branches of *S. touretii* specimens are often strongly curved, especially distally. *Scleropodium obtusifolium* also exhibits this feature, but it can be useful in differentiating *S. touretii* from *S. cespitans*, which tends to have the strongest curvature near the base of branches.

Under the microscope, the species can be recognized by a combination of large, generally concave leaves, long lamina cells, and a distinct acumination on some leaves. On a robust specimen, leaf size will differentiate *S. touretii* from all but *S. obtusifolium* and perhaps *S. occidentale*, but both of these species typically lack serrations or serrulations in the apex.

ECOLOGY & DISTRIBUTION (Fig. 2.13). In the Old World, this species is common in western Europe and throughout the Mediterranean basin and Macaronesia. Specimens from the Azores and Cape Verde Islands were not examined in this study, but the species has been reported from those island groups (Vanderpoorten et al. 2007, and references therein). The species also occurs in a single population in the vicinity of Hobart, Tasmania that was introduced from Europe (Carter 2010, Seppelt et al 2011).

In North America, the species is very common throughout cismontane California and northward to the coast of British Columbia. It extends southward to the Mediterranean climatic region of northwestern Baja California, Mexico and has been reported from islands off the Pacific coast of Baja California (Koch & Crum 1950). In California, it is very common in a wide range of plant communities and is frequently the dominant bryophyte species along trail banks in oak woodlands, chaparral, and mixed evergreen communities in mostly sunny to deeply shaded conditions. It is most common on bare earth, but does occur on rock outcrops and, less commonly, roots and tree bases. Morphological variation is extensive in the species and appears to be related to very local microsite conditions rather than broader scale variables (e.g. elevation, latitude). Varieties of *S. touretii* have been described, including *S. touretii* var. *colpophyllum* in North America and *S. touretii* var. *teneriffae* from the Canary Islands, but molecular data (Carter in press) and an ecological analysis (González-Macebo & Hernández-García 1996) have failed to provide convincing evidence that morphologically diagnosable entities exist within the species, although there is some phylogeographic structure (Carter *in press*).

NOMENCLATURAL AND TAXONOMIC NOTES. Problems with the nomenclature of *Scleropodium touretii* originated with the adoption of Hedwig's Species Muscorum Frondosorum (1801) as the starting point for bryophyte nomenclature. The problem is briefly summarized here based on a detailed synopsis presented by Koch (1949). European *S. touretii* was first recognized by Vaillant (1727) and was called *Hypnum illecebrum* by Linnaeus (1753). A different moss from eastern North America (currently *Bryoandersonia illecebra* (Hedw.) H.

Rob.) was included in the concept of *H. illecebrum* L. by Dillenius (1777). Hedwig, in 1801, referenced the eastern North American specimen for the name *H. illecebrum* Hedw. This created the problem that *H. illecebrum* L. (from Europe) was a different entity (and based on a different type) from *H. illecebrum* Hedw. from eastern North America. For the *H. illecebrum* of Linneaus, the first validly published name was *H. touretii* Brid. 1812, so the new combination *Scleropodium touretii* (Brid.) Koch was made in 1949.

Unfortunately, along with the majority of Bridel's herbarium at Berlin, the type specimen collected by Tourette was apparently lost in World War II. The protologue (Bridel 1812, p. 185) cites the type locality of the Tourette collection as near Lyon, France. This differs from earlier citation of the type locality as near Paris, which was based on the type of *Hypnum illecebrum* L. (Grout 1899).

Scleropodium australe Hedenäs, originally segregated from *S. cespitans* based on two morphological characters, was represented from only the type locality in Hobart, Tasmania. A recent analysis using molecular and morphological data demonstrated that this population does not differ from European collections of *S. touretii* and represents a recent human introduction from Europe (Carter 2010).

Scleropodium touretii (Brid.) Koch var. *teneriffae* Winter was described to accommodate material from the Canary Islands and Madeira that differed from typical *S. touretii* (which also occurs on those islands) in having a smaller size, growing in looser mats, with branches shorter and less tumid, and leaves lanceolate (as opposed to ovate). González-Mancebo & Hernández-García (1996) conducted a study examining niche preferences among *S. touretii* var. *touretii*, *S. touretii* var. *teneriffae*, and intermediate individuals and were able to demonstrate that these three morphotypes are associated with different environmental conditions. My own interpretation is that the *teneriffae* morphotype is the extreme along a spectrum of morphological plasticity associated with different habitats. A molecular phylogenetic study (Carter *in press*) demonstrated some molecular differentiation among some individuals from Macaronesia, but these specimens are not morphologically different from typical *S. touretii* and do not correspond to *S. touretii* var. *teneriffae*.

A key to the species of *Scleropodium*

When identifying species of *Scleropodium*, both microscopic leaf characters as well as the general appearance and habit of the plant without magnification should be considered. For example, *S. cespitans* has shoots that tend to be much more attenuate than those of other species. Branch curvature tends to be proximal in *S. cespitans*, but distal in *S. touretii*. Earlier keys have used the extent to which shoots are julaceous as a discriminating character. This character is highly plastic in all species and depends on microhabitat. With some species pairs, especially *S. touretii* versus *S. cespitans*, the habit of the plant can, at times, be more diagnostic than the microscopic characters.

1. Alar cells in >10 rows near margin, +/- isodiametric and thick walled, leaves <0.8mm wide …… 2
1' Alar cells in <9 rows near margin, 1-4:1, inflated or thick walled, leaf width various …… 3
    2. Leaf apex long acuminate, leaves decurrent …… *S. californicum*
    2' Leaf apex obtuse to short acuminate, leaves not decurrent …… *S. julaceum*
3. Plants aquatic. Leaf apex entire (rarely minutely serrulate) …….. 4
3' Plants terrestrial. Some leaf apices serrulate to strongly serrate …….. 5
    4. Spine at tip of costa present, costa of some leaves >90% leaf length, leaves typically <1.2mmwide …… *S. occidentale*
    4' Spine at tip of costa absent, costa shorter, leaves typically >1.2mm wide …… *S. obtusifolium*
5. Cell dimensions at mid-leaf 1:7-12, leaves typically <0.9mm, sporophyte capsules erect …… *S. cespitans*
5' Cell dimensions at mid-leaf 1:8-20, leaves typically >0.9mm, sporophyte capsules horizontal …….. *S. touretii*

New or Important State/Province Records

Scleropodium californicum
OREGON: *Bilderback DB-2010-M2* (UC): Coos Co., near Bandon, on rock ca. 80m from beach. 43.115° N, 124.434° W

Sceropodium julaceum
OREGON: *Bilderback DB-2010-M5* (UC): Coos Co., near Bandon, on coastal bluffs. 43.085 N, 124.436 W; *BilderbackDB-2009-M2* (UC): Curry Co., Saddle Rock, offshore from Crook Point, on coastal bluffs 42.250 N, 124.415 W; *Wagner 8684* (UBC): Lane Co., Eugene, Univ. of Oregon campus, common on base of street trees 44° 03' N, 123° 05' W; WASHINGTON: *Suksdorf s.n. (22 Dec 1891)* (UBC): Klickitat Co., Bottomlands, Bingen, on willow trunks 45° 40' N, 121° 25' W. *Schofield 36340* (UBC): Cowlitz Co., near Kalama, on boulder; BRITISH COLUMBIA: *Boas 215* (UBC): Victoria, Colquitz River near Beaver Lake. 5 May 1962.

Scleropodium obtusifolium
The species is common throughout California, Oregon, Washington, and southeastern British Columbia. Flowers (1973) and Lawton (1971) indicated the presence of the species in several

Rocky Mountain states but did not cite vouchers. Specimens away from the Pacific states region are much less common, so vouchers are listed here.
ALASKA. Prince of Wales Island (55° 33' N, 133° 03' W), *Worley 6765;* near Ketchikan, *Worley 6220* (UBC); ARIZONA: Mohave Co., *Norris 81478*; Gila Co., *Haring 3485*
IDAHO: Idaho Co., *Dewey 850* (UC); Elmore Co., *MacFadden 5726* (UC); Latah Co., *Anderson 1709* (UC), Clearwater Co., *Gray 1842* (UBC); MONTANA: Flathead Co. *Williams ex herb. NY No. 9,* near Kalispell (48° 12' N,114° 82 W); Missoula Co., Mill Creek Rd., *Schofield 120999* (UBC); NEVADA. Washoe Co., *Shevock 22671* (UC); Carson City Co., *Shevock 22043* (UC), *Shevock 21966* (UC); Elko Co., *Shevock 22812* (UC); UTAH. Salt Lake Co., *Flowers ex herb UT No. 29;* Summit Co., *Flowers 1766* (UBC); Duchesne Co., *Flowers 1680* (UBC); Juab Co., *Flowers 2856* (UBC).

Excluded taxa

Scleropodium brachyphyllum Cardot Bull. Soc. Bot. Geneve 4:379. 1912.
Type: Faurie 367. Fusan [=Busan], North Korea. May 1906
Holotype not located, possibly at PC
Isotype at HIRO (!)

Original protologue from Cardot (1912):

> ***Scleropodium brachyphyllum*** Card. Sp. nov. – Dense cespitosum lutescens, habitu et magnitudine S. caespitoso (Wils.) Br. Eur. Subsimile. Rami obtuse et breviter attenuati subjulacei. Folia patenti-sulave, marginibus planis, ubique serratis, costa ad ¾ vel Paulo ultra evanida, reti laxiusculo, cellulis rhomboidali-sublinearibus, superioribus oblongis, alaribus parvis, subquadratis, sat numerosis, subobscuris. Caetera desunt. A praecedente foliis multo latioribus et brevioribus, a S. caespitoso foliis serratis prima scrutatione distinctum.
> Coree: Fusan (n. 267).

NOTES: See notes below *S. coreense*.

Scleropodium coreense Cardot Bull. Soc. Bot. Geneve 4:380. 1912.
Type: Faurie 597. Ouen-san [=Wonsan], South Korea. 2 July 1906
Holotype not located, possibly at PC
Isotype at HIRO (!)

Original protologue from Cardot (1912):

> ***Scleropodium coreense*** Card. sp. nov – *S. caespitoso* (Wils.) Br. [= *S. cespitans* (Mull.)Koch] eur. Statura et habitu sat simile, foliis autem longioribus, margine serrulatis, acumine latiore, sobobtuso, costa angustiore, et reti laxiore diversum. Folia oblonga, late et breviter acuminata, obtusula, marginibus planis, fere e basi serrulatis, costa ad ¾ evanida, cellulis laxiusculis, rhomboidali-sublinearibus, superioribus brevioribus, alaribus sat numerosis, minutis, subquadratis, obscure viridibus. Caetera desiderata.
> Coree: Ouen-San (n. 597).

NOTES: Below the diagnoses, Cardot provided the following note, translated here from the original French (Cardot 1912, p. 380). *Scleropodium caespitosum*, as referenced here, is synonymous with *S. cespitans* (Müller) Koch. No illustrations were included in the protologues.

> In the absence of sporophytes, it is with some doubt that I place these two plants in the genus *Scleropodium*; it is possible that they are *Eurhynchium*. However, they are reminiscent of *Scleropodium caespitosum* so I felt obliged to classify them, at least temporarily, next to this species.

The next reference in the literature to these two taxa is in Takaki's (1955) treatment of east Asian Brachytheciaceae. In the treatment, he provided new descriptions in English and good illustrations. Along with these, he made the following note:

> An examination of the original specimens has revealed that they are both sterile and any characters of sporogone cannot be seen. We can hardly induce the general characters of the genus from these specimens only. As the generic characters of this genus, A. J. Grout states in his "Moss flora of North America" that "Closely allied to *Brachythecium* and included in it by some authors; differing slightly in the general habit and in the julaceous branches with concave, often obtuse leaves; leaf-cells very long and narrow, 10-20:1. Stem leaves abruptly and slenderly acuminate in most species. Seta rough; capsule as in *Brachythecium*." On these generic characters the two Korean species agree almost with that, except the form of leaf cells. There are some doubts as to placing these species in *Scleropodium*. But the author is not sure of it, until the more sufficient materials are at his disposal [sic].

To explore the basis of these concerns, I obtained isotypes from HIRO, as well as several other collections from HIRO and IFP for examination. The holotypes presumably originally went to PC, but Ignatov & Huttunen (2002) were unable to find them there, or at BM, H, NICH, or KYO. Because of the importance of sporophytic characters for some generic concepts in Brachytheciaceae (e.g. operculum shape differentiating *Scleropodium* from *Eurhynchium* in Robinson's (1962) key to North American Brachytheciaceae), the sterility of the types is problematic. However, several gametophytic characters clearly separate the two type specimens from *Scleropodium*.

Leaves of the types of both *S. brachyphyllum* and *S. coreense* are serrate from apex to the point of attachment. *Scleropodium* species range from completely entire to serrate in the distal ½ of the leaf. Serration proximal to mid-leaf is absent in *Scleropodium* (serrulation proximal to midleaf is rare, but present, in most species), but is typical in other genera in the Brachytheciaceae. Even when the serrations are small toward the leaf base, the marginal cells are shorter and more rhomboidal than the nearby lamina cells. While common in other Brachytheciaceae, this characteristic is unknown in *Scleropodium*. In *S. julaceum*, it is commonly observed in the distal 1/3 of the leaf, but never toward the leaf base.

Two more subtle characters are the lack of enlarged, irregularly rounded cells adjacent to the base of the costa, which is typical in *Scleropodium*, as well as the lamina cell shape. Most species in *Scleropodium* have cells that are vermicular to long-vermicular (5-20:1), whereas the cells in the two Korean types are linear and shorter. *Scleropodium julaceum* has short lamina cells, but in well-developed leaves, there is still a hint of S-shaped curvature in the cells, as compared to the straight cells of the Korean specimens.

While it is clear that these type specimens do not represent species of *Scleropodium*, it is unclear (to me at least) which genus should accommodate them, and whether they represent unique entities or whether they should be synonomized with species in a separate genus (or genera). Generic concepts in Brachytheciaceae are still being actively reinterpreted, and at this time I am only able to state with conviction that they are in the family, but not in *Scleropodium*. Hopefully future studies by a researcher more familiar with east Asian Brachytheciaceae will resolve this problem.

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Figures

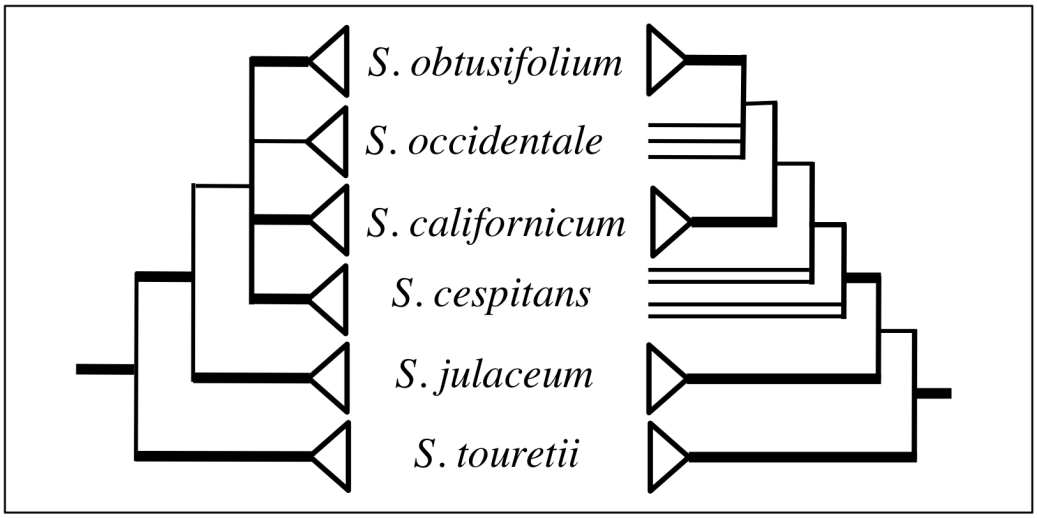

Figure 2.1. Summary of phylogenetic relationships among *Scleropodium* species estimated from 104 accessions using a combined dataset of ITS, *rps4*, *psbA2* & *trnG* sequence data from Carter (ms accepted). Topology on left is from a parsimony analysis and on the right from a Bayesian analysis. Heavy branches indicate strong support (parsimony bootstrap > 75 or posterior probability > 0.95, respectively). The unsupported paraphyly of *S. cespitans* in the Bayesian analysis is caused by molecular divergence among North American and European populations of that species.

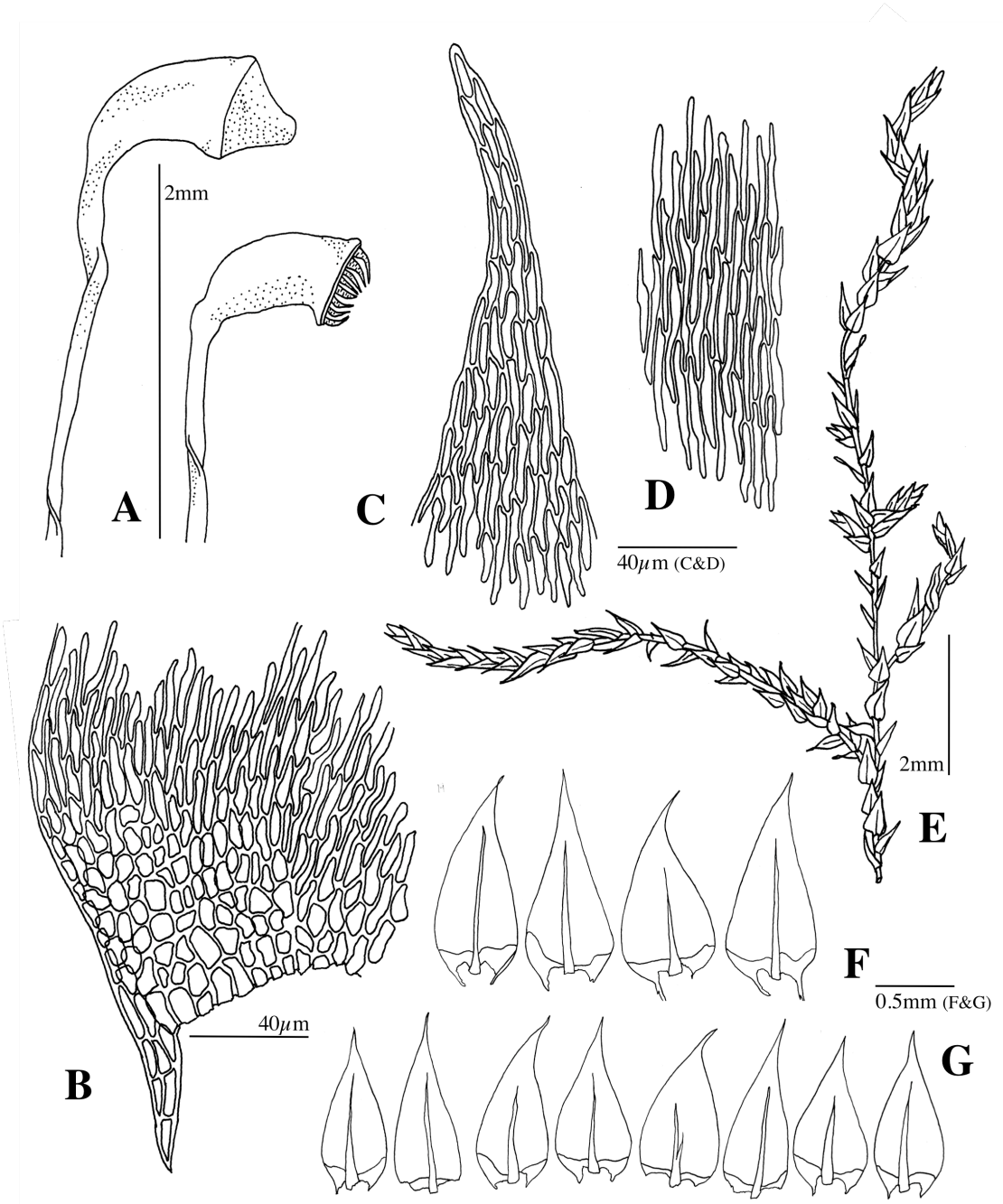

Figure 2.2. *Scleropodium californicum*. A) Sporophytes, B) Alar region, C) Leaf apex, D) Cells at mid-leaf, E) habit, F) Stem leaves, G) Branch leaves.

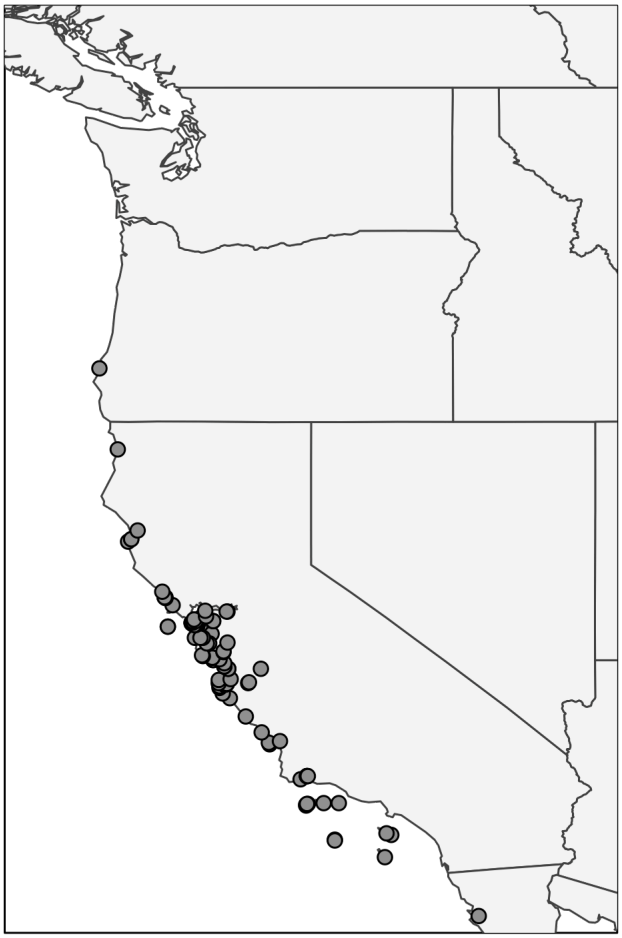

Figure 2.3. Distribution of *Scleropodium californicum*

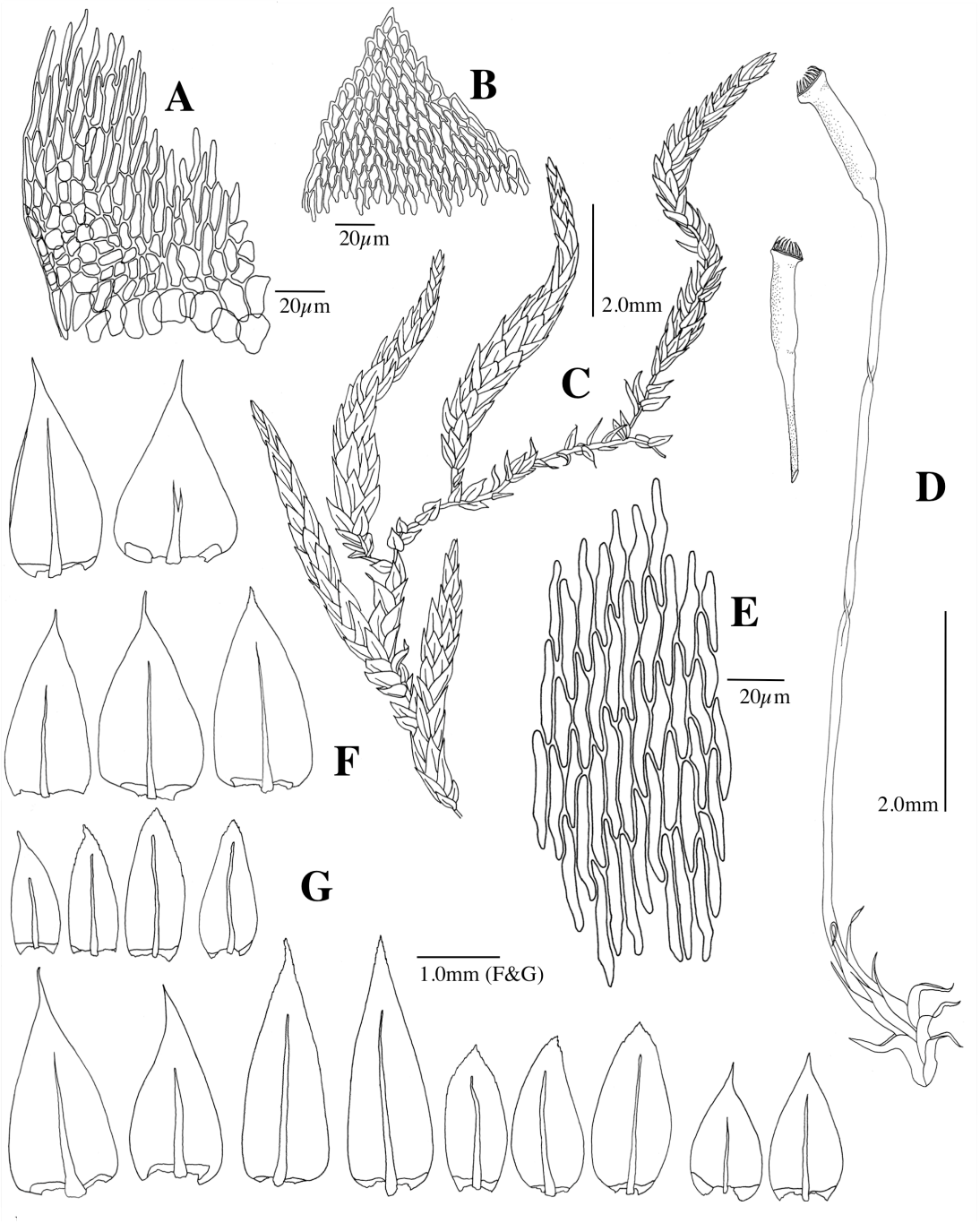

Figure 2.4. *Scleropodium cespitans*. A) Alar region, B) Leaf apex, C) Habit, D) Sporophytes, E) Cells at mid-leaf, F) Stem leaves, G) Branch leaves

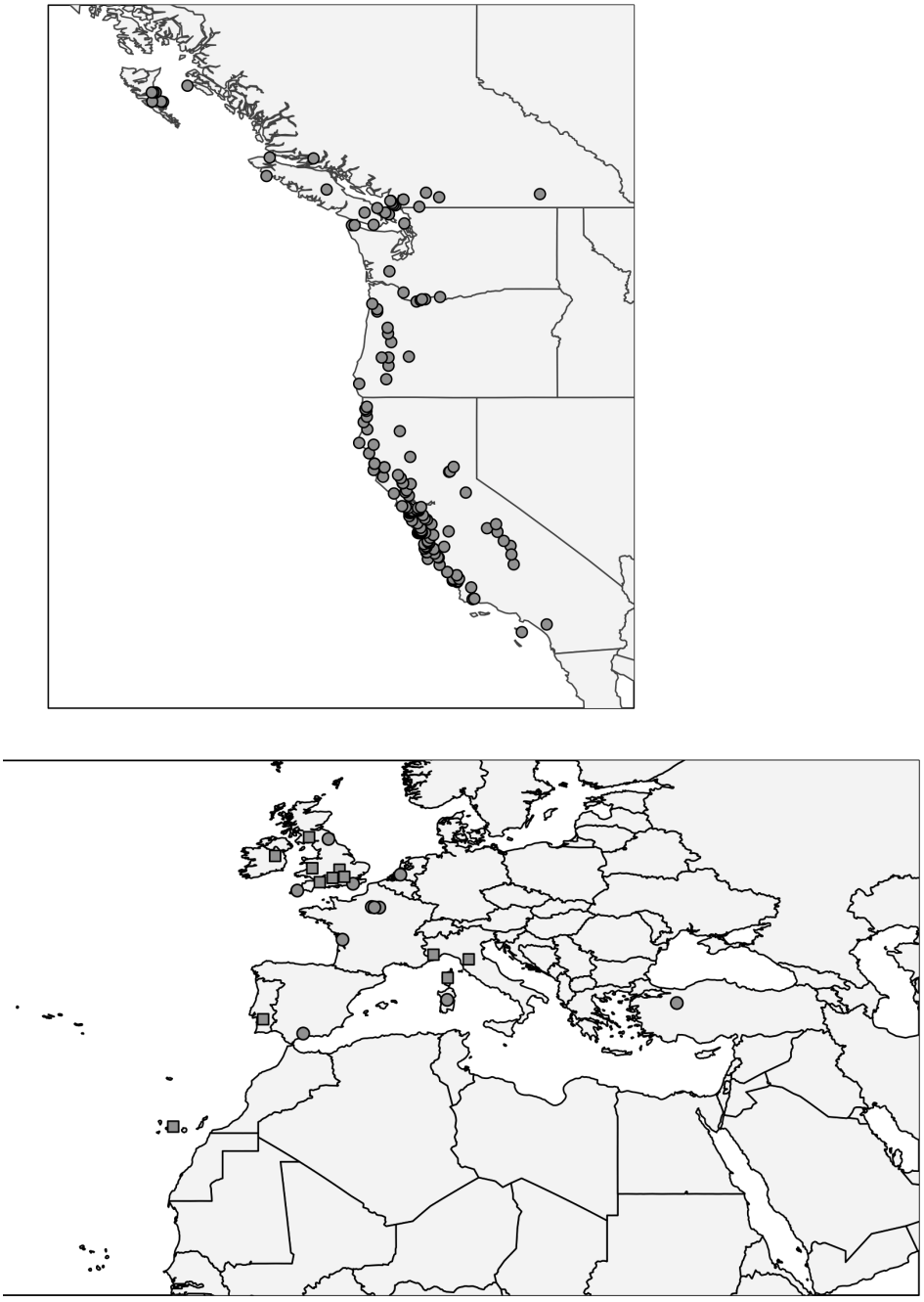

Figure 2.5. Distribution of *Scleropodium cespitans*. Squares are reliable reports from the literature.

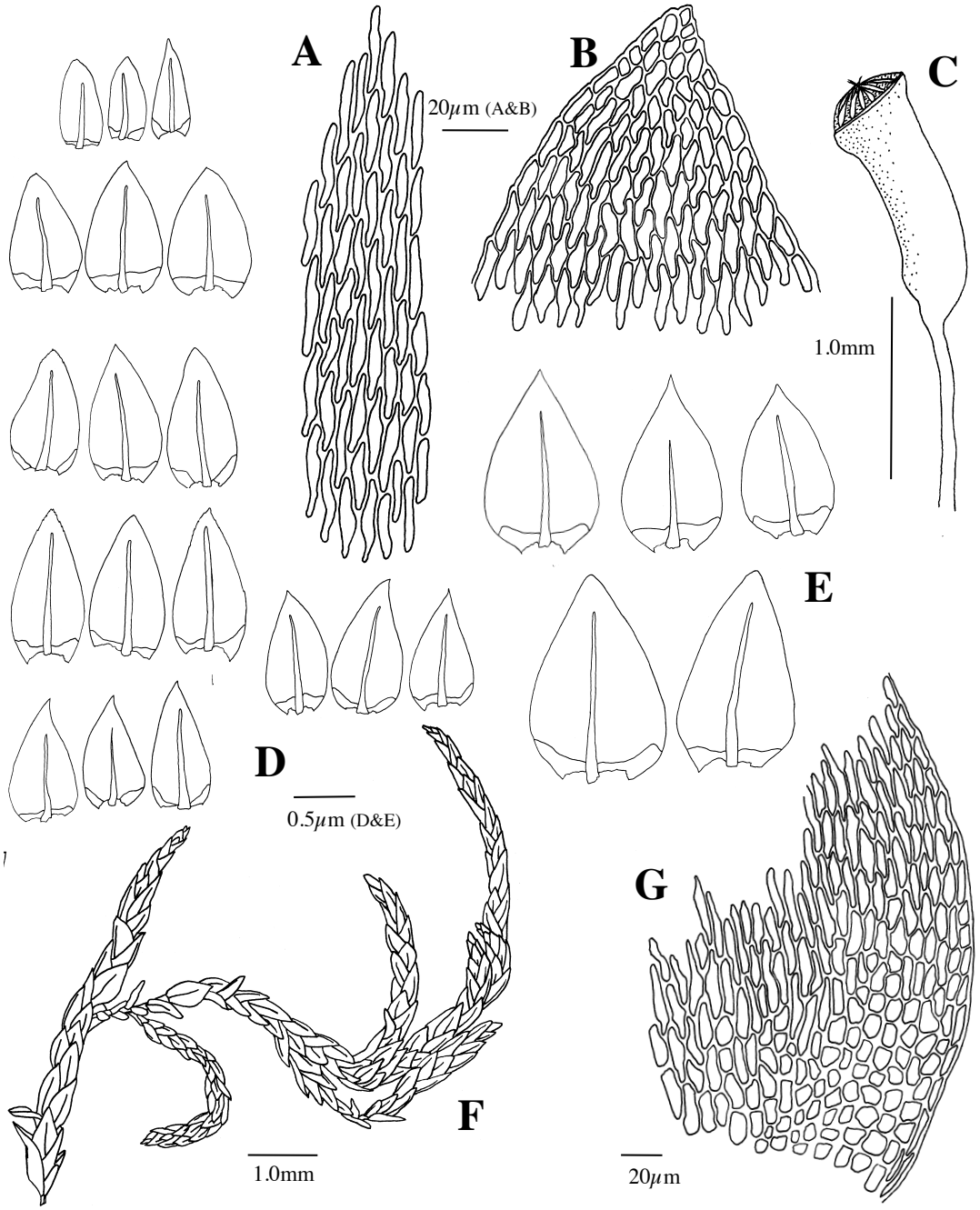

Figure 2.6. *Scleropodium julaceum*. A) Mid-leaf cells, B) Leaf apex, C) Sporophyte, D) Branch leaves, E) Stem leaves, F) Habit, G) Alar region.

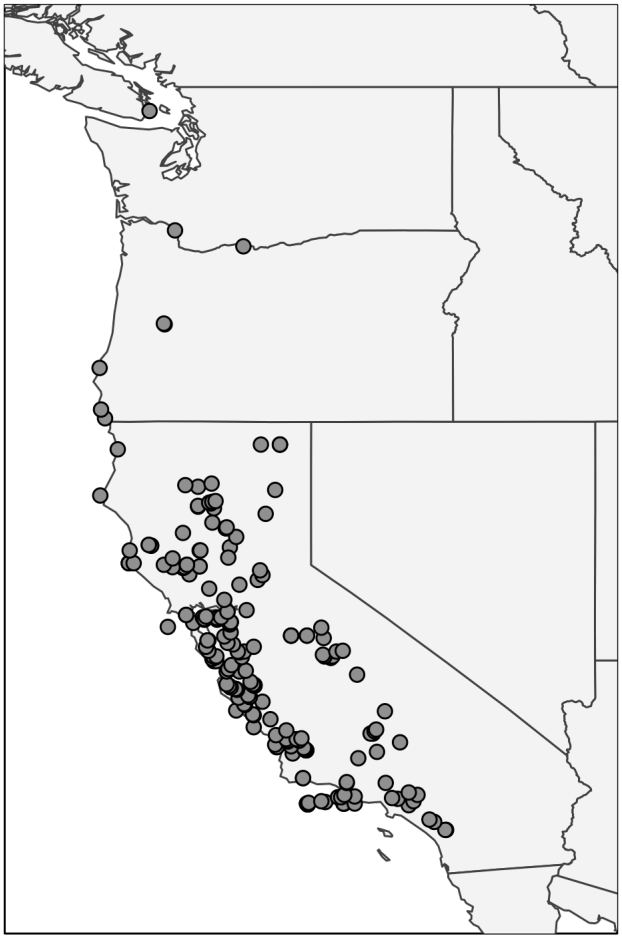

Figure 2.7. Distribution of *Scleropodium julaceum*.

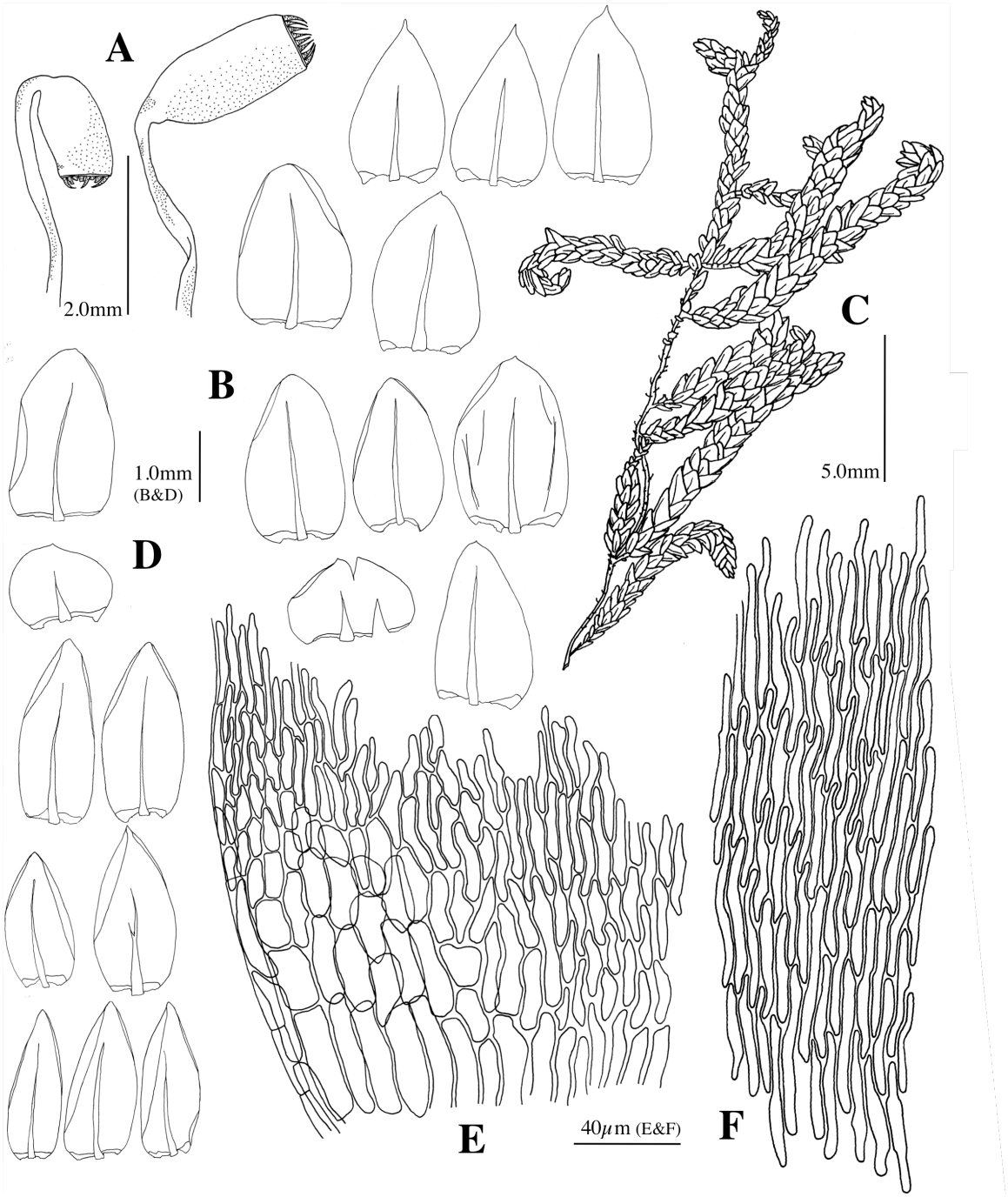

Figure 2.8. *Scleropodium obtusifolium*. A) Sporophytes, B) Stem leaves, C) Habit, D) Branch leaves, E) Alar region, F) Mid-leaf cells

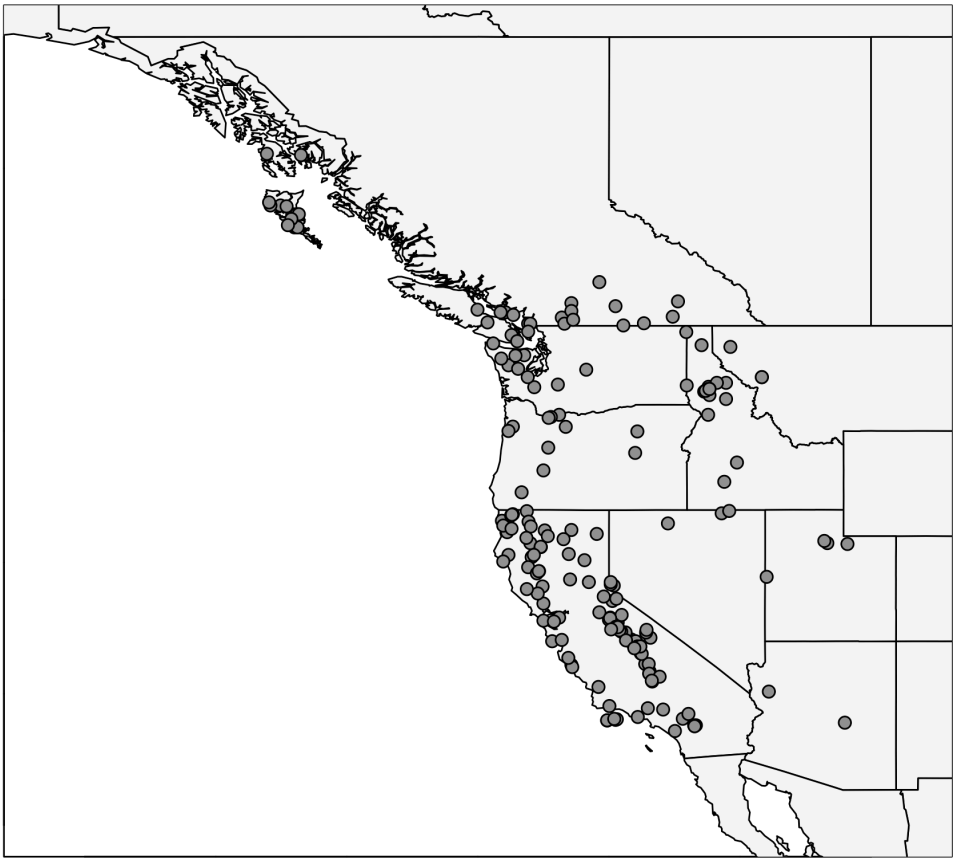

Figure 2.9. Distribution of *Scleropodium obtusifolium*.

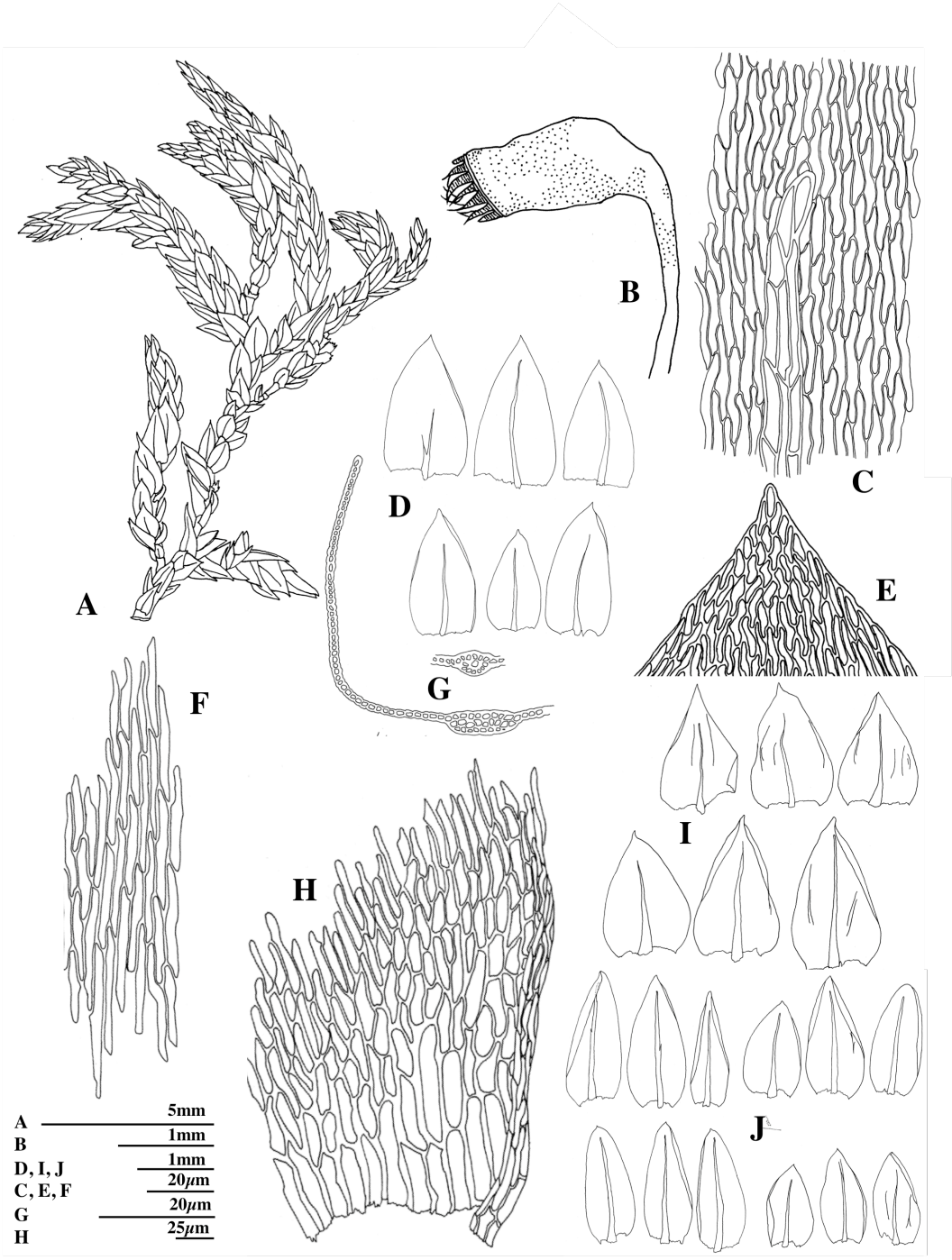

Figure 2.10. *Scleropodium occidentale*. A) Habit, B) Sporophyte, C) Costa apex, D) Stem leaves (above) and branch leaves (below), E) Leaf apex, F) Cells at mid-leaf, G) Costa cross sections, H) Alar region, I) Stem leaves, J) Branch leaves.

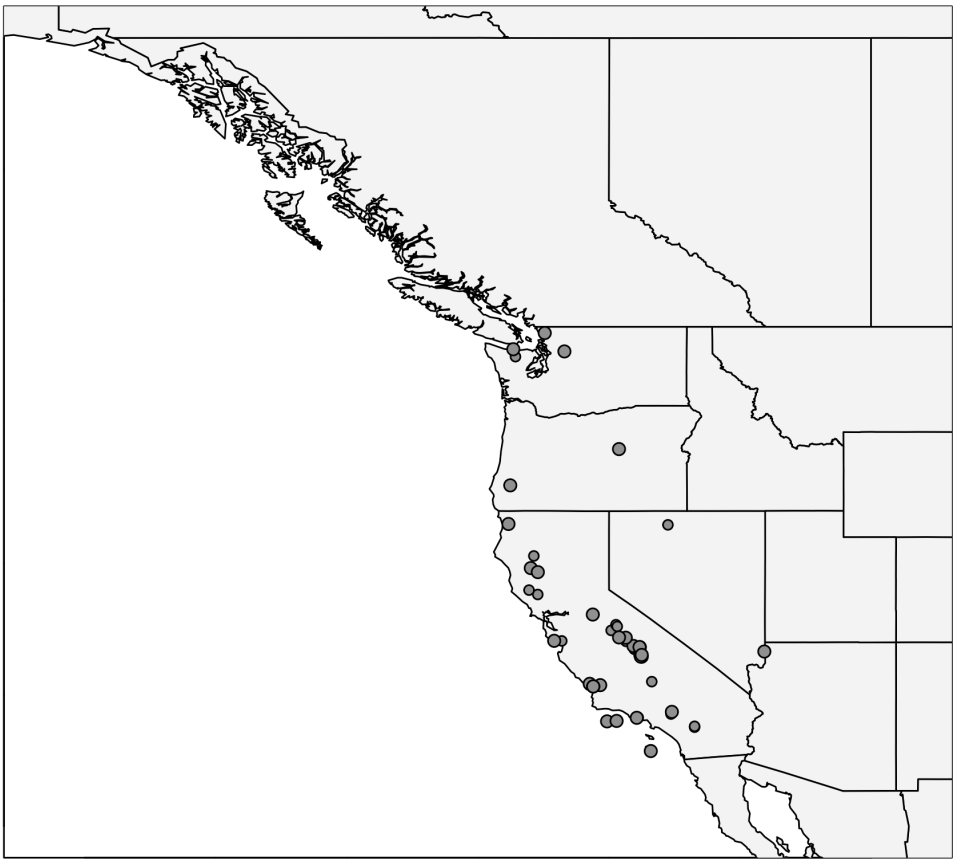

Figure 2.11. Distribution of *Scleropodium occidentale*.

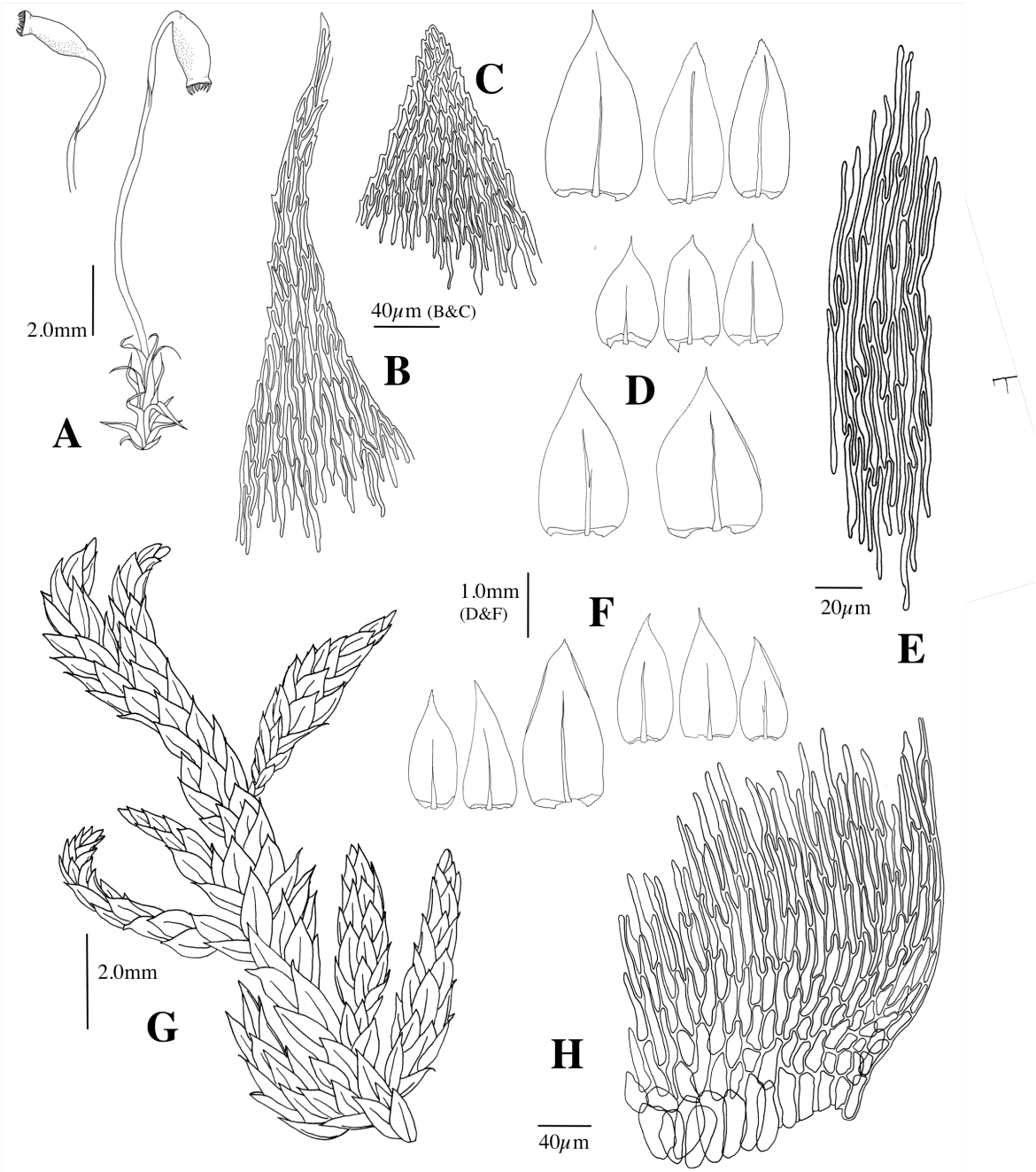

Figure 2.12. *Scleropodium touretii*. A) Sporophytes, B,C) Leaf apex, D) Stem leaves, E) Mid-leaf cells, F) Branch leaves, G) Habit, H) Alar region.

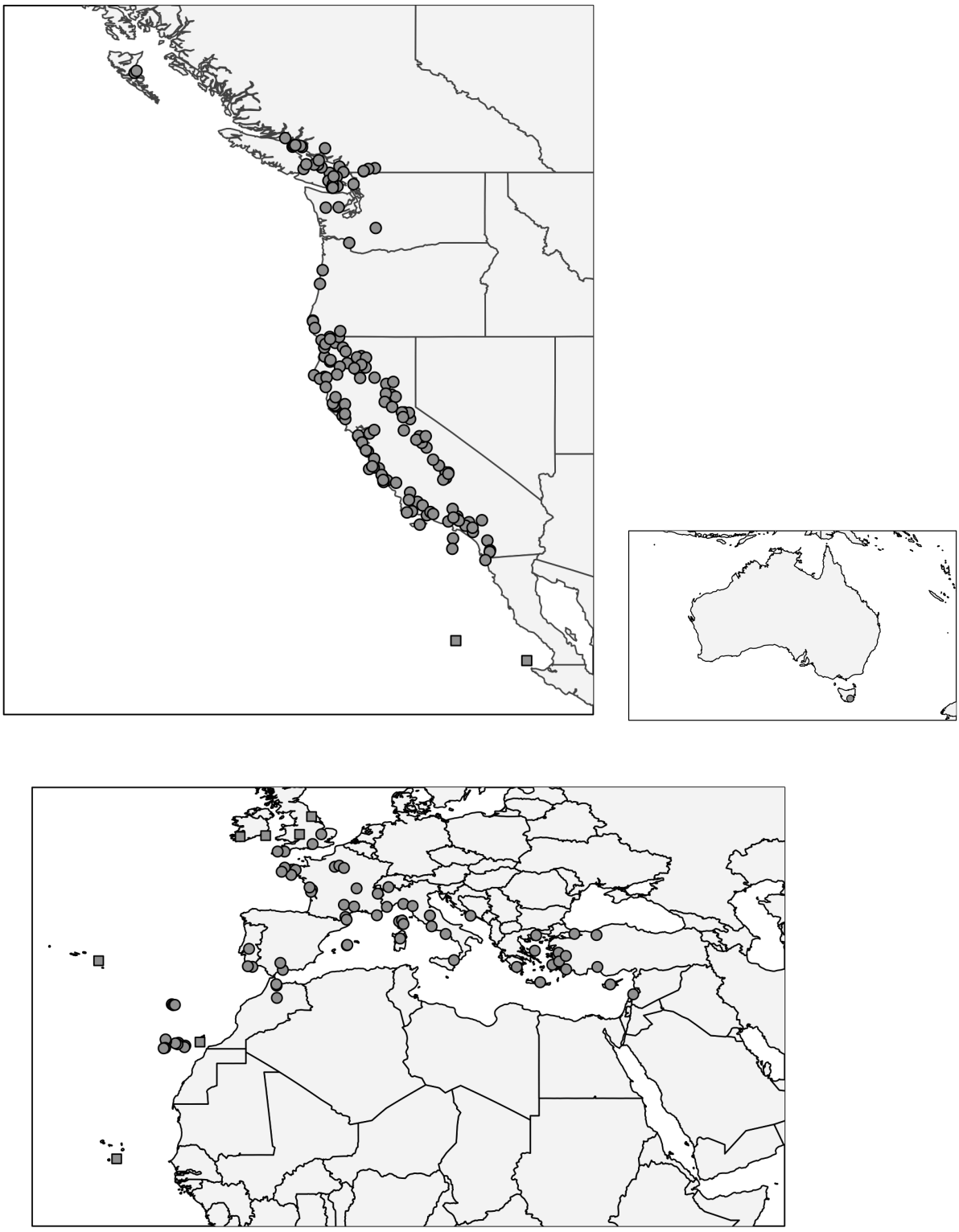

Figure 2.13. Distribution of *Scleropodium touretii*. Squares are reliable reports from the literature.

CHAPTER 3. Niche differences among species and sexes in the moss genus *Scleropodium* (Brachytheciaceae)

Abstract

The study of niche differences and niche breadth among closely related species provides important information about the diversification process. In this study, ecological niche parameters were quantified for five sympatric species of the moss genus *Scleropodium* in California at two spatial scales: individual moss patches (typically 10-1000cm^2) and 100m^2 plots. All patches were examined to determine sex and sex expression to test for niche differences among sexes and nonexpressing patches within each species. At the plot scale, important environmental variables associated with relative abundances of the five species were slope, drainage size, and canopy cover. At the patch scale, canopy cover of evergreen trees, substrate (rock, tree base, soil) and distance from drainage center were important. Canopy cover was also correlated with the distribution of sexes for some species at both spatial scales, with males and/or sporophytes being restricted to shadier sites. Niche differences among species were more clear at the patch scale, but at both spatial scales, niche overlap was very high among most species pairs, suggesting that selection maintaining a wide niche breadth may override specialization in these species.

Introduction

When closely related species co-occur at small spatial scales, they tend to parse the environment along one or more ecological axes (MacArthur & Levins 1967, Schluter 2000). This niche partitioning is especially evident in plants, which often partition local resources such as soil moisture or nutrients with remarkable precision (Cavender-Bares et al. 2004). Understanding of fine-scale ecological niche differences in plants is pivotal to understanding the evolutionary processes at play, however these niche differences are often complex. For example, traits associated with edaphic specialization and herbivore defense can interact to jointly drive the diversification process (Fine et al. 2004). An understudied but fascinating group of plants for studying fine-scale niche differentiation is the bryophytes (mosses, liverworts, horworts). These organisms are small and exhibit niche differences at the scale of centimeters to meters. They also lack roots, flowers, fruits, and, in most cases, herbivores. This simplicity (i.e. reduction in the number of ecological axes to consider) allows for the isolation of factors driving niche differentiation and perhaps diversification.

Despite their simplicity, bryophytes often exhibit striking patterns of fine-scale niche differentiation (Smith 1982, Slack 1990). Much of what is known about bryophyte niche breadth and overlap come from wetland systems, in particular *Sphagnum* bogs (e.g. Slack 1984, Vitt & Slack 1984) and riparian stream corridors (e.g. Slack & Glime 1985, Vitt et al. 1986, Glime & Vitt, 1987). One benefit of bog and riparian systems is that they allow niche axes including water chemistry and position relative to water level to be easily quantified. Studies of niche breadth and overlap among terrestrial bryophytes species are less common (Watson 1981, Horton 1988, Sagar & Wilson 2009), but have also documented niche differences including pH, soil chemistry, substrate texture, and light availability. An important similarity between aquatic and terrestrial systems is that both show repeated patterns of clear niche differentiation, differences in niche breadth among species, and often extensive niche overlap among species.

An independent but complementary research trajectory examining bryophyte niches is the study of intraspecific niche differentiation among sexes and among individuals which produce gametangia and those that do not. In dioicous species (i.e. those with separate male and female gametophytic individuals), a growing body of literature has documented that sexes can behave differently in different environments (e.g. Cameron & Wyatt 1990, Bowker et al. 2000, Fusleier & McLetchie 2004). In the desert moss *Syntrichia caninervis* male individuals (and consequently sporophytes) are restricted to mesic sites below shrubs while female individuals occur both in the mesic sites and in harsher microsites away from shrub canopies (Bowker et al. 2000, Stark et al. 2005). A similar pattern at a much larger spatial scale was found in the cosmopolitan moss *Bryum argenteum* with males becoming increasingly rare in arid regions (Benassi et al. 2011). Similarly, environmental gradients have been linked to production of gametangia, with non-expressing individuals more common in harsher environments (Stark et al. 2005). These studies are part of a much larger body of work that has documented and attempted to explain the strongly female biased sex ratio observed in many bryophyte species (reviewed in Bisang & Hedenas 2005).

To date, there are no empirical studies linking niche partitioning among closely related species and intraspecific niche partitioning among sexes in mosses. In strongly moisture-limited environments, niche partitioning among species and among sexes within a species both occur largely along a single environmental axis: moisture. This has interesting evolutionary implications, especially in environments where the most common members of the community (i.e. those engaged most heavily in resource partitioning) are very closely related. For example, if diversifying selection among two species acts more strongly on the more ecologically restricted males, the realized niches where sex occurs could become more distant without a corresponding shift in the realized niche of the species, as females of both species retain broad ecological niches. In addition, if males of one species occupy a different niche space than females of another sympatric species, this could serve as an effective reproductive isolating mechanism, even if the ecological niches of the two species (aggregating male, female and non-expressing individuals) are overlapping.

In this study, the frequencies of five sympatric species in the moss genus *Scleropodium* (Brachytheciaceae) were quantified along important moisture-related environmental axes to test for niche differentiation among species and among sexes within species. *Scleropodium* is a genus of six dioicous (i.e. with separate male and female gametophytic individuals) species, including one recently discovered (Carter, unpublished data) and not yet described (called *Scleropodium* 'species B' here to avoid confusion with the *S.* 'species A' of Norris & Shevock [2004]). All are all very common in the Mediterranean climatic region of California and are frequently dominant species in their preferred habitats. They range in microsite preference across a broad moisture gradient from seasonally submerged boulders in streams to soil banks, boulders and tree bases in shaded habitats, to soil banks in sunny grasslands. The study describes patterns of co-occurrence among species and sexes at two spatial scales, $100m^2$ plots and individual moss patches, and documents environmental factors that correlate with the patterns of co-occurrence.

Methods

Field site

Fieldwork was conducted at Blue Oak Ranch Reserve, a unit of the University of California Natural Reserve System, in the winter and spring of 2011. The reserve is located in the hills east of San Jose, CA (37° 22' N, 121° 43'W) and experiences a strongly Mediterranean climate (hot, dry summers and cool, wet winters) with a mean annual precipitation of ca. 60cm. The topography consists of steep hills ranging from ca. 500m to 800m in elevation. The vegetation at the site is primarily oak woodland dominated by one or more of *Quercus douglasii*, *Q. lobata*, *Q. agrifolia* and *Q. kelloggii* that vary greatly in density and relative abundance according to slope and slope aspect. Patches of coastal scrub dominated by *Artemisia californica*, *Baccharis pilularis* and *Mimulus aurantiacus* are common, and canyon bottoms feature dense woodlands of *Q. agrifolia*, *Umbellularia californica*, *Platanus racemosa*, and in the deepest canyons *Q. chrysolepis*.

Sampling

In bryophyte ecology, as in all plant ecology, it is well understood that spatial scale is an important consideration in any discussion of coexistence (Vitt et al. 2003, Newmaster et al. 2005). While earlier bryophyte ecological studies tended to focus on sampling at very fine spatial scales (reviewed by Slack 1990), more recent work has highlighted the importance of capturing data at both the microsite scale and the mesosite scale (Sagar & Wilson 2009). Microsites are small areas at the millimeter to centimeter scale defined by parameters including substrate, shade, and inclination (Vitt & Belland 1997); mesosites are areas on the order of meters to hundreds of meters which support relatively homogenous vegetation dictated by environmental parameters including slope, slope aspect, elevation, and tracheophyte vegetation type (Sagar & Wilson 2009). Researchers have tended not to use spatially constrained sampling methods (plots) when quantifying diversity in mesosites due in part to the variation of mesosite size and in part to difficulties with capturing rare species in plot-based sampling schemes (Vitt et al. 2003, Newmaster et al. 2005, Sagar & Wilson 2009).

In this study, $100m^2$ plots were used to sample mesosites because a spatially bounded design was needed for comparison of plots, and because none of the species in question are rare at the study site. Ecological data were also collected at the scale of individual moss patches (i.e. continuous mats of a single species not connected to mats of the same species). Patches can be made up of one or more individuals of the same species. Plots were established by randomly selecting longitude and latitude coordinates, then using aerial photographs to select the nearest point within the nearest drainage to each of the randomly selected points. A handheld GPS unit was then used to navigate to the plot center. A plot consisted of a 5m wide belt situated perpendicular to the drainage and extending from the drainage to 10m up both slopes at 90 degrees from the direction of the drainage. Data collected for each plot included the azimuth and slope of the drainage, the azimuth and slope of each of the two 5x10m halves of the plot, and the width of the scoured portion of drainage. Six estimates of canopy cover were obtained for each plot, at 0m, 5m and 10m up from the drainage for each half plot. Canopy estimates for each tree species were made using a handheld convex densiometer and the six values were averaged for plot level analyses. Cover classes for thatch and leaf litter were also recorded for each half of the plot (0=absent, 1=0-25%,2=25-50%,3>50%). Twenty-seven plots were surveyed.

Within each plot ecological data for each patch of *Scleropodium* were collected. This included the substrate (rock, soil, tree root, litter), canopy cover of each tree species above the patch using a handheld convex densiometer, distance from the drainage, co-occuring bryophyte species, and the patch size. Because of the large variation in patch size from $<5cm^2$ to over 5000

cm^2, patch sizes were estimated to the nearest $25cm^2$ using a transparent grid. Only patches $>10cm^2$ were surveyed. Each patch was subsampled and the subsamples were brought back to a laboratory where they were examined microscopically to ensure accurate species identification and to determine whether each patch contained male individuals, female individuals, both, if neither sex was expressing, or if the patch had sporophytes.

Analysis

The matrix of species abundance (number of patches) in each plot was subjected to Principal Components Analysis to determine the similarity of plots based on the distribution of each species in each plot. Permutation tests were then performed with the environmental variables collected for each plot to determine which, if any, environmental variables were significantly correlated with the structure of the ordination produced with species abundances. Permutation tests were performed using 'envfit' in the vegan package for R (Oksanen et al. 2008). All other analyses were also performed using R (R Development Core Team 2010). For the three species with large sample sizes (*S. julaceum, S. touretii* and *S. obtusifolium*), the procedure was repeated to determine whether plot level environmental variables were significantly correlated with an ordination structure of abundances of male, female, sporophyte bearing and nonexpressing patches for each species.

To determine whether ecological differences were also present at the level of individual moss patches, Kruskal-Wallis tests were used to test for differences among species in the percent canopy cover (evergreen, deciduous and total) as well as distance from drainage. ANOVAs could not be used because the data could not be coerced to normality through a number of transformations. A chi-squared analysis was used to determine if differences observed in occupancy of different substrates was significant.

To determine whether niche differences occurred among sexes and non-expressing individuals within each species, Kruskal-Wallis tests were used to test for significantly different distributions along the continuous niche axes that were important for differentiating the species, and a chi-squared analysis was used for the categorical substrate variable. For these analyses, patches were coded as male only, female only, sporophytes present (male and female individuals present), or non-expressing (no gametangia present). In the dataset there were three patches (less than one percent of the total number) that included male and female individuals but did not posses sporophytes. These patches were not included in the analyses.

Results

Summary of Data

Data were collected from 27 plots and included a total of 431 individual patches (*S. cespitans*, 40; *S. julaceum*, 125; *S. obtusifolium*, 78; *S.* species B, 13; *S. touretii*, 175). The mean number of patches per plot was 15.3 (range 2:62). The mean number of species occurring in each plot was 2.6 (range 1:5). The mean cover of *Scleropodium* (pooled across species) for the plots was 0.28 m^2, or 0.0028% of the area of an entire $100m^2$ plot. However, the mean cover within one meter in each direction of the plot midline (the middle $10m^2$ centered on the drainage) was $0.16m^2$, or 0.16% of the area. Six plots contained a single species (either *S. julaceum* or *S. touretii* only), with the rest of the plots containing at least two (6 plots with 2 spp, 9 plots with 3 spp, 4 plots with 4 spp and 2 plots with all 5 spp).

Of the 431 patches, 36 (8.3%) were touching and/or intermingled with a patch of another species of *Scleropodium*. These 18 mixed species patches included the following species pairs: *S. julaceum* & *S. touretii* (N=13), *S. obtusifolium* & *S. cespitans* (2), *S. obtusifolium* & *S. julaceum* (1), *S. julaceum* & *S. cespitans* (1), *S. touretii* & *S. cespitans* (1). For each of these mixed species patches, each species was considered separately in the analyses.

Species niches at the plot scale

A PCA of species abundance (number of patches) in each plot was able to effectively separate the plots along axes that correlated with moisture-related environmental variables, with PC1 accounting for 66 % of the variation and PC2 accounting for an additional 24 % (Fig. 3.1). The ordination was able to differentiate plots dominated by *S. julaceum*, *S. touretii* and *S. obtusifolium*, with abundances of *S. cespitans* and *S.* species *B* being less important. Permutation tests revealed that four environmental variables were significantly correlated with the ordination structure of the plots: scour (width of drainage with vegetation removed by scouring) (P= 0.001), slope of the drainage (P= 0.044), evergreen canopy cover (P= 0.001) and total canopy cover (P= 0.001) (Fig. 3.1). Along these niche axes, *Scleropodium julaceum* was most prevalent in upland (small scour, steep slopes) sunny plots, *S. touretii* was more common in upland shadier plots, and *S. obtusifolium* was most common in plots in flatter, larger drainages. At the plot scale, niche overlap was high. Plots were as likely to contain either four or five of the five species (N=6, 22%) as they were to contain a single species (N=6, 22%). Therefore differences in the niche preferences were mostly expressed as differences in abundance rather than discrete niche differences.

Species niches at the patch scale

At the scale of individual patches, important environmental variables for differentiating among species were distance from drainage (Fig. 3.2), canopy cover of evergreen tree species (Fig. 3.3) and substrate (Fig. 3.4). Statistical tests indicated that distributions along these niche axes differed significantly among species (Canopy cover: Kruskal-Wallace $P < 0.00001$; Distance from drainage: Kruskal-Wallis $P < 0.00001$; Substrate: chi-squared $P < 0.00001$).

For distance from drainage, *Scleropodium obtusifolium* and *S.* species B were restricted to within 0.5m from the edge of drainages, while the other species occurred in the drainages but also occurred well away from drainages. Among the three species that were not restricted to the drainages, *S. cespitans*, *S. julaceum* and *S. touretii*, differences were observed in substrate preference and evergreen canopy cover. *Scleropodium julaceum* and *S. touretii* both occurred on soil and rocks but *S. julaceum* was more common in open microsites and *S. touretii* was more common in shaded microsites. *Scleropodium cespitans* was distinct from both of these species in its preference for tree bases and its absence on soil substrates.

Sex niches at the plot scale

Ordinations of the abundances of male, female, sporophyte bearing and nonexpressing individuals in each plot for *S. julaceum, S. touretii* and *S. obtusifolium* demonstrated similar patterns among the three species. In all three species, ordination structure was driven by differences in the abundance of male, female and nonexpressing patches, with little emphasis on sporophyte bearing patches (results not shown). Permutation tests indicated that for *S. julaceum* and *S. touretii*, evergreen tree canopy was negatively correlated with the abundance of female

patches (*S. julaceum* P=0.015, *S. touretii* P=0.022). There were no significant correlations of environmental variables with the *S. obtusifolium* abundances.

Sex niches at the patch scale

A tabulation of the number of patches that were male only, female only, with sporophytes, or non-expressing yielded results within the range of other bryophyte species (Fig. 3.5, Table 3.1). Sex ratios were similar among species, varying from 0.9 to 2.4 (Female:Male), and the proportion of non-expressing individuals varied among species from 8% to 46%. Sporophyte production varied over an order of magnitude, from 7% to 65%. Sex expression, sexuality, and sporophyte presence were only very weakly related to the size of the patch, i.e. little evidence was found for a threshold for gametangia production or sporophyte production (Table 3.2). Tests for differences among habitat preferences within species indicated, where sufficient sample sizes allowed, that habitat preferences among male, female, sporophyte bearing, and nonexpressing patches were generally weak (Table 3.2). The only exception was the distribution of sporophyte bearing patches of *S. julaceum* along the evergreen canopy cover axis. While most individual patches of *S. julaceum* were located in areas with low evergreen canopy cover, patches bearing sporophytes were concentrated in more heavily shaded areas.

Comparisons of niche preferences within species at the microsite scale did not show meaningful patterns that were generalizable across species or across environmental variables, but several specific differences were found (Table 3.2). Significant differences ($P<0.01$) were found for relationships within *S. cespitans* for distance from drainage and within *S. julaceum* for evergreen canopy. Both of these patterns were driven by the distribution of sporophyte bearing patches, with *S. cespitans* sporophytes being disproportionately found in or near drainages and with *S. julaceum* sporophytes being found disproportionately in shady microsites.

Earlier work has indicated that patch size is important for sex expression and sporophyte production (Rydgren & Okland 2002). Three of the five species in this study did not show significant differences in patch size among female, male, sporophyte bearing and nonexpressing patches. *Scleropodium julaceum* and *S. touretii*, the two species that occurred frequently outside of deeply shaded areas and away from drainages, demonstrated a modest threshold pattern in log patch size ($0.05<P<0.01$). In both cases differences were driven by sporophyte bearing patches tending to be larger than average and nonexpressing patches tending to be smaller than average.

Discussion

Field studies of closely related plant species growing together in nature provides a crucial first step in understanding the ecological and evolutionary processes that maintain diversity. While this approach is very effective, it has been underutilized in the study of terrestrial mosses (Watson 1981, see Slack 1985 and Glime & Vitt 1987 for aquatic studies). *Scleropodium* provides a good system for studying niche partitioning because all species in the genus occur within the same ecosystems. While this may seem trivial to a plant ecologist, a significant hurdle to disentangling bryophyte niche evolution is that closely related bryophyte species distributions often occur on different continents.

Niche differences among species (at the plot and patch scale)

Three important results emerged from quantifying the species niches in this study. First, niche preferences do occur although two spatial scales and multiple environmental axes were

necessary to characterize the niche differences. Second, niche breadths vary among species. Third, niche overlap among species is very broad. Each of these three results has important implications in understanding the diversification process in *Scleropodium*.

The assumption that co-occurring, closely related species occupy different niches is a basic tenet of classical ecology (Gause 1936), however other models exist that may better explain bryophyte distributions at fine spatial scales (e.g. the lottery model, Chesson & Warner 1981). The results presented here indicate that all species pairs with the exception of *S. obtusifolium* and *S.* species B occupy measurably different niches on axes related to either moisture (evergreen canopy cover, distance from drainage) or stability (scour, substrate). *Scleropodium obtusifolium* and *S.* species B are restricted to seasonally submerged streambeds subjected to high disturbance. Kimmerer and Allen (1982), in a study of stream bryophytes, also found that niche differences were not important among bryophytes low in stream channels. Their interpretation was that fine scale distributions in that microsite were dictated by disturbance rather than competition. This is a plausible explanation for the patterns uncovered in this study, although the low sample size of *S.* species B is undoubtedly a factor as well.

Scleropodium obtusifolium and *S.* species B also demonstrated much narrower niche breadth than the other three species in their restriction to streambeds. This relationship hints at a tradeoff between specialization and niche accessibility. The three generalist species here (*S. cespitans, S. julaceum, S. touretii*) were widespread throughout the plots but were subject to potentially competitive interactions with other species (each other, as well as ecologically similar species including *Homalothecium arenarium*, *Claopodium whippleanum* and *Bestia longipes*). On the other hand the two streambed specialists were the sole occupants of the larger streambeds. The streambed environment requires special adaptations for bryophytes which must be able to tolerate both periodic submergence and also the scouring of fast moving streams. The smallest streambeds, perhaps not submerged enough to prevent colonization by the terrestrial species, were occupied by all species and displayed patterns more in line with a stochastic dispersal model than one of ecological filtering.

Across the range of habitats sampled, niche overlap tended to be very broad, both at the patch scale (see Summary of Data section in Results) and at the plot scale (Fig. 3.1). One plausible interpretation of this is that the habitats are unstable relative to the lifespan of an individual clone. The preferred habitat across all species in the genus is relatively bare substrates away from dense grasses and forbs. These areas, by their very nature, are easily eroded. A hypothesis consistent with the results presented here is that selection for life-history characteristics associated with this unstable habitat may be counteracting pressure toward ecological specialization, especially in *S. julaceum* and *S. touretii*. These two species show distinct niche preferences at both the patch and plot scales (Fig. 3.1, Fig. 3.3), but are often also frequently found growing intertwined with one another (see see Summary of Data section in Results).

Niche differences among species (at the plot and patch scale)

To date no studies have examined the ecological distribution of sexes relative to the distributions of congeners in the same environment in mosses. Because sexual reproduction requires male and female individual gametophytes to be essentially touching, differences in the niches of male individuals of a species and the female individuals of a closely related species could render the two reproductively isolated. The results of this study indicate that this can be ruled out as a mechanism for reproductive isolation in *Scleropodium*.

The general trend across bryophytes is that there is a female biased sex ratio (Bisang & Hedenas 2005). This ratio tends to be stronger with increasing environmental aridity, and female gametophytes often have broader ecological niches with males (and consequently sexual reproduction) restricted to more mesic microenvironments (Bowker et al. 2000, Stark et al. 2005). The results of this study mostly support both of these patterns. The sex ratio varied among species and the stronger biases were for species occupying more arid microclimates, although this result was obscured by differing sex expression among species. In species that had significant niche differences among sexes, males and sporophytes tended to be in more mesic microclimates and non-expressing individuals tended to be in the more arid microclimates, though statistical support was not strong in most cases.

Sex ratios and the extent to which a species relies on sexual reproduction may be important foci for divergence in bryophytes of arid regions. In this study, substantial variation was found among species in both these traits (Fig. 3.5), although it is unclear whether differences are under selection or are simply artifacts of growing in slightly different microenvironments. The relative number of patches that were non-expressing versus with sporophytes (the result of successful sexual reproduction), for example, is very striking. At this site, *S. cespitans* maintained a notably different substrate preference for tree bases and aversion to soil (Fig. 3.4), which may be associated with its similarly unique profusion of sporophytes (Fig. 3.5). *Scleropodium obtusifolium*, on the other hand, relies much less on gamete production (Fig. 3.5), a characteristic which may be associated with its streambed habitat (Fig. 3.2). Sexual reproduction in mosses and other bryophytes requires a film of water through which the sperm must swim from a male to a female gametophyte (at least in dioicous species like *Scleropodium*). This close tie between water and sex, in conjunction with the interspecific variation in sexual traits and the niche differences of species along moisture gradients, suggest that moisture gradients may be important in maintaining and perhaps generating diversity in arid regions.

An important alternative hypothesis is that genetic differences, for example different chromosome numbers, are responsible for diversification in *Scleropodium* and other moss groups with ecologically similar species living in sympatry. If this is the case, then ecological differences among sexes and species are simply a result of reproductive isolation followed by selection on the independent lineages. The results presented here provide support for this idea in that the ecological niche space where all five species produce sporophytes (i.e. have successful sexual reproduction) is essentially the same: shady creek banks. The niche differences seen among species are predominantly driven by females and non-expressing individuals. This suggests that, in addition to future field studies linking niches of species and sexes, a better understanding of genetic isolating mechanisms in bryophytes will be prerequisite to explaining the role of niche differences in the diversification process of these organisms.

Conclusions

Niche differences among the five closely related moss species studied here are detectable at two spatial scales, $100m^2$ and individual moss patches, with stronger patterns at the patch scale. This provides empirical evidence that, while the microsite is very important, environmental factors at larger spatial scales are also important. Niche overlap was very high among most species pairs, indicating that reproductive isolation due to ecological differences is unlikely. This is true even considering that niche differences were detectable not only among species but among sexes within species. Niche differences among sexes, when present, supported the established pattern that males are restricted to mesic sites while females and non-expressing individuals occupy

broader niches.

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Figures and Tables

	non-expressing	S	M only (M + S)	F only (F +S)	M:F including S
S. cespitans	8%	65%	15% (80%)	13% (78%)	1.0
S. julaceum	31%	7%	18% (25%)	44% (51%)	2.0
S. obtusifolium	46%	18%	8% (26%)	28% (46%)	1.8
S. species B	8%	38%	31% (69%)	23% (62%)	0.9
S. touretii	25%	17%	10% (27%)	48% (65%)	2.4

Table 3.1. Tabulation of the number of patches that possessed antheridia (male), archegonia (female), sporophytes (S, implying presence of males and females) and non-expressing individuals. Values presented are percentages of the number of individuals of each species

	Log patch size	Distance from drainage	Percent Evergreen Canopy	Substrate
S. cespitans	0.112	0.002	0.697	0.034
S. julaceum	0.033	0.525	<0.001	0.623
S. obtusifolium	0.086	0.032	0.044	<0.001
S. species B	---	---	---	---
S. touretii	0.025	0.059	0.092	0.761

Table 3.2. P values for tests of differences among male only, female only, sporophyte bearing and non-expressing patches within species. Sample sizes for *S.* species B were too low to perform statistical tests. Tests performed for each column were: log patch size (ANOVA), distance from drainage & percent evergreen canopy (Kruskall Wallace), and substrate (chi-squared).

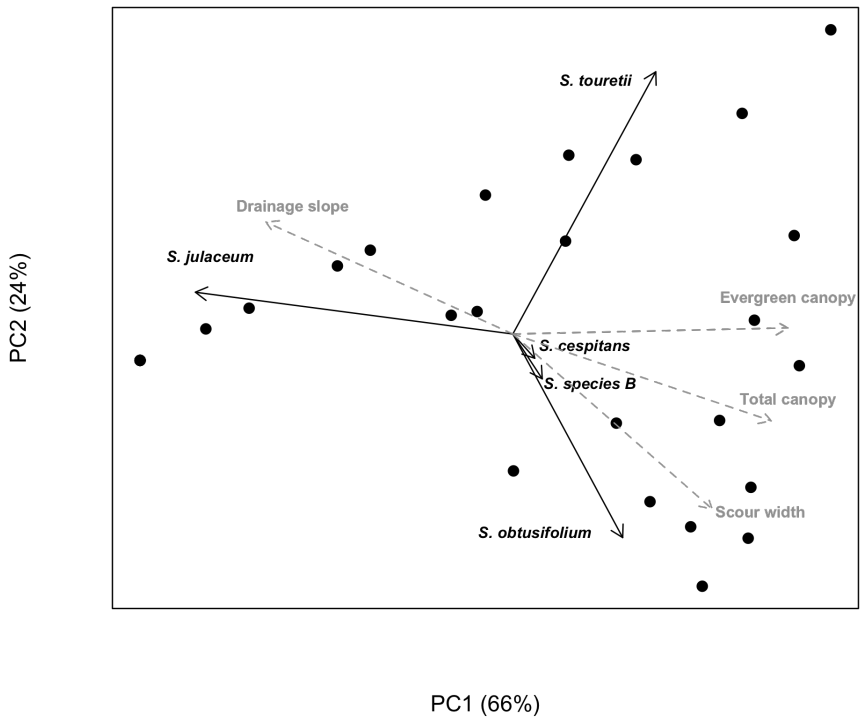

Figure 3.1. PCA of plots using abundance of each species in each plot. PC1 explains 66% of variance & PC2, 24%. Black vectors represent species abundances. Grey dashed vectors represent environmental axes that correlate significantly with the structure of the abundance matrix.

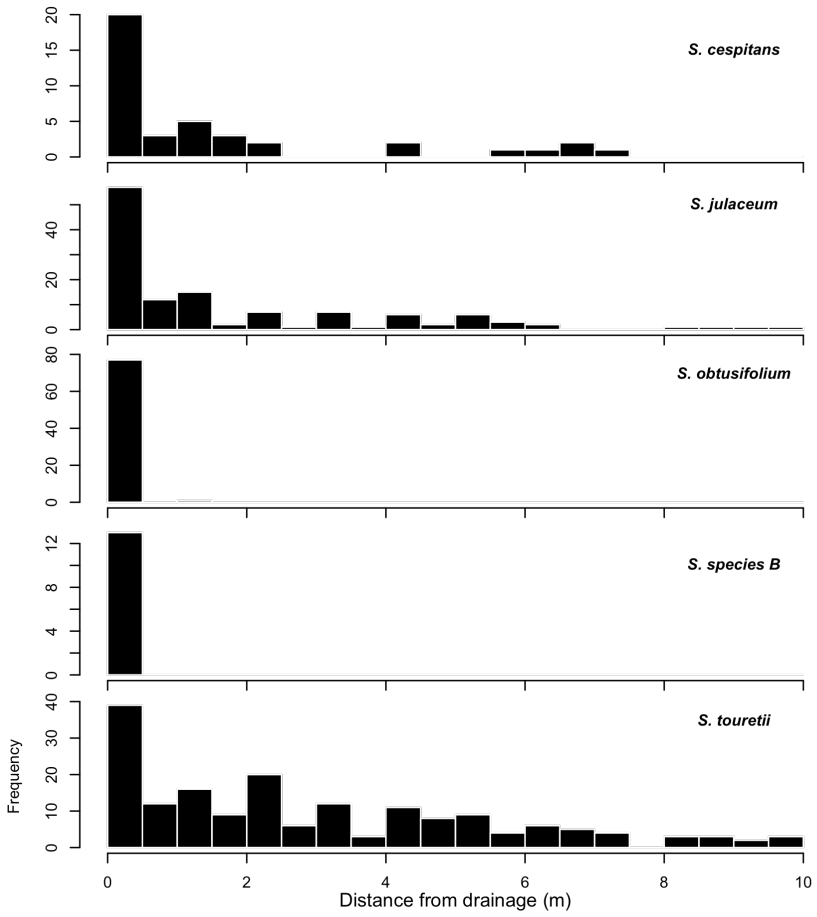

Figure 3.2. Frequency distributions showing the position of individual patches relative to the middle of the drainage (0m). Note different Y-axis for each species.

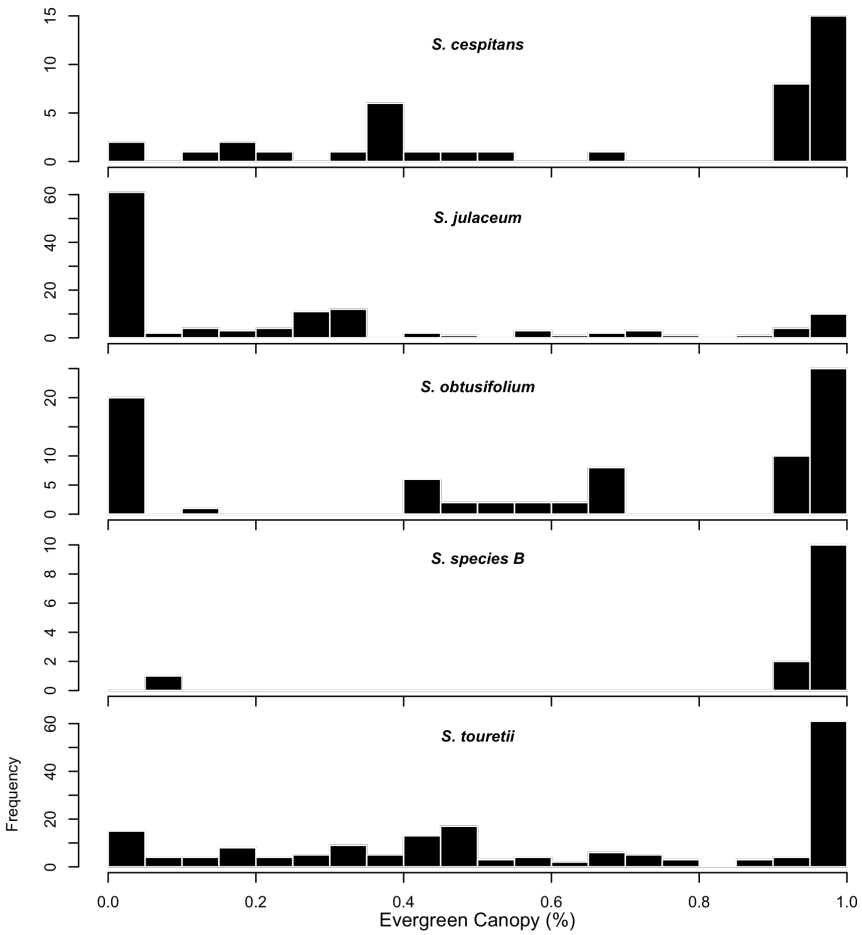

Figure 3.3. Frequency distributions showing the estimated canopy cover of evergreen tree species above individual moss patches. Values range from 0% (no evergreen cover) to 100% (complete evergreen cover). Note different Y-axis for each species.

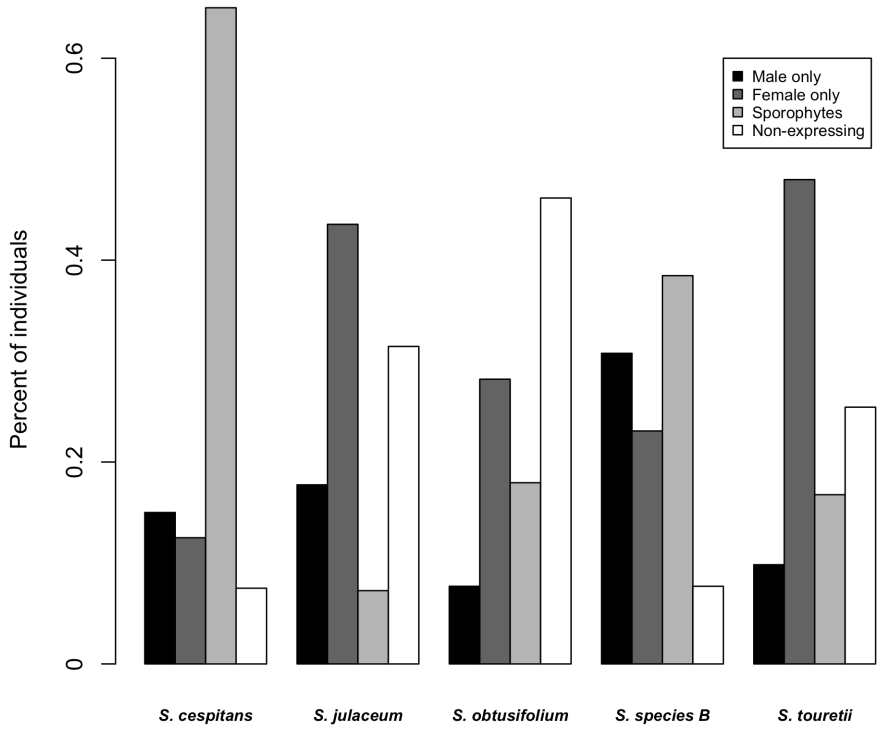

Figure 3.4. Sex ratios for each species. Bars represent the percent of patches for each species that were male only, female only, bearing sporophytes or nonexpressing.

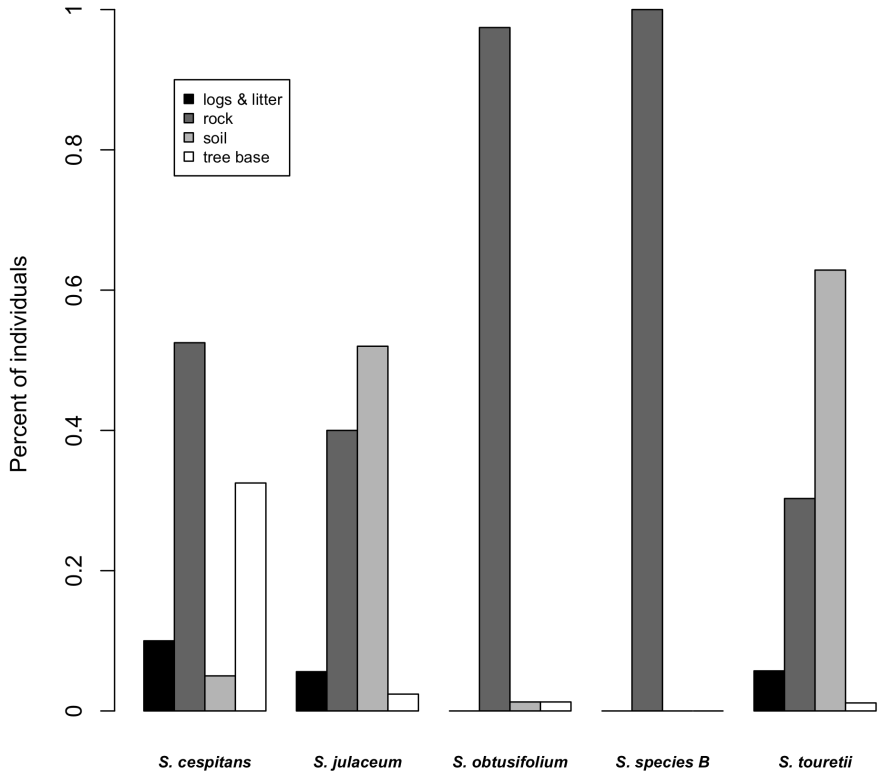

Figure 3.5. Occurrence of each species across substrate types. Bars indicate the percent of individual patches of a species that were found on each substrate type.

CHAPTER 4. *Scleropodium occidentale (Brachytheciaceae)*, a new moss species from western North America

Abstract

A new species of *Scleropodium* (Brachytheciaceae) is described from western North America. Initially recognized in a recent molecular phylogenetic study of the genus, *Scleropodium occidentale* B.E. Carter sp. nov. is very similar in morphology, ecology, and distribution to *S. obtusifolium*. A morphological investigation of *S. obtusifolium* and *S. occidentale* was conducted using 62 specimens assigned to species independently based on analyses of DNA sequences of nuclear ribosomal ITS and the chloroplast *trn*G region. Morphological differences between the two species are subtle, but include relatively robust and commonly spine-tipped stem and branch leaf costae in *S. occidentale* versus weaker and without a spine in *S. obtusifolium*, slightly longer lamina cells in *S. obtusifolium* than in *S. occidentale,* and a strangulate capsule in *S. occidentale* in contrast to the cylindrical capsule in *S. obtusifolium*. Fieldwork and herbarium study indicate that *S. occidentale* is common in California and is present in Oregon, Washington, Nevada, and southern British Columbia. All herbarium specimens of *S. occidentale* were previously labeled as *S. obtusifolium*.

Introduction

Aquatic and semi-aquatic environments vary in the length of periodic submersion and drought, as well as water temperature, chemistry, and flow rate. As a result, mosses occupying these environments, especially pleurocarps, are well known for extensive phenotypic plasticity within species (Lodge, 1959; Hedenäs, 1996). This plasticity is a well documented complication to morphological circumscription and delimitation of aquatic and semi-aquatic moss species (Andrews, 1957; Vanderpoorten & Jaquemart, 2004). Increasingly, molecular data are becoming important for investigating the high degree of plasticity in pleurocarpous mosses by allowing elucidation of the relationship between genetic variation and morphological variation within and among species (Draper & Hedenäs, 2009; but see Vanderpoorten & Shaw, 2010).

Scleropodium obtusifolium (Mitt.) Kindb. in Macoun (Brachytheciaceae) is a common seasonally submerged rheophyte (specialist on substrates in fast-moving streams) in western North America. The species, like other rheophytes, displays a broad range of phenotypes that putatively reflect abiotic variation within its habitat. Because *S. obtusifolium* is easily identified to genus and was thought to be the only rheophyte in *Scleropodium*, thorough study of its morphological variation has not been undertaken. However, three attempts have been made to segregate morphological variants from *S. obtusifolium*. Grout (1899, 1928) recognized *S. obtusifolium* var. *homomallum* as a very robust form with secund leaves and long, strongly distally curved branches. He also recognized *S. obtusifolium* forma *laxum* as having flaccid stems with less closely appressed leaves. More recently, Norris and Shevock (2004b) suggested *S.* "species A" as a segregate with very small orbicular leaves and somewhat erect, rigid branches. These proposals, however, have not been tested by rigorous investigation of the breadth of morphological variation throughout the range of the species.

A recent molecular phylogenetic study demonstrated that *Scleropodium obtusifolium*, as currently circumscribed, comprises two genetically distinct entities, *S. obtusifolium* sensu stricto and *S.* "species B" (Carter in press). None of the genetic structure observed in that study corresponded to any of the three morphological segregates previously recognized (*S.*

obtusifolium var. *homomallum*, *S. obtusifolium* forma *laxum*, *S.* "species A" sensu Norris & Shevock [2004b]). Of twenty-nine accessions originally labeled *S. obtusifolium* in the molecular study, seventeen accessions represented *S. obtusifolium* sensu stricto and twelve represented *S.* "species B". Depending on the type of molecular dataset (ITS, chloroplast, or a combined ITS and chloroplast dataset) and the phylogenetic criterion (parsimony or Bayesian), the two species were either reciprocally monophyletic and placed in an unresolved polytomy with *S. californicum* (Lesq.) Kindberg and *S. cespitans* (Müll. Hal.) Koch or were sister to one another with very low support, or only *S. obtusifolium* was monophyletic and was sister to the unresolved *S.* "species B" accessions (i.e. no support for monophyly or for paraphyly of *S.* "species B"). A statistical parsimony network analysis indicated that, although phylogenetic resolution was generally poor, accessions of *S. obtusifolium* and *S.* "species B" formed two clusters that were as distinct as any other species pair in the genus. Diagnostic differences were found between *S. obtusifolium* and *S.* "species B" in all four of the molecular markers used in the study (ITS and the chloroplast *rps*4, *trn*G and *psb*A2 regions), and subsequent examination of DNA voucher specimens suggested that the presence or absence of a spine at the terminus of the costa might be a useful diagnostic morphological character.

The absence of a costa spine and the specific habitat preference for seasonally submerged microsites have historically been important for the delineation of *S. obtusifolium*, with the other species in the genus occupying terrestrial habitats and possessing a costa spine (Grout, 1899; 1928; Robinson, 1962; Lawton, 1967; 1971; Flowers, 1973; Norris & Shevock, 2004b; Ignatov, in prep.). While these differences are consistent in the literature, identification of herbarium specimens has typically been made according to the ecological affinity regardless of the presence or absence of a costa spine or other phenotypic variation. The presence of two genetically distinct species within the morphological and ecological circumscription of *S. obtusifolium*, in conjunction with the uncertainty around the delineation of these two from other species in the genus prompted the current study.

The new species is described and illustrated, and results of a morphological study investigating delineation of *S. obtusifolium* and the new species are presented below. In addition, the diagnostic molecular characters, distribution maps for the new species and for *S. obtusifolium*, and a key to the species are presented. Because of the high phenotypic plasticity in both species, the morphological analyses were conducted using the twenty-nine DNA vouchers identified in the previous study along with thirty-three new specimens identified using ITS and *trn*G.

Scleropodium occidentale* B.E. Carter *sp. nov. **Fig. 4.1**

TYPE: *Carter 1838*. California, San Luis Obispo County, Los Padres National Forest, Cerro Alto Campground along Highway 41 between Morro Bay and Atascadero. Cerro Alto Trail heading east from the back of the campground along east fork of Morro Creek. *Quercus agrifolia- Umbellularia californica* forest along riparian corridor with dense understory of ferns, *Rubus*, *Toxicodendron*, *Cornus* and chaparral shrubs from surrounding steep hillsides. Growing on rocks along creek with *Kindbergia praelonga*. 35° 25.49'N, 120° 44.12'W, elevation 340 m. 4 May 2007. (holotype: UC; isotype NY)

Description

Plants yellow green to dark green, forming dense mats. Branching sympodial and irregular, with stems and branches similar. Stems typically < 25 mm, occasionally to 50 mm, branches typically

< l5mm. Shoots strongly julaceous and slightly to strongly curved, with strongest curvature near middle or proximal, not distal. Mature shoots typically attenuate, 0.8—1.2 mm in diameter at widest point (dry). Rhizoids inserted below leaf costa insertion, brown, smooth. Pseuodparaphyllia broadly triangular; paraphyllia absent. Axillary hairs mostly 2—4 per axil, each hair 2—4 cells in length, cells elongate rectangular, clear to light brown. Branch and stem leaves similar. Leaves ovate, occasionally lanceolate to elliptic, 1.0—1.7 mm × 0.5—1.2 mm, slightly to strongly concave, not plicate; apices acute to rounded or cuspidate, occasionally short-acuminate; distal margins often widely incurved, margins typically entire or less commonly with serrulations restricted to cusp/acumination. Costa typically robust throughout and ending abruptly in an abaxial spine, less commonly tapered, (50—)70—95% leaf length. Cells of lamina 4-7 μm × 30-60 μm, 4—12:1, vermicular with rounded ends; cells in apex shorter, 2-5:1; juxtacostal cells irregular, enlarged, 2—3 rows from leaf base; alar cells irregular or rectangular 1—5:1, wider than lamina cells, in 2—5 rows, with walls thickened or cells slightly inflated. Dioicous, with male and female plants not differentiated. Calyptra cucullate, smooth, naked. Seta 11—15 mm, sinistrose, roughened throughout by prorate cells or becoming smooth distally. Capsule 1.8—2.5 mm × 0.5—0.9 mm, horizontal to pendant, constricted slightly to strongly below mouth. Stomata few, near base of capsule, pores broadly elliptic, 2:1. Exothecial cells irregularly rounded to rectangular with rounded ends, 10—22 μm × 30—65 μm. Annulus of 2—3 rows of cells. Peristome double. Exostome dark orange-red, striate below, papillose above. Endostome pale yellow, basal membrane 50—55% of endostome height, cilia as long as processes, sparsely nodose, often fused near apex. Operculum short-conic. Spores 11—18 μm, minutely papillose. Genbank accessions: ITS HM771721, *rps4* HM771815, *trnG* HM771909, *psbA2* HM772003.

Representative specimens examined

California specimens are listed geographically, by Jepson Region (Baldwin et al., 2012; see also Norris & Shevock, 2004a), then by county. Specimens at UC are listed by the collector number only. Specimens from other herbaria include herbarium code in parentheses. Specimens with an asterisk (*) were sequenced by Carter (in press) and specimens with two asterisks (**) were sequenced in this study (see Appendix A for GenBank numbers).

UNITED STATES: CALIFORNIA. Northwestern California (NW) Colusa Co.: North Fork Campground, Mendocino NF, *Shevock 20988*; Del Norte Co.: Smith River, *Schofield 93105* (UBC); Glenn Co.: Wildcat Canyon, *Laeger 2061***; near Elk Creek, *Shevock 15801*; Mendocino Co.: near Covelo, Mendocino National Forest, *Carter 2749*; Shasta Co.: Whiskeytown National Recreation Area, *Norris 105353*; Sonoma Co.: near Cloverdale, *Mueller 6598*; Tehama Co.: Tomhead Gulch, Mendocino NF, *Norris 56890*; Trinity Co.: Hall City Caves, Shasta-Trinity NF, *Norris 71677*. **Central western California (CW)** Contra Costa Co.: Mt. Diablo, *Shevock 24519***; Monterey Co.: northwest of Fort Hunter Liggett, *Shevock 29324*; San Luis Obispo Co.: Black Mountain, *Carter 1659**; Cuesta Ridge, *Carter 1402**, *1414**; Santa Cruz Co.: South Fork Falls Creek, Henry Cowell Redwoods State Park, *Shevock 31791*. **Southwestern California (SW)** Los Angeles Co.: San Gabriel Mountains, *Shevock 21667**; San Clemente Is., *Villasenor sn [26 Mar. 2011]***; Riverside Co.: San Jacinto SP, *Harpel 560*, Riverside Co. Park, *Harpel 1494* (UBC); Santa Barbara Co.: Santa Rosa Is., *Carter 6231***; Santa Cruz Is., *Carter 4631***; Ventura Co.: Santa Monica Mountains, *Sagar 580**. **Sierra Nevada (SN)** Butte Co.: Oroville Dam, *Janeway 7773*; Calaveras Co.: Angels Camp, *Shevock 19323***; Fresno Co.: San Joaquin River Canyon, Sierra NF, *Shevock 20628***; Monarch

Wilderness, Sequoia NF, *Shevock 12697*, 13608***; Kern Co.: near Democrat Station, Sequoia NF, *Norris 80834*; Madera Co.: O'Neals, *Shevock 17095**; Mariposa Co.: South Fork Merced River, Sierra NF, *Shevock 21547*; Placer Co.: near Dutch Flat, *Norris 101597*; Plumas Co.: Feather River Canyon, Plumas NF, *Norris 52600*; Tulare Co.: Potwisha Campground, Sequoia NF, *Shevock 12952*, 12957***; Squirrel Creek, Sequoia NF, *Shevock 17645**; Mineral King Road, *Shevock 14602**; Buckeye campground, Sequoia NF, *Shevock 15211**; Tuolumne Co.: Tuolumne River Bridge, Stanislaus NF, *Shevock 19569*. **Cascade Ranges (CaR)** Shasta Co.: Shasta Lake, Shasta NF, *Norris 84822*. **Great Central Valley (GV)** Butte Co.: Bidwell Park, *Norris 70025*. **NEVADA,** Clark Co.: Virgin Mountains, *Shevock 23639***. **OREGON,** Curry Co.: near Bill Moore Creek, *Schofield 93023* (UBC); Douglas Co.: Bear Creek Rec. Area, *Shevock 26366*; Grant Co.: Black Canyon, Ochoco NF, *Norris 79093*; Josephine Co.: Kalmiopsis Wilderness, *Becking 960400*; Multnomah Co.: Oneonta Gorge, *Flowers 8997* (UBC). **WASHINGTON,** Columbia Co.: Tucannon River drainage, Umatilla NF, *Schofield 116781* (UBC); King Co.: Mt. Baker, *Schofield 71299* (UBC); Snohomish Co.: Stillaguamish River, *Schofield 120743* (UBC)

CANADA: BRITISH COLUMBIA, Vancouver Island, Englishman River Falls, *O. Lee sn (6 Apr. 1980)* (UBC); Vancouver Island, Fair Harbor, *F. Boas sn (Jul. 1969)* (UBC); Queen Charlotte Islands, Moresby Island, *Schofield 31400* (UBC).

Methods

Specimens were selected to represent both the geographic distribution and the morphological variation of the species complex. DNA extraction, PCR, and sequencing of ITS and the chloroplast *trn*G region were performed using the protocols and primers used by Carter (in press). Thirty-three sequences were aligned by eye with the alignment of 29 sequences (17 *S. obtusifolium*, 12 *S. occidentale*) used in the earlier study. By comparing the new sequences with the existing alignment, all new sequences could unambiguously be assigned to one of the two species.

For morphological dimensions, mature stem leaves were measured using an ocular micrometer in a standard light microscope. Based on the results of Carter (in press), the presence of a costa spine and the length of the cells of the mid-lamina were suspected to have utility in delineating the species so these were quantified for the new specimens. Leaf length, width, length:width ratio, relative costa length, cell width, and cell length:width ratio were also quantified. For quantifying the presence or absence of the costa spine, ten leaves were randomly selected and the value presented is the percentage of the ten leaves possessing a spine. For cell measurements, 10 cells were selected randomly from approximately half way between the leaf apex and base and approximately half way between the costa and the leaf margin. Cells were selected from three to five leaves from a single stem.

Statistical analyses were performed using the R statistical software (R Development Core Team, 2010). Cell measurements of the two species were compared using a standard two-tailed t-test, but the costa data were not normally distributed so a Mann-Whitney test was used.

Results & Discussion

Morphology

Scleropodium occidentale is only likely to be confused with *S. obtusifolium*. All of the 85 *S. occidentale* specimens examined for this study, the 54 cited here and an additional 31 from within the geographic range of the cited specimens, were originally labeled *S. obtusifolium*. With *S. obtusifolium*, the new species shares the ecological preference for intermittently submerged habitats, and an entire (or minutely serrulate) apex and lack of a strong acumination on both stem and branch leaves. This combination of characters easily distinguishes these two species from any other in the genus.

In earlier treatments of the genus, Lawton (1967, 1971) used the absence (*S. obtusifolium*) or presence (all other species of *Scleropodium*) of an abaxial costa spine to help differentiate *S. obtusifolium* from the rest of the genus. In *Scleropodium* the 'spine' is typically a single cell at the tip of the costa that is attached to the lamina proximally, but is unattached to, and often is angled away from, the lamina in the distal 1/2 to 1/3 of the cell. In *S. julaceum* Lawton and *S. cespitans*, there are occasionally two or more spines near the tip of the costa. The costa spine and the mean length of the lamina cells are the most reliable diagnostic characters to differentiate between *S. occidentale* and *S. obtusifolium* (costa spines: Mann-Whitney test, $W=81$, $P<0.00001$; cell length: T-test, $t=6.28$, $df=51.03$, $P<0.00001$) but the two species overlap substantially in both characters (Figs. 4.2, 4.3). All other morphological characters quantified (leaf length, width and length:width ratio, relative costa length, cell width, and width:length ratio) were not significantly different and had similar ranges and variances between the two species.

The holotype of *S. obtusifolium* (Drummond's Musci Americani no. 193. [BM!]) lacks a costa spine and has long lamina cells not found in *S. occidentale*. It was collected from the Rocky Mountains in southeastern British Columbia (Bird, 1967). From this geographic area, specimens with no costa spines and long cells are common and no specimens have been seen with costa spines and shorter cells. Based on those observations, the name *S. obtusifolium* applies to the species without the spines and with longer lamina cells and the species with costa spines and shorter cells (i.e., *S. occidentale*) is the one that previously was unnamed.

Several other characters are less useful, but contribute to the overall differences between the two species. The leaf apex of *S. obtusifolium* is typically rounded or occasionally cuspidate. In *S. occidentale*, the leaf apex typically ranges from obtuse to acute, with the distal leaf margins often slightly inrolled. The costa in *S. occidentale* is variable in length, but in at least some branch leaves it will usually extend to 90% or more of the leaf length. The costa length in *S. obtusifolium* is also variable, but very rarely achieves that length. The costa in *S. occidentale* is also more robust. On at least some branch leaves it tapers only slightly until ending abruptly in a spine, whereas in *S. obtusifolium* the costa tapers gradually to the tip.

Sporophytes are rarely collected in both *S. occidentale* and *S. obtusifolium*. However, if present, fully mature capsules are useful for distinguishing the two species. The fully mature capsule of *S. obtusifolium* is cylindrical. In *S. occidentale*, the fully mature capsules are at least slightly strangulate, sometimes strongly, although young capsules can be found in which the strangulation is not yet evident.

As with many species in the Brachytheciaceae, and especially in aquatic and semi-aquatic species, morphological plasticity is high in both *S. occidentale* and *S. obtusifolium*. With the use of sequence data to identify specimens, it was clear in this study that some specimens cannot be

confidently identified using only the currently recognized morphological characters. Future work may uncover more reliable characters; however, morphological plasticity driven by the semi-aquatic habitat may be sufficient to require common garden experiments for detecting additional diagnostic traits.

Diagnostic molecular characters and incongruence

Scleropodium occidentale and *S. obtusifolium* could be diagnosed by at least one character using any of the four molecular regions used by Carter (in press). Figure 4.4 displays the alignments with diagnostic sites for each of the four molecular regions. In the current study, ITS and *trn*G were sequenced for an additional 33 accessions because the diagnostic characters in those regions are nucleotide substitutions rather than indels and may be less subject to homoplasy.

In the 33 specimens sequenced for this study, four individuals (6% of the total 62) were found with a chloroplast sequence of one species and an ITS sequence of the other species. Similar incongruence was found in accessions of *S. californicum*, *S. cespitans*, and *S. touretii* (Brid.) Koch by Carter (in press). Morphologically, the four accessions from the present study fall in the range where the two species overlap (Figs. 4.2, 4.3) and so could not be confidently placed within a species using either molecular or morphological data. Geographically, the specimens were collected from areas where the two species are sympatric and both very common (**Fig. 5**). This ambiguous placement of several accessions is consistent with an earlier interpretation (Carter in press) that divergence among species of *Scleropodium* may not be complete. However, an alternative hypothesis that hybridization is occurring between *S. obtusifolium* and *S. occidentale* is not ruled out by the data presented here.

Ecology and distribution

Scleropodium occidentale is common throughout the California Floristic Province, and extends northward to British Columbia and east to Nevada (Fig. 4.5). North of California, herbarium records suggest that it decreases substantially in abundance. This may be due to collection bias, but the Pacific Northwest is a relatively well-collected region for mosses, especially by comparison with California, so this is likely to be an accurate reflection of the species range. This distribution is very similar to that of *S. obtusifolium*, with the main difference being that there are no records for *S. occidentale* in the northern Rocky Mountains (Fig. 4.6).

Although *S. occidentale* has an ecological preference much more similar to *S. obtusifolium* than to the other species in the genus, herbarium label data suggest a degree of differentiation between the two species. In California, where both species are most common, *S. obtusifolium* is found from sea level to approximately the transition from white fir (*Abies concolor* (Gordon & Glen) Hildebr.) forest to red fir (*A. magnifica* A. Murray bis) forest, at roughly 2000 m elevation in the southern Sierra Nevada, and lower to the north. It is common in many vegetation types throughout California and the Pacific Northwest, but always occurs only in microhabitats that are seasonally submerged.

Scleropodium occidentale has a more restricted ecological preference. Like *S. obtusifolium*, it is restricted to seasonally submerged sites mostly below 2000 m elevation, but it appears to be more prevalent in drier regions. It is most common in oak dominated foothills and represented by only a few collections from coniferous forests. From specimens with adequate label data, 25% were collected in foothill woodland communities (dominants including various combinations of: *Quercus douglasii* Hook. & Arn., *Q. wislizeni* A. DC., *Q. agrifolia* Née, *Q. chrysolepis* Liebm., *Pinus sabiniana* D. Don, *P. coulteri* D. Don, *Aesculus californica* (Spach)

Nutt.), 21% were collected in canyon live oak woodland (dominated by *Q. chrysolepis*, associates: *Pseudotsuga menziesii* (Mirbel) Franco, *Q. kelloggii* Newb.), 19% were from coast live oak woodlands (dominated by *Q. agrifolia*, associates: *Umbellularia californica* (Hook. & Arn.) Nutt., *Q. douglasii, Platanus racemosa* Nutt.), with the remainder (35%) collected from chaparral, montane coniferous forest, and riparian woodlands. Although verification is needed, label data suggest that *S. occidentale* may occupy smaller and/or steeper drainages with lower water volumes. If true, this would suggest that *S. occidentale* may be physiologically intermediate between *S. obtusifolium*, which can occupy large creeks with perennial flow, and the other species in the genus, which do not tolerate any submergence.

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Figures and Tables

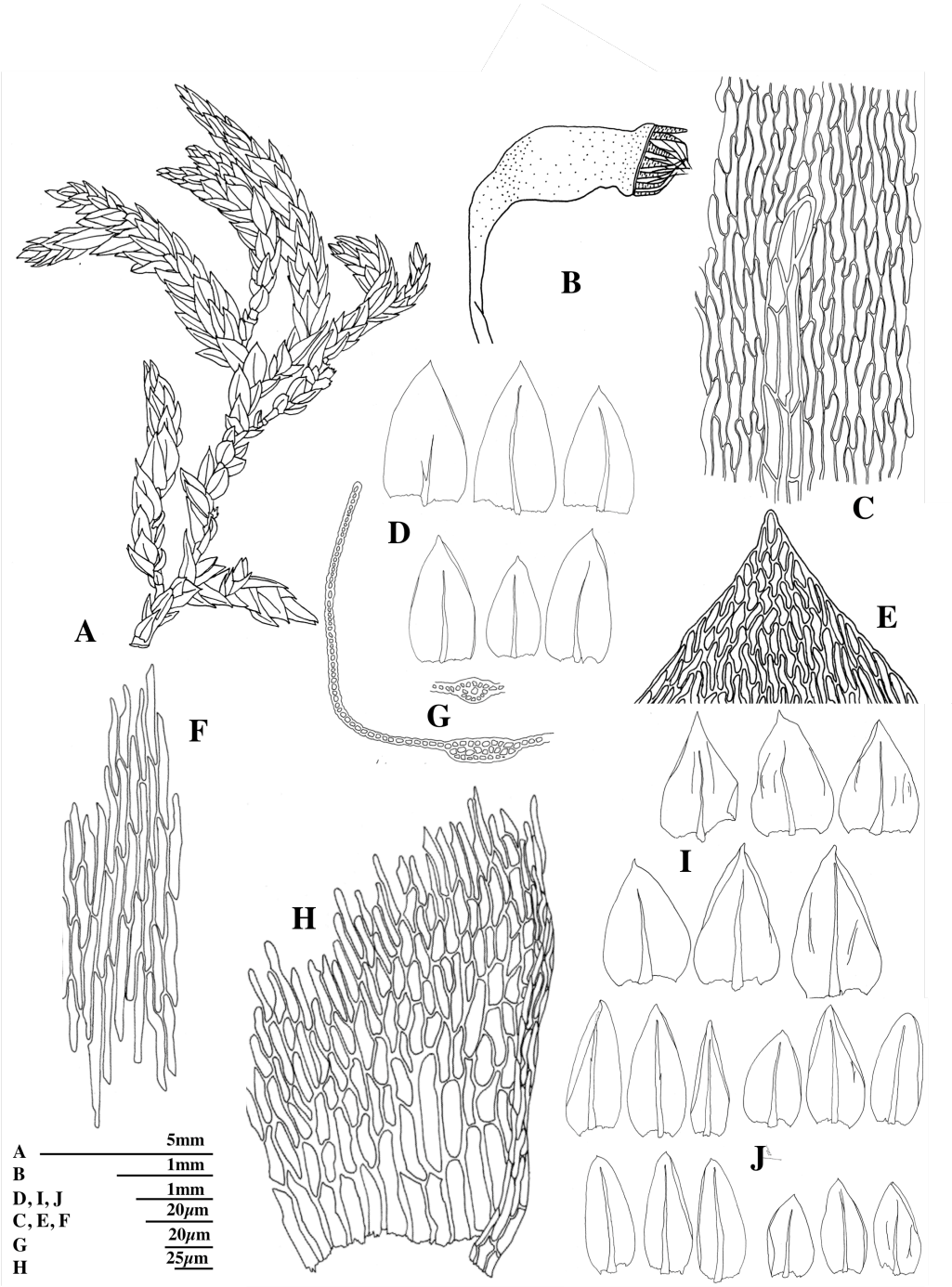

Figure 4.1. *Scleropodium occidentale* B.E. Carter *sp. nov*. All illustrations except I & J were drawn from the type. A) habit, B) sporophyte, C) costa tip, D) stem leaves (above) and branch leaves (below), E) leaf apex, F) lamina cells, mid-leaf, G) costa cross-section distal to mid-leaf (above) and proximal to mid-leaf (below), H) alar region, I) stem leaves, J) branch leaves. I & J are included to demonstrate the range of variation in the species and are drawn from *Carter 1659* and *Shevock 17645*.

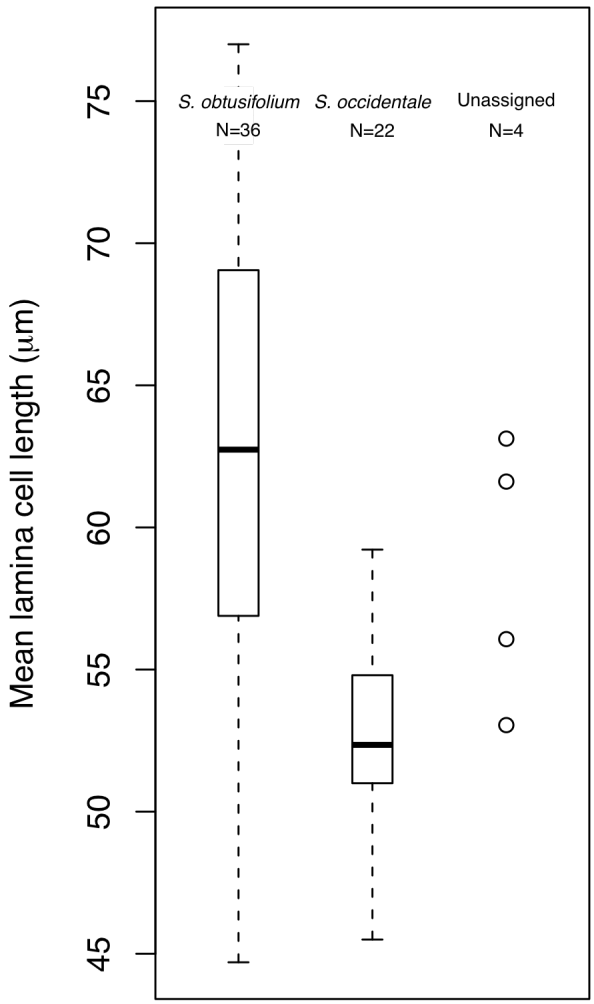

Figure 4.2. Cell lengths from mid-leaf of *Scleropodium obtusifolium*, *S. occidentale*, and four specimens that could not be assigned to species based on ITS & trnG sequences. The distributions of *S. obtusifolium* and *S. occidentale* are significantly different (T-test $P < 0.00001$).

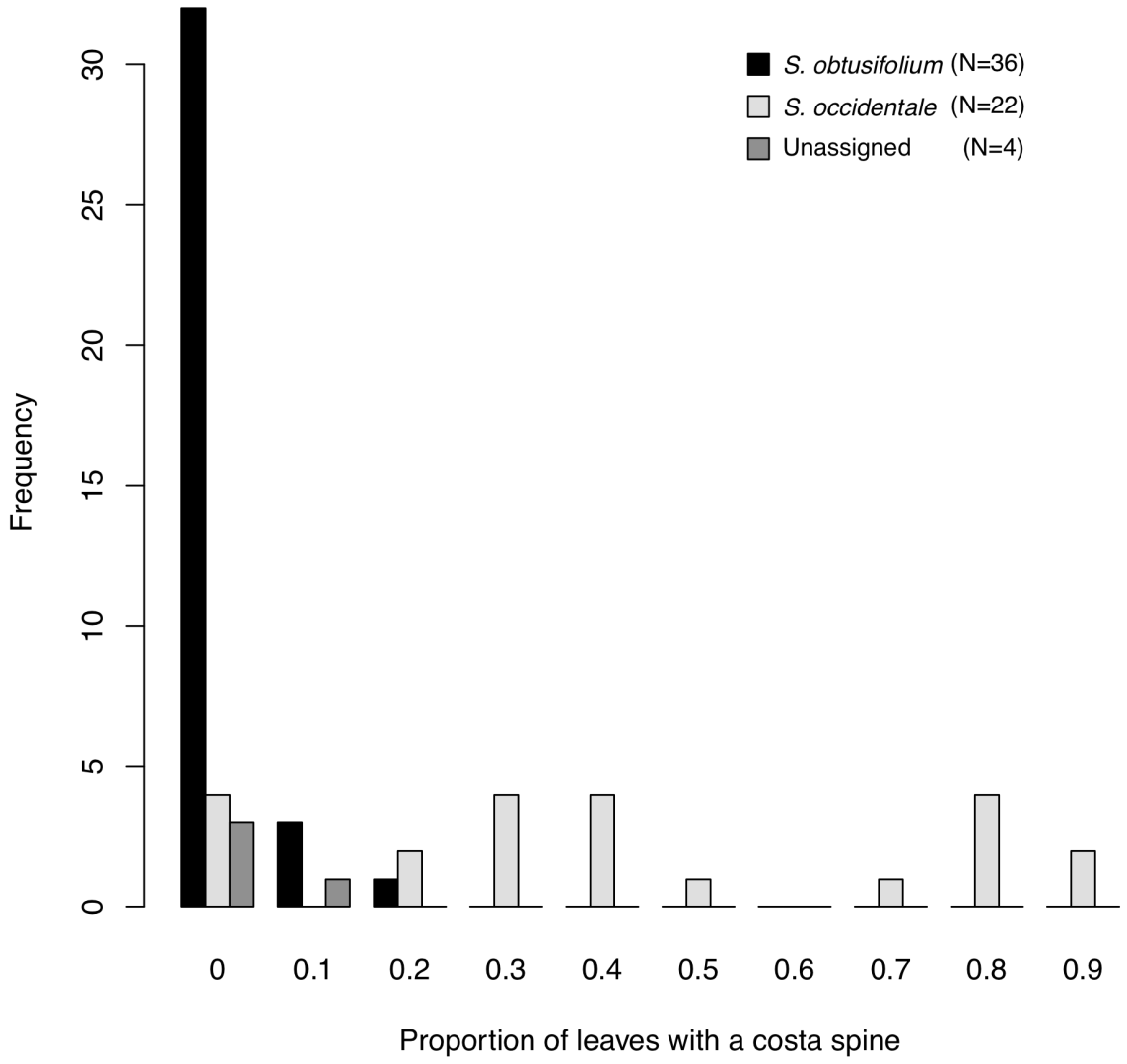

Figure 4.3. Frequencies of plants with different proportions of leaves bearing a costa spine for *Scleropodium obtusifolium*, *S. occidentale*, and four specimens that could not be assigned to species based on ITS & trnG sequences. The distributions of *S. obtusifolium* and *S. occidentale* are significantly different (Mann-Whitney $P < 0.00001$).

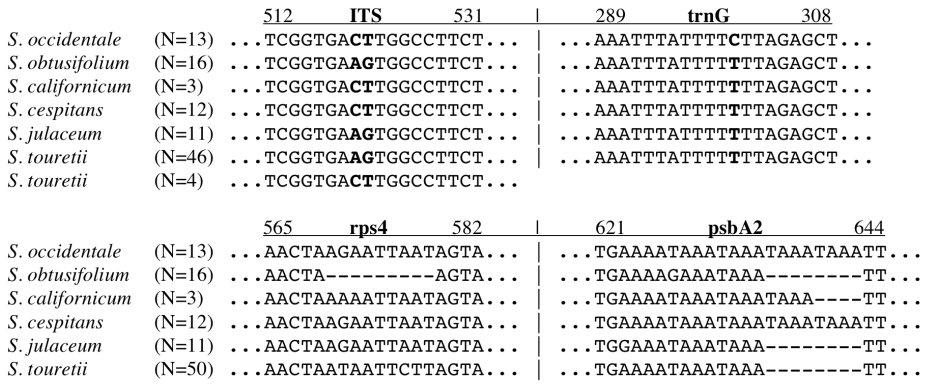

Figure 4.4. Alignments indicating nucleotide positions (in bold) differentiating *S. obtusifolium* and *S. occidentale*. Data from Carter (in press).

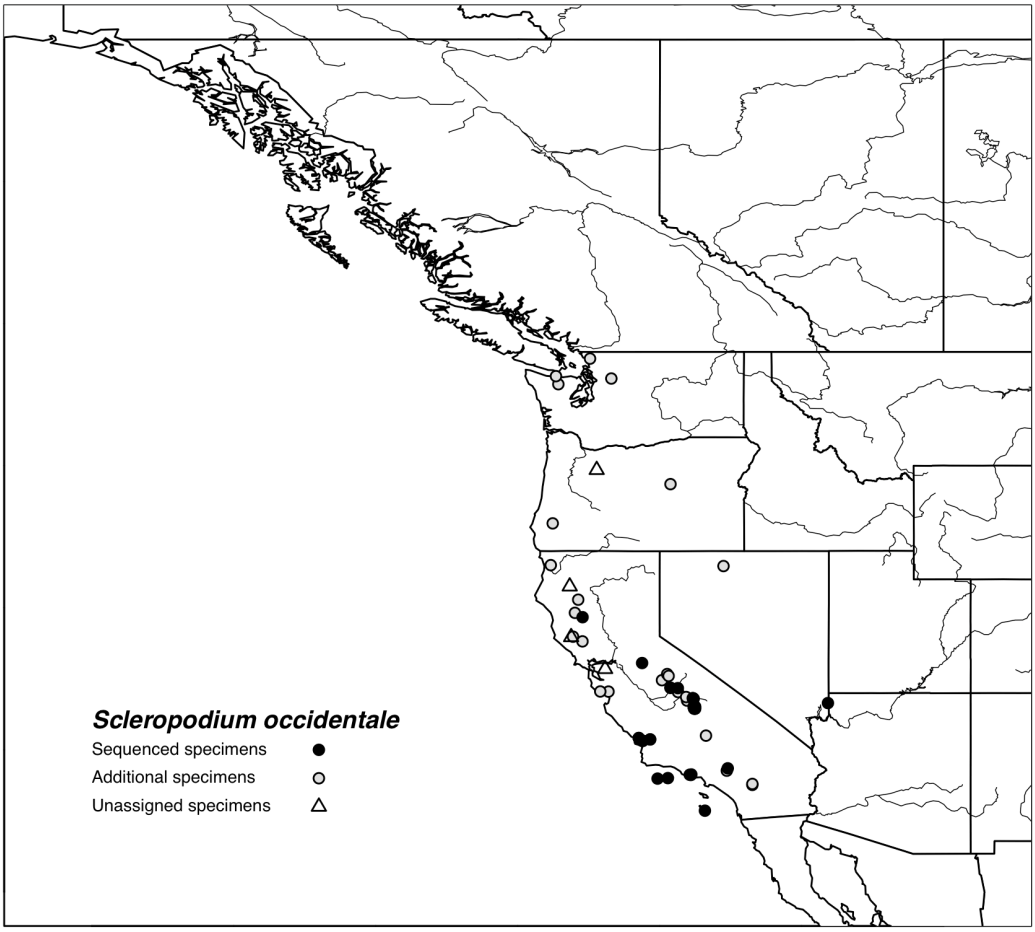

Figure 4.5. Geographic distribution of *Scleropodium occidentale* and four specimens that could not be assigned to species based on ITS & trnG sequences.

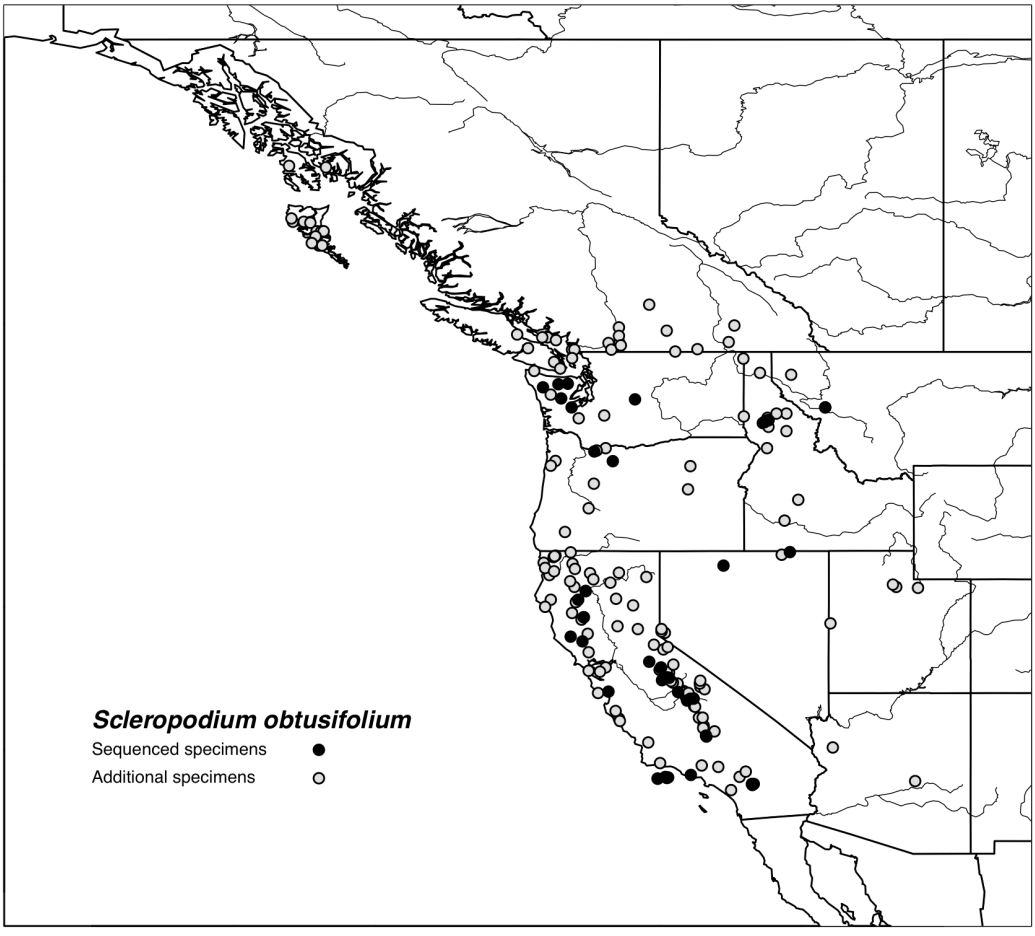

Figure 4.6. Geographic distribution of *S. obtusifolium*.

Appendix A. GenBank accession numbers (ITS, *trnG*) for the specimens used in this study. Except where noted, voucher specimens are at UC.

Scleropodium occidentale*:** *Carter 6231* (JQ421733, JQ421766); *Carter 4631* (JQ421734, JQ421767); *Villasenor sn (26 Mar 2011)* (JQ421735, JQ421768); *Shevock 20628* (JQ421736, JQ421769); *Shevock 19323* (JQ421737, JQ421770); *Shevock 13608* (JQ421738, JQ421771); *Shevock 23639* (JQ421739, JQ421772); *Shevock 14646* (JQ421740, JQ421773); *Laeger 2061* (JQ421741, JQ421774); *Shevock 12957* (JQ421742, JQ421775); ***Scleropodium obtusifolium: *Gray 1678* [UBC] (JQ421747, JQ421780); *Gray 1842* [UBC] (JQ421748, JQ421781); *Schofield 120999* [UBC] (JQ421749, JQ421782); *Carter 6243* (JQ421750, JQ421783); *Carter 4721* (JQ421751, JQ421784); *Carter 6047* (JQ421752, JQ421785); *Carter 6000* (JQ421753, JQ421786); *Norris 103682* (JQ421754, JQ421787); *Shevock 23755* (JQ421755, JQ421788); *Carter 2612* (JQ421756, JQ421789); *Shevock 19569a* (JQ421757, JQ421790); *Shevock 19569* (JQ421758, JQ421791); *Shevock 22804* (JQ421759, JQ421792); *Norris 95271* (JQ421760, JQ421793); *Norris 94911* (JQ421761, JQ421794); *Norris 104477* (JQ421762, JQ421795); *Norris 98593* (JQ421763, JQ421796); *Norris 83659* (JQ421764, JQ421797); *Norris 98773* (JQ421765, JQ421798); ***Scleropodium* sp. (not assignable based on ITS/trnG sequence data)*:*** *Shevock 24519* (JQ421743, JQ421776); *Carter 2801a* (JQ421744, JQ421777); *Carter 512* (JQ421745, JQ421778); *Norris 109412* (JQ421746, JQ421779)

CHAPTER 5. The taxonomic status of the Tasmanian endemic moss, *Scleropodium australe* (Brachytheciaceae)

Abstract

Scleropodium australe is known from a single population in a suburb of Hobart, Tasmania and is the only southern hemisphere representative in an otherwise north temperate genus. It was originally segregated from *S. cespitans* based on two quantitative characters, median lamina cell width and relative costa length. Molecular data presented here (ITS*, rps4, trnG, psbA2*) indicate that *S. australe* is nested within a well supported clade of *S. touretii*. Morphologcial measurements also fail to demonstrate any differences between *S. australe* and *S. touretii*. Based on the lack of any molecular or morphological differences between *S. touretii* and *S. australe*, and considering the ruderal habitat of the latter, *S. australe* is reduced to synonomy with *S. touretii*.

Introduction

Brachytheciaceae is a diverse family of mosses with members that often exhibit a challenging amount of morphological variation (Huttunen & Ignatov 2004, Robinson 1962). Increasingly, molecular analyses are becoming very useful in not only inferring evolutionary relationships within the family (Huttunen & Ignatov 2004, Vanderpoorten et al. 2005), but also in understanding biogeographic patterns (Huttunen et al. 2008), recognition of morphologically distinct but previously unrecognized taxa (Draper & Hedenäs 2009a, Draper & Hedenäs 2009b, Hedenäs et al. 2009), and as new evidence in evaluation of currently recognized taxa that are putatively rare or endemic (Blokeel et al. 2005).

Within Brachytheciaceae, the small genus *Scleropodium* is typical of the family in its difficult array of morphological variation. Specimens are generally assigned to the genus without difficulty, however differences among species are often subtle. The genus consists of eight currently recognized species with a primarily north temperate distribution (Grout 1899, Lawton 1967, Lawton 1971, Norris & Shevock 2004a, Smith 2004). The center of diversity is along the Pacific coast of North America where as many as six of the species can be found in sympatry. The genus also occurs in Europe (*S. cespitans* (Müll. Hal.) Koch & *S. touretii* (Brid.) Koch*,* both disjunct from North America), Korea (*S. coreense* Cardot & *S. brachyphyllum* Cardot) and Tasmania (*S. australe* Hedenäs).

Scleropodium australe, endemic to Tasmania, is unique within the genus in having a southern hemisphere distribution. The species was described from a collection made by Ann Ratkowsky (now deceased) in 1980 from a single population in a somewhat ruderal area in a suburb of Hobart, Tasmania (Hedenäs 1996). A thorough discussion of the morphology, distribution, and ecology of the species has been presented by Hedenäs (1996, 2002). The original diagnosis of *S. australe* is based on two quantitative differences from *S. cespitans*, namely a longer costa (*S. australe*: 3/5–4/5 leaf length; *S. cespitans*: 1/2–2/3) and narrower median lamina cells (*S. australe*: 4.0–6.5µm; *S. cespitans*: 5.0-8.0(9.0)µm). The first determination applied to the holotype was *Pseudoscleropodium purum* (Hedw.) Fleisch. but, as discussed by Hedenäs (1996), the similarity of *S. australe* to that species is only superficial.

Given the peculiar biogeography, narrow distribution, and potential risk of habitat destruction, additional information was desired to assess the status of *S. australe* as an independent species so that its conservation priority could be assessed.

Methods

Efforts to resample the population

Discussion with R. Seppelt at HO prompted his successful attempt to find the location of the population upon which this species was named. Although attempts to find the species at the type locality were unsuccessful due to the vagueness of the collection notes, individuals were discovered within several hundred meters of where it was believed the original material was collected (R. Seppelt & L. Cave, pers. comm., Dec. 2009). The present collection was found in a sheltered, moist site on soil and leaf litter beside a walking trail on the north side of Guy Fawkes Rivulet, beneath a canopy of *Eucalyptus viminalis, Acacia dealbata,*and *Crataegus* sp. Associated mosses were *Kindbergia praelonga, Wijkia extenuata, Hypnum cupressiforme* with scattered *Pseudoscleropodium purum*. After comparing the newly collected specimens to the isotype at HO, Seppelt sent duplicates (*Seppelt 27568*) to J. Shevock at CAS and UC, and material from the duplicate at UC was used in the analyses presented here.

Molecular data

Examination of the *S. australe* specimen revealed a stronger overall resemblance to *S. touretii* rather than to *S. cespitans* from which it was originally segregated. *Scleropodium cespitans* and *S. touretii* intergrade morphologically and data gathered for an ongoing global revision of the genus (Carter, in prep.) has revealed that many specimens of these two species are misidentified in herbaria. Due to the difficulty of correctly identifying *S. cespitans* and *S. touretii*, individuals of both species were sequenced to compare with *S. australe* (Table 5.1 and TREEBASE accession 10594: http://purl.org/phylo/treebase/phylows/study/TB2:S10594).

Based on preliminary analyses of the entire genus, ITS and the chloroplast *rps4*, *trnG*, and *psbA2* sequences are known to vary among species within the genus. Outgroup taxa were selected with the help of earlier Brachytheciaceae phylogenies that included representatives of *Scleropodium* (Huttunen & Ignatov 2004, Vanderpoorten et al. 2005).

Isolation of DNA was conducted using the DNEasy Plant Mini-prep kit (Qiagen, Germantown, Maryland, USA) following the manufacturer's protocol with subsequent dilution to 1:100. PCR amplifications were performed using AccuPower PCR premix tubes (Bioneer Co., South Korea). Primers and PCR protocols were obtained from Shaw et al. (2003). PCR amplicons were cleaned using Exonuclease I and Antarctic Phosphotase (New England Biolabs Inc., Ipswich MA, USA). Clean PCR amplicons were cycle sequenced at the UC Berkeley DNA sequencing facility (http://mcb.berkeley.edu/barker/dnaseq/index.html).

Sequences were aligned manually in Mesquite version 2.5 (Maddison & Maddison 2008). No regions needed to be excluded on account of ambiguity. Chloroplast and ITS datasets yielded the same topologies, so the datasets were concatenated. Several indels were found to be phylogenetically informative so gaps were coded into a separate data matrix and included in phylogenetic analyses. All gaps were coded as binary characters (present/absent) regardless of length (Simmons & Ochoterena 2000).

Phylogenetic analyses were performed using maximum parsimony in PAUP* version 4.10b10 (Swofford 2002) and using Bayesian inference in MrBayes (Huelsenbeck & Ronquist 2001). A heuristic search was implemented for the parsimony analysis, and support was assessed with 100 bootstrap replicates. For the Bayesian analysis, the Akaike criterion was used to select the (GTR + I) substitution model for the concatenated sequence data using MrModeltest version

2.3 (Nylander 2004). A separate model with identical forward and reverse transition rates was used for the indel matrix (Lewis 2001). The Bayesian analysis was run for 1,000,000 generations with sampling every 100 generations. Stationarity of the likelihood values was checked by plotting and the initial 2500 trees from the burn-in phase were removed, with the remaining trees combined to represent the posterior probability distribution.

Morphological data

To determine whether *S. australe* falls outside of the range of normal morphological variation of *S. touretii*, a dataset of 8 continuous characters was measured using the *S. australe* specimen and 33 specimens representing *S. touretii* (15 specimens from western North America, 18 specimens from the Old Word, including northwestern Europe, the Mediterranean Basin and the Canary Islands). The characters measured were selected based on putative usefulness in differentiating species within the genus based on published keys and descriptions (Grout 1899, Lawton 1967, Norris & Shevock 2004b). *Scleropodium australe* is known only from sterile material so only gametophytic characters could be used. The characters measured were 1) leaf length, 2) leaf width, 3) leaf length/width ratio, 4) leaf length/costa length ratio, 5) number of differentiated cells along the margin, 6) lamina cell length, 7) lamina cell width, and 8) lamina cell length/width ratio. *Scleropodium* stems often demonstrate sympodial branching, so designation of true branches (as opposed to immature stems) can be tenuous. Because of this only well developed stem leaves were used. Cells of the lamina were measured at approximately 1/3 of the way from the leaf base and midway between the costa and margin. For all eight characters, ten measurements were made for each individual. The mean of the ten measurements was then used to represent the individual in the subsequent multivariate analysis.

The eight characters were checked for univariate normality and colinearity. Cell width and leaf width were omitted from the multivariate analysis because they were strongly correlated with cell length and leaf length, respectively. The remaining six variables were converted to z-scores and subjected to a Principal Components Analysis. All data analysis was done using the R statistical software (http://www.r-project.org/).

Results

Phylogeny

The aligned lengths for the four regions were: ITS 729bp, *rps4* 568bp, *psbA2* 662bp, and t*rnG* 532bp. Of these, 87 positions were variable but uninformative, and 34 positions were parsimony informative (13 from ITS, 7 each from the three chloroplast regions). An additional 8 gaps from the indel matrix were parsimony informative. The 50% majority rule consensus tree from the Bayesian analysis is presented in Figure 5.1. The parsimony analysis yielded a single best tree (length= 142, CI= 0.9366, RI=0.9135) with a topology fully congruent with that of the Bayesian analysis. In these analyses, *Scleropodium australe* is nested within a well supported clade of Old World *S. touretii*, and *S. cespitans* is monophyletic. Within the clade comprising Old World *S. touretii* and *S. australe*, each of the three *S. touretii* individuals have a single autapomorphic substitution within ITS, and all four individuals are identical across the three chloroplast regions.

Morphology

The first two principal components capture 86% of the variation within the original dataset of six characters (Fig. 5.2). Based on these six characters, *S. australe* falls within the multivariate range

of variation of *S. touretii*. To verify that no single character could be used to diagnose *S. australe*, the single value of *S. australe* for each of the eight characters was compared to the distribution of values for the *S. touretii* sample. Statistical tests were deemed inappropriate given that only a single specimen of *S. australe* was available, but for all 8 characters the *S. australe* specimen fell within one standard deviation of the mean of the *S. touretii* distribution (Table 5.2).

Discussion

In the original diagnosis, Hedenäs (1996) seggregated *Scleropodium australe* from *S. cespitans* while making no reference to *S. touretii*. Differences between *S. touretii* and *S. cespitans* have been the source of some confusion, and many specimens of *S. touretii* and *S. cespitans* are misidentified in both European and North American herbaria. Researchers have generally separated the two by defining *S. touretii* as having larger, more concave leaves, a shorter costa, longer and narrower median lamina cells and fewer rows of differentiated basal cells (but see discussion of 'alar region' in Hedenäs (1996)). In addition, *S. touretii* tends to have recurved leaf apices and often has stems that are distally arcuate. This combination of characters, in conjunction with the large size, is unique in the genus. For all of these characters, *S. australe* is indistinguishable from *S. touretii.*

Molecular data presented here indicate that *S. cespitans* and *S. touretii* are separate entities, but that *S. australe* is nested within *S. touretii* (Fig. 5.1). A subsequent morphological analysis of quantitative characters used to differentiate these and other species of *Scleropodium* also failed to identify any features with which *S. australe* could be differentiated from *S. touretii* (Fig. 2 and Table 2). Furthermore, *S. australe* was known from just a single population in a ruderal area, with the new collection from close by. The recently discovered individuals were found with *Pseudoscleropodium purum*, an introduced species, and in the vicinity of several introduced vascular species (R. Seppelt & L. Cave, pers. comm., Dec. 2009). Based on the morphological, molecular and ecological evidence presented here, it appears that *S. australe* is a population of European *S. touretii* recently introduced to Tasmania. Therefore *Scleropodium australe* is here reduced to synonomy with *S. touretii*. This eliminates the need to consider *S. australe* as a conservation priority, and also changes the native range of the genus *Scleropodium* to a strictly northern hemisphere distribution.

Nomenclatural amendment:
Scleropodium touretii (Brid.) L.F.Koch, Rev. Bryol. Lichénol. 18: 177. 1949.
Basionym: *Hypnum touretti* Brid., Musc. Recent. Suppl. 2: 185. 1812.
Synonym: *Scleropodium australe* Hedenäs, Nova Hedwigia 62: 457. fig.1: A, F–I. 1996. *A.V.Ratkowsky H372*. (CBG 8206591; Isotypes: AD, CBG, HO, L.) *syn. nov.*

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Figures and Tables

Taxon	Collection (Herbarium)	Origin	Accession Numbers (*ITS, rps4, trnG, psbA2*)
Scleropodium australe	Seppelt 27568 (UC)	Hobart, Tasmania	GU306129, GU329706, GU329716, GU329726
Scleropodium cespitans	Nyholm 876b/71 (S)	Izmir, Turkey	GU306130, GU329707, GU329717, GU329727
Scleropodium cespitans	Bisang 930106 (S)	Sardinia, Italy	GU306131, GU329708, GU329718, GU329728
Scleropodium cespitans	Een sn, 14 Mar 1998 (S)	Andalucia, Spain	GU306132, GU329709, GU329719, GU329729
Scleropodium cespitans	Carter 1839 (UC)	California, USA (1)	HM367712, HM367722, HM367727, HM367717
Scleropodium cespitans	Carter 667 (UC)	California, USA (2)	HM367713, HM367723, HM367728, HM367718
Scleropodium obtusifolium	Shevock 17188 (UC)	California, USA	GU306133, GU329710, GU329720, GU329730
Scleropodium touretii	Een sn, 2 Jun 1994 (UC)	Sancta Clara-a-Velha, Portugal	GU306134, GU329711, GU329721, GU329731
Scleropodium touretii	Long 38021 (E)	Umbria, Italy	GU306135, GU329712, GU329722, GU329732
Scleropodium touretii	Een G027 (S)	Crete, Greece	GU306136, GU329713, GU329723, GU329733
Scleropodium touretii	Carter 2867 (UC)	California, USA (1)	HM367714, HM367724, HM367729, HM367719
Scleropodium touretii	Carter 1825 (UC)	California, USA (2)	HM367715, HM367725, HM367730, HM367720
Scleropodium touretii	Carter 2459 (UC)	Oregon, USA	HM367716, HM367726, HM367731, HM367721
Homalothecium nuttallii	Carter 336 (UC)	California, USA	GU306137, GU329714, GU329724, GU329734
Kindbergia praelonga	Carter 1506 (UC)	California, USA	GU306138, GU329715, GU329725, GU329735

Table 5.1. Vouchers and Genbank accession numbers for specimens used in the molecular analyses.

	S. touretii	*S. australe*
Alar cells along margin	4.7—7.4	5.4
Leaf length (mm)	1.2—1.7	1.3
Leaf width (mm)	0.61—0.93	0.78
Leaf length/width	0.46—0.62	0.60
Costa length/lf length	0.64 —0.79	0.72
Lamina cell length (μm)	60—82	67
Lamina cell width (μm)	4.7—5.5	5.0
Lamina cell length/width	11.7—16.9	13.4

Table 5.2. Comparison of stem leaf measurements for *S. australe* and 33 individuals representing *S. touretii*. Values for *S. touretii* are the mean (+/-) one standard deviation.

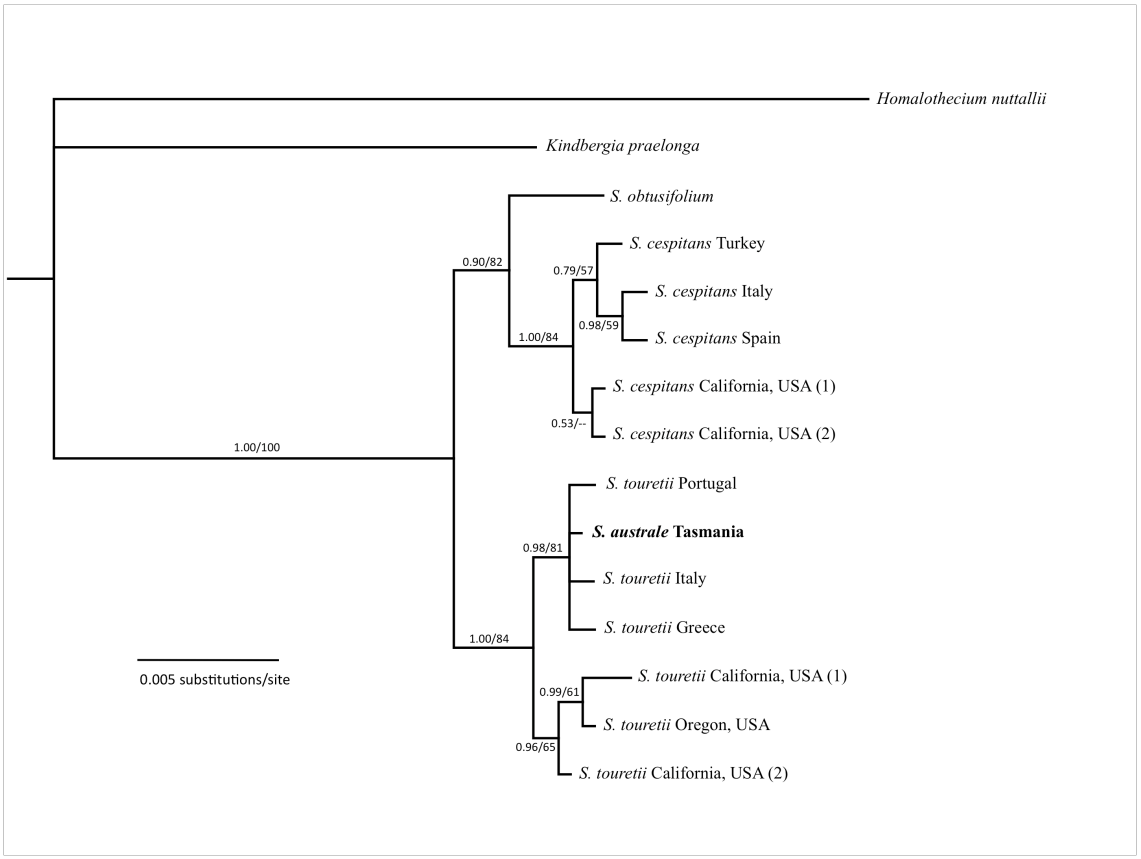

Figure 5.1. Fifty percent majority-rule consensus of 7500 trees sampled from the posterior probability distribution generated by a Bayesian analysis of the combined ITS, *rps4*, *psbA2* and *trnG* dataset. Support values are Posterior Probabilities/Maximum Parsimony bootstrap values. Values less than 0.50/50% are indicated by "--".

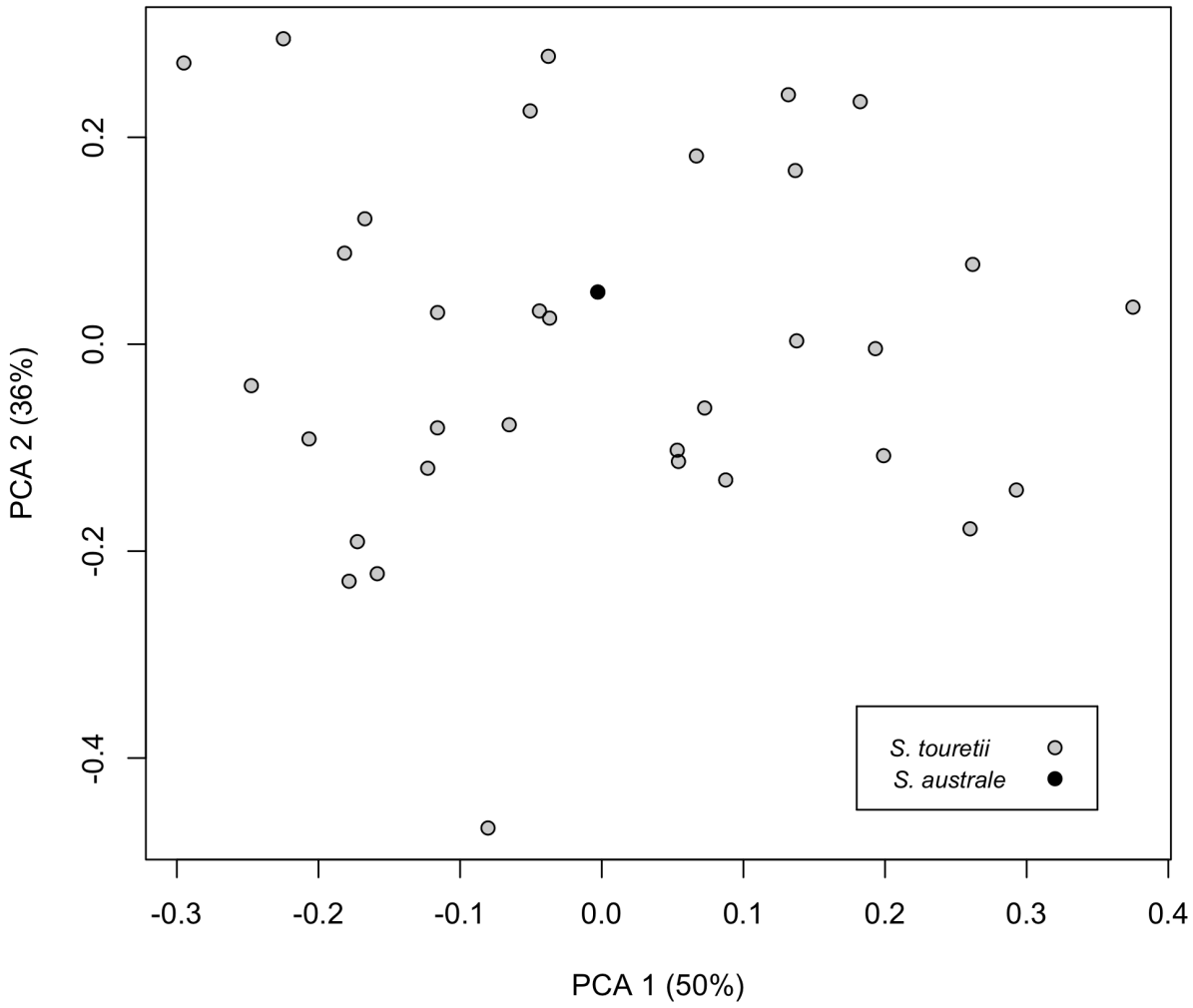

Figure 5.2. Principal Components Analysis of 6 morphological characters for *S. australe* and 33 individuals representing *S. touretii*.