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Assessing Traditional Morphology- and Chemistry-Based Species Circumspections in Lichenized Ascomycetes: Character Evolution and Molecular Species Delimitation in Common Western North American Lichens

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Assessing traditional morphology- and chemistry-based species circumspections in lichenized ascomycetes: character evolution and species delimitation in common western North American lichens

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ABSTRACT

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Accurate species delimitation has critical implications for ecological and conservation studies; and for understanding factors driving diversification. However, a growing body of evidence indicates that morphology-based species circumspection in lichenized ascomycetes often fails to accurately represent the number of fungal species. The use of molecular data in lichen systematics provides an important alternative to traditional morphological characters for identifying natural groups and assessing evolutionary histories in challenging lichen taxa. In this work, I examined two common lichen-forming genera in western North America, *Rhizoplaca* and *Xanthoparmelia,* as models for investigating character evolution, species delimitation in morphologically and chemically diverse species, and identification of lineages in the early stages of divergence. Phylogenetic hypotheses were reconstructed to assess character evolution using sequence data from four nuclear ribosomal markers and fragments from two nuclear loci. I applied a multifaceted approach to delimit species in *Rhizoplaca* and *Xanthoparmelia* by assembling multiple lines of evidence using DNA sequence data, and genealogical and population genetic analyses. I have found that traditionally circumscribed species are not supported by molecular data. For example, in *Rhizoplaca* previously unrecognized lineages were identified within what has thus far been considered a single species. In contrast, morphologically and chemically distinct species within *Xanthoparmelia* were not supported by molecular data. Distinct medullary chemistries, growth forms, and the production of vegetative diaspores appear to have evolved independently multiple times in *Xanthoparmelia*. This work clearly indicates that morphological and chemical characters do not always accurately reflect lichen species diversity within even the best known and studied genera. My study of the *Rhizoplaca melanophthalma* species complex demonstrates that the genus *Rhizoplaca,* as presently circumscribed, is more diverse in western North American than previously thought. I present these analyses as a working example of species delimitation in morphologically cryptic lichenized fungi. In *Xanthoparmelia* diagnostic morphological and chemical characters have evolved in a highly homoplasious manner. In contrast to other studies documenting previously undiscovered fungal lineages masked within lichen species circumscribed by traditional morphological and chemical characters, my work suggests that species diversity has been overestimated in the lichen genus *Xanthoparmelia*.

Keywords: character evolution, convergence, lichens, morphology, Parmeliaceae, *Rhizoplaca*, secondary metabolites, speciation, species concepts, species delimitation, vagrant lichens, *Xanthoparmelia*

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CHAPTER ONE

Complex patterns of speciation in cosmopolitan "rock posy" lichens - an integrative approach to discovering and delimiting fungal species in the lichen-forming *Rhizoplaca melanophthalma* species-complex (Lecanoraceae, Ascomycota)

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Abstract

A growing body of evidence indicates that morphology-based species circumspection of lichenized ascomycetes greatly misrepresents the number of existing species. Recently it has been demonstrated that population-level processes operating within diverging populations can facilitate the identification of lineages in the early stages of species divergence. The cosmopolitan "rock posy" lichen (*Rhizoplaca melanophthalma*) species-complex includes a number of morphologically distinct species that are both geographically and ecologically widespread, providing a model system to evaluate speciation in lichen-forming ascomyctes. In this study, we assembled multiple lines of evidence from ribosomal and nuclear DNA sequence data, morphology, and biochemistry for species delimitation in the *Rhizoplaca melanophthalma* species-complex. Using multiple analytic approaches, we recover a total of ten candidate species in this study, four of which were described as distinct taxa and six previously unrecognized lineages found within what has been thus far considered a single species. Multiple instances of sympatry support the view that these lineages merit recognition as distinct taxa. Generally, we found little corroboration between morphological and chemical characters and previously unidentified lineages defined in this study, as most candidate species were morphologically polymorphic. However, secondary metabolite data supported one cryptic saxicolous lineage, characterized by orsellinic-derived gyrophoric and lecanoric acids, which we consider to be taxonomically significant. Our study of the *R. melanophthalma* species-complex indicates that the genus *Rhizoplaca*, as presently circumscribed, is more diverse in western North American than originally perceived, and we present our analyses as a working example of species delimitation in morphologically cryptic and recently diverged lichenized fungi.

Key words: lichen species concepts, *Rhizoplaca*, secondary metabolites, speciation, species delimitation, sympatry, vagrant lichens

Introduction

Lichens are obligate symbiotic systems consisting of a filamentous fungus, a photosynthetic partner (eukaryotic alga and/or cyanobacterium), and, at least in some cases, non-photosynthetic bacteria (Cardinale et al., 2008; Grube et al., 2009; Hodkinson and Lutzoni, 2009; Selbmann et al., 2010). The lichenized condition has been extremely successful for many fungal lineages, with an estimated 40% of all ascomycetes forming lichens (Lutzoni, Pagel, and Reeb, 2001). Traditionally, morphology and the expression of signature secondary metabolites have been used to define taxonomic boundaries for lichenized fungi (Culberson, 1972; Hale, 1990; Huneck and Yoshimura, 1996; Huneck, 1999). However, these characters are often widely variable, and their homology has proven difficult to assess between and within taxonomic groups (LaGreca and Lumbsch, 2001; Lumbsch and Schmitt, 2001; Blanco et al., 2004a; Ott et al., 2004; Crespo et al., 2007). A growing body of evidence suggests that in many cases lichen species diversity has been misrepresented (Kroken and Taylor, 2001; Buschbom and Mueller, 2006; Wirtz, Printzen, and Lumbsch, 2008; Crespo and Pérez-Ortega, 2009; O'Brien, Miadlikowska, and Lutzoni, 2009; Printzen, 2009; Wedin et al., 2009), and morphology/chemistry-based species circumspections may underestimate lichenized ascomycete diversity, especially within morphologically similar species with cosmopolitan distributions (Hawksworth, 2001; Crespo et al., 2002; Molina et al., 2002; Murtagh et al., 2002; Dettman, Jacobson, and Taylor, 2003; Divakar et al., 2005).

Because species represent fundamental units of analysis in various sub-disciplines of biology, accurate species diagnoses are critical. Therefore, reassessing current species delimitation is particularly relevant in lichenized fungi, especially in cases when well-established morphological and chemical characters used to define species boundaries are uninformative or

incongruent. One of several challenges associated with empirical species delimitation in lichenized fungi is finding and applying the appropriate character sets and analytical tools (Wirtz, Printzen, and Lumbsch, 2008; Crespo and Pérez-Ortega, 2009). In spite of the complicated issues associated with attempts to empirically define species, all contemporary species concepts share the common view that species are segments of separately evolving metapopulation lineages (de Queiroz, 1998, 1999; Mayden, 1999; de Queiroz, 2007). This concept allows researchers to investigate species delimitation using different empirical properties and facilitates the development of new methods to test hypotheses of lineage separation (de Queiroz, 2007). A rapidly growing interest in species delimitations has resulted in novel approaches to investigate species boundaries (Sites and Marshall, 2004; Knowles and Carstens, 2007; O'Brien, Miadlikowska, and Lutzoni, 2009; Vieites et al., 2009; Carstens and Dewey, 2010; O'Meara, 2010; Weisrock et al., 2010; Yang and Rannala, 2010), and more properties (lines of evidence) supporting putative lineages are associated with a higher degree of corroboration (de Queiroz, 2007). Methods identifying lineages in the early stages of species divergence are particularly informative in understanding the processes driving speciation (Wiens, 2004; Weisrock et al., 2010).

An integrative approach to species delimitation is recognized as an essential strategy for rigorously testing species boundaries, particularly among cases involving recent speciation events (Will, Mishler, and Wheeler, 2005; Knowles and Carstens, 2007; Roe and Sperling, 2007). Reliance on a single type of data, such as molecular, morphological, or chemical, often provides an incomplete or inaccurate view of true relationships. Although different data sets and different operational criteria may give conflicting or ambiguous results due to multiple evolutionary processes occurring within and between populations, the use of several independent

suites of characters, such as morphology, geographic range, host preference, and cross-validation using inferences from multiple empirical operational criteria have been shown to establish robust species boundaries (Hey et al., 2003; Sites and Marshall, 2004; Dayrat, 2005; Duminil et al., 2006a; Roe and Sperling, 2007; O'Brien, Miadlikowska, and Lutzoni, 2009; Ruiz-Sanchez and Sosa, 2010; Weisrock et al., 2010).

As traditional characters used to delimit lichen species tend to misrepresent mycobiont diversity, we feel it is important to address lichen species boundaries using an integrative approach based on multiple independent datasets and operational criteria to effectively identify and delimit lichen species. We selected the rock posy *Rhizoplaca melanophthalma* species-complex (Ascomycota, Lecanorales, Lecanoraceae) as a model system to assess species diversity for this study because of its broad ecological and geographical distribution, morphological, chemical and genetic diversity, and its importance as a sensitive indicator of environmental health (Leuckert, Poelt, and Hahnel, 1977; Dillman, 1996; Arup and Grube, 2000; Aslan, Budak, and Karabulut, 2004; Ugur et al., 2004; Zhou et al., 2006). This group was identified as a well-supported monophyletic lineage and includes the placodiod crustose taxon, *Lecanora novomexicana* H. Magn., the umblicate taxon *R. melanophthalma* (DC.) Leuckert & Poelt, and at least 4 vagrant, obligatory unattached, species (Arup and Grube, 2000).

The green rock posy lichen *R. melanophthalma* sensu lato (s. l.) has a worldwide distribution, and in North America it ranges from the northern boreal zone to Mexico along the Rocky Mountain corridor. It is commonly found in the Intermountain Western United States growing in large populations on rocky substrates. Specimens are generally umblicate (fixed to the substrate by a single point of attachment), but often appear squamulose or pulvinate (polyphyllous), and considerable chemical variation is found within the species (McCune, 1987;

Ryan, 2001). However, the assignment of taxonomic rank to distinct morphologies and chemotypes within *R. melanophthalma* s. l. remains uncertain. The vagrant, obligatory unattached, taxa in North America, including *R. cylindrica* (not formally described), *R. haydenii* (Tuck.) W. A. Weber, *R. haydenii* subspecies (ssp.) *arbuscula* Rosentreter, *R. idahoensis* Rosentreter & McCune, *R. melanophthalma* subsp. *cerebriformis* Rosentreter & B. D. Ryan, *R. melanophthalma* ssp. *crispa* Rosentreter & B. D. Ryan, and *R. subidahoensis* (not formally described), are endemic to the high plains and mountains of the central and northern Rocky Mountains in western North America and are particularly susceptible to habitat fragmentation, altered fire dynamics, and agricultural conversion (Rosentreter, 1993). The relationships of the closely related taxa within this group, including the placodiod *Lecanora novomexicana* and vagrant *Rhizoplaca* species remains unclear.

Speciation in lichenized fungi is, in general, understudied, and we present our analyses of the *R. melanophthalma* species-complex to represent the larger focus of this study, which is robust species delimitation in morphologically cryptic and recently diverged lichenized fungi. In this study we followed the general lineage concept (GLC; de Queiroz, 1998, 1999) as our non-operational species definition using an integrative approach to assess diversity within the *R. melanophthalma* species-complex. We analyzed molecular data within a phylogenetic framework to identify candidate species by examining monophyletic groups recovered in the topology, and assessed the putative lineages across individual gene trees to identify lineages that exhibited genealogical exclusivity, an expected pattern for divergent lineages (Avise and Ball, 1990; Baum and Shaw, 1995; Hudson and Coyne, 2002). Candidate species were also evaluated within a population-level framework to assess gene flow and genetic differentiation (O'Brien, Miadlikowska, and Lutzoni, 2009), and we used multi-locus sequence data to identify genetic

clusters without a priori assignment of individuals (Groeneveld et al., 2009; Weisrock et al., 2010). Finally, we investigated patterns in morphological and chemical variation and geographical and ecological distributions for each candidate species. The use of multiple data sets and the combination of analytical methods provides a robust approach to detect and evaluate unidentified lineages within the *R. melanophthalma* species-complex.

Materials and Methods

Taxon Sampling—Sequence data were analyzed from 170 individual posy rock lichens. The focal group was represented by four species from the *R. melanophthalma* species-complex, including *R. melanophthalma* (127 specimens from 37 localities), *Lecanora novomexicana* (6 from 4 localities), *R. haydenii* (6 from 4 localities), and *R. idahoensis* (4 from 2 localities); three formally described subspecies (ssp.), *R. haydenii* ssp. *arbuscula* (2 from a single locality), *R. melanophthalma* ssp. *cerebriformis* (1), *R. melanophthalma* ssp. *crispa* (1); and two undescribed species, *R. cylindrica* (1) and *R. subidahoensis* (1). Figure 1 depicts the high degree of morphological variation within the sampled *R. melanophthalma* species-complex in western North America. The present study emphasized umblicate saxicolous forms; therefore sampling of the lobate taxon *L. novomexicana* and vagrant taxa were relatively limited. Collections of *R. melanophthalma* s. l. were initially made in 1997 at ten, 9 x 15 m plots along an altitudinal gradient (2200 – 3400 m) at Thousand Lakes Mountain (TLM), Wayne County Utah, USA (Porter, 1998), and three additional 9 x 15 m plots (2200 m, 2800 m, and 3300 m) were collected on the neighboring Boulder Mountain Plateau (BM), Wayne and Garfield Counties, Utah, in 2008. Seven individual thalli were randomly chosen from each plot to assess ecological trends in distributions and reproductive isolation between candidate species identified in this study (see

section 3.3). We also sampled 39 additional specimens from the *R. melanophthalma* species-complex, collected from 24 populations throughout the Intermountain West, USA. Available internal transcribed spacer sequences obtained from GenBank, representing 20 individuals, were included to assess relationships within a broader taxonomic and phylogeographic context. *Rhizoplaca subdiscrepans* (Nyl.) R. Sant. (3 specimens) and *R. chrysoleuca* (Sm.) Zopf (18 specimens) were selected as outgroups, as identified in previous studies (Arup and Grube, 2000; Cansaran et al., 2006; Zhou et al., 2006). Collection information for all included specimens is summarized in Supplementary Table S1, and new voucher material generated for this study is housed at the Brigham Young University Herbarium of Nonvascular Cryptogams (BRY), Provo, Utah, USA.

Molecular data and sequence alignment—Total genomic DNA was isolated using either the E.Z.N.A. Plant DNA Kit (Omega Bio-Tek, Norcross, GA), following manufacturer's instructions, or the Prepease DNA Isolation Kit (USB, Cleveland, OH), following the plant leaf extraction protocol. We generated new sequence data via polymerase chain reaction (PCR) for five fungal nuclear markers including three nuclear ribosomal loci, the entire internal transcribed spacer region (ITS), a fragment of the intergenic spacer (IGS), and a group I intron located within nuclear SSU ribosomal DNA (Gutiérrez et al., 2007); and fragments from two low-copy protein-coding loci, *MCM7* and β-tubulin. The nuRNA gene tandem repeat exists in large copy numbers (100-200 copies) facilitating the amplification of the selected markers from older specimens (Thousand Lake Mountain collections made in 1997). Although low levels of intragenomic variation in fungal rDNA repeats suggest convergent evolution in which homogenization is very rapid and effectively maintains highly similar repeat arrays (Ganley and Kobayashi, 2007), previous studies have confirmed the utility of the sampled ribosomal loci for

species- and population-level studies in lichenized ascomycetes (Thell, 1999; Kroken and Taylor, 2001; Blanco et al., 2004b; Blanco O and et al., 2004; Buschbom and Mueller, 2006; Lindblom and Ekman, 2006; Brunauer et al., 2007; Gutiérrez et al., 2007; Wirtz, Printzen, and Lumbsch, 2008; O'Brien, Miadlikowska, and Lutzoni, 2009; Wedin et al., 2009). Although a gene duplication of β-tubulin has occurred within Ascomycota, the paralogs are easily distinguishable within the analyzed group, and the marker has been successfully employed to investigate α-level relationships in other lichenized ascoymycetes (Buschbom and Mueller, 2006; O'Brien, Miadlikowska, and Lutzoni, 2009; Wedin et al., 2009).

Standard polymerase chain reactions (PCR) were used to amplify targeted loci. Fungal-specific primers used in PCR amplifications and in the cycle sequencing reactions are shown in Table 1. PCR cycling parameters used for amplifying the ITS, group I Intron, and β- tubulin loci followed the methods of Blanco et al (2004); cycling parameters for amplifying the IGS followed the 66-56° touchdown reaction described in (Lindblom and Ekman, 2006); and PCR cycling parameters for amplifying the *MCM7* fragment followed Schmitt et al. (2009). PCR products were quantified on 1% agarose gel and stained with ethidium bromide. In cases where no PCR product was visualized for the β-tubulin and *MCM7* loci, internally nested PCR reactions were performed using 0.3µl of PCR product from the original reaction and newly developed internal primers 'BT-RhizoF' and 'BT-RhizoR' for the β-tubulin fragment, and 'Lec*MCM7*f' and 'Lec*MCM7*r' for the *MCM7* fragment. Nested PCR reactions followed the touchdown PCR cycling parameters described above used to amplify the IGS fragment. PCR fragments were cleaned using the PrepEase PCR Purification Kit (USB, Cleveland, OH), following manufacture's protocol, and complementary strands were sequenced using the same primers used for amplification. Sequencing reactions were performed using the Big Dye3 Termination

Sequencing Kit (Applied Biosystems, Foster City, CA), and products were run on an AB 3730xl automated sequencer at the DNA Sequencing Center, Brigham Young University Provo, Utah, USA.

Sequences were assembled and edited using Sequencher version 3.1.1 (Gene Codes Corporation, Ann Arbor, MI) and Se-Al v2.0a11 (Rambault, 1996), and sequence identity was confirmed with the 'megaBLAST' search in Genbank (Wheeler et al., 2006). Sequences were aligned in Muscle version 3.6 (Edgar, 2004), using default settings.

Nucleotide Polymorphism analyses and gene-flow estimation—We used DnaSP 5.10 (Librado and Rozas, 2009) to calculate basic nucleotide polymorphism statistics, including numbers of haplotypes (*H*), total number of polymorphic sites (N_{poly}), average pairwise diversity per site, (π; Nei, 1987) for each candidate species (see section 3.3). In addition, gene flow between candidate species was assessed by calculating F_{ST} values using DnaSP and counting the number of fixed nucleotides for all pairwise comparisons (O'Brien, Miadlikowska, and Lutzoni, 2009). *F*-statistic calculations were estimated from specimens with complete ITS, IGS, ß-tubulin, and *MCM7* dataset (the ribosomal group I intron was missing in all specimens assigned to a single candidate species, and this marker was therefore excluded from F_{ST} calculations). Aligned sequences were scanned for fixed characters between each candidate species and the remaining data matrix in DnaSP, and the total number of fixed nucleotide positions was tabulated for each candidate species.

Phylogenetic analyses—Preliminary phylogenetic reconstructions were performed for each sampled marker independently. However, overall weak phylogenetic signal was identified in the ribosomal group I intron and both protein-coding gene trees, and we preferred to concatenate all markers for phylogenetic reconstructions to improve topology and increase nodal

support (Wiens, 1998). Although potential pitfalls of concatenating independent nuclear genes in phylogenetic analyses exist (Degnan and Rosenberg, 2009; Edwards, 2009), coalescent-based methods using multilocus data to simultaneously indentify independently evolving lineages and infer relationships among these are limited (O'Meara, 2009). Furthermore, coalescent-based phylogenetic methods are still very sensitive to deviations from assumptions, especially post-divergence introgression (Leache, 2009; Liu et al., 2009). Heterogeneity in phylogenetic signal among the sampled markers was assessed before combining the datasets (Lutzoni et al., 2004). We performed maximum likelihood (ML) analyses of the concatenated ribosomal dataset (ITS, IGS, and group I intron), β-tubulin, and *MCM7* markers separately in RAxML version 7.0.4 (Stamatakis, 2006; Stamatakis, Hoover, and Rougemont, 2008), using the 'rapid bootstrapping' option as implemented in the CIPRES Web Portal. RAxML allows partitioned analyses implementing the general time reversible (GTR) model of evolution for all partitions, and in the ribosomal dataset individual loci were treated as separate partitions. We used the GTRGAMMA model, which includes a parameter (Γ) for rate heterogeneity among sites, and chose not to include a parameter for estimating the proportion of invariable sites following recommendations of (Stamatakis, 2006). Support values for the ribosomal, β-tubulin, and *MCM7* phylogenies were examined for well-supported (≥ 70%) conflicts between data sets (Lutzoni et al., 2004).

GenBank accessions were represented solely by ITS sequences, and exploratory phylogenetic reconstructions of all combined accessions and sequence data resulted in reduced nodal support across the topology and important ambiguous relationships. Therefore we chose not to include accessions represented solely by ITS sequences in the complete combined data in order to minimize the effect of missing data (Baurain, Brinkmann, and Philippe, 2007).

Phylogenetic relationships were estimated from the combined data set using mixed-model Bayesian inference (BI) as implemented in Mr.Bayes version 3.1.2 (Huelsenbeck and Ronquist, 2001). We used MrModeltest version 2.3 (Nylander et al., 2004) to identify the appropriate model of evolution for each marker using the Akaike Information Criterion (AIC; Posada and Crandall, 2001), and we treated each marker as a separate partition. Four independent replicate searches were executed with eight chains; each run started with randomly generated trees and consisted of sampling every 1000 generations for 20,000,000 generations. To evaluate stationarity and convergence between runs, log-likelihood scores were plotted using TRACER version 1.5 (Rambaut and Drummong 2003), ESS statistics, and the average standard deviation in split frequencies were assessed following (Hall, 2007). Trees generated prior to stationarity were discarded as "burn-in" (Huelsenbeck et al., 2001). The results were summarized with a majority-rule consensus tree from the remaining trees from the four independent runs. Bayesian posterior probabilities (PP) were assessed at all nodes, and clades with PP $\geq$ 0.95 were considered strongly supported (Huelsenbeck and Rannala, 2004).

Because BI may resolve bifurcations with strong support when relationships are really unresolved (Kolaczkowski and Thornton, 2007), we conducted an ML analysis using RAxML 7.0.4, permitting each locus to evolve independently under the GTR substitution model (Stamatakis, 2006; Stamatakis, Hoover, and Rougemont, 2008). We used the GTRGAMMA model, which includes a parameter (Γ) for rate heterogeneity among sites. Following the recommendations of Stamatakis (2006), we did not include a parameter for the proportion of invariable sites, because Γ mathematically account for this source of rate heterogeneity by using 25 rate categories. A search combining 200 separate maximum likelihood searches (to find the

optimal tree) and 1000 “fastbootstrap” replicates to evaluate nodal support was conducted on the complete dataset.

In order to assess relationships within a broader geographic context we reconstructed the ITS gene tree using both BI and ML inference from all available ingroup ITS sequences, including 20 sequences retrieved from the GenBank database, with *R. chrysolueca* selected as the outgroup (Arup and Grube, 2000; Zhou et al., 2006). We implemented MrModeltest version 2.3 (Nylander et al., 2004) to identify the appropriate model of evolution using the AIC, and the ITS gene was treated as a single partition. BI and ML reconstructions were performed for the complete ITS dataset as described above.

The combined topology indicated strong phylogentic subdivision within the *R. melanophthalma* species-complex, and the topology was used to guide the identification of candidate species for this study. We chose to define a total of 10 putative species to represent four currently accepted taxa and six phylogenetic lineages identified within the topology representing *R. melanophthalma* s. l. (section 3.3) Following the recommendations of Sites and Marshall (2004) and de Queiroz (2007), we implemented multiple analytical approaches to assess species boundaries for independent corroboration of the candidate species identified in the current study. We emphasized species delimitation criteria that identify lineages exhibiting the population genetic patterns of cohesion through gene flow to identify recently diverged species (Duminil et al., 2006b; Shaffer and Thomson, 2007; Weisrock et al., 2010).

Haplotype network reconstructions and genealogical concordance—Although topologies generated by concatenation are often reasonable approximations of reality (Weins 1998), concatenated datasets may potentially be misleading because they can generate unexpected phylogenetic signals, in particular those from DNA sequences sampled from rapidly

diverging clades (Kolaczkowski and Thornton, 2004; Edwards, Liu, and Pearl, 2007; Kubatko and Degnan, 2007; Matsen and Steel, 2007; Kolaczkowski and Thornton, 2008). Furthermore, in cases of low levels of divergence and non-bifurcating relationships, tree representation may fail to accurately portray a reasonable genealogy (Clement, Posada, and Crandall, 2000). In these cases, network approaches provide an important alternative to phylogenetic reconstructions. We used statistical parsimony to assess the genealogical relationship of every individual and compare relationships of candidate species between genes. Because recombination within nuclear genes can lead to errors in the estimated topology (Posada, Crandall, and Holmes, 2002), we tested for recombination events in the low-copy protein-coding markers using methods implemented in Recombination Detection Program RPD3 (Martin, Williamson, and Posada, 2005; Heath et al., 2006). Networks were constructed under a 95% parsimony probability criterion (Templeton, Crandall, and Sing, 1992) from concatenated ribosomal sequences (ITS, IGS, intron), the β-tubulin, and the *MCM7* fragments using the program TCS v1.21 (Clement, Posada, and Crandall, 2000). Gaps were treated as missing data for the ribosomal network reconstruction to include voucher specimens missing one of the three ribosomal loci. All protein-coding sequences were trimmed to the length of the fragment resulting from nested PCR reactions and a single sequence missing approximately half the fragment was removed from the β-tubulin network analysis. All network uncertainties (i.e. closed loops) were treated following Templeton and Sing (1993). Relationships of candidate species were evaluated between individual gene trees to identify lineages that exhibited genealogical exclusivity across multiple loci (Avise and Ball, 1990; Hudson and Coyne, 2002). The presence of the same clades in the majority of single-locus genealogies is taken as evidence that the clades represent reproductively isolated lineages (Dettman, Jacobson, and Taylor, 2003; Pringle et al., 2005),

Bayesian population structure analysis—Individual-based approaches provide an alternative for identifying population structure and barriers to gene flow (Saisho and Purugganan, 2007), as analyses based on predefined delineations of groups may obscure patterns of differentiation (Latch et al., 2006; Rowe and Beebee, 2007). We used a Bayesian population assignment test implemented in STRUCTURE version 2.32 (Pritchard, Stephens, and Donnelly, 2000; Falush, Stephens, and Pritchard, 2003) to infer population structure based on a combined genotypic matrix from all five loci (ITS, IGS, group I intron, β-tubulin, and *MCM7*), without using known geographic location or putative species classification of the individual as priors. The five selected loci were estimated to be sufficient to provide an overview of the highly differentiated groups (Saisho and Purugganan, 2007; Groeneveld et al., 2009; Weisrock et al., 2010). An admixture model was used with correlated allele frequencies. We implemented 15 replicate runs for each number of assumed populations (*K*), with a range of *K* from 1 to12. Based on preliminary runs, all analyses used 30,000 MCMC generations to estimate the posterior distribution following a burn-in period of 15,000 generations. In some cases, independent runs for *K* values 3 through 12 appeared to converge on different parameter space, and longer burn-in or MCMC did not significantly improve convergence. Therefore, we calculated the median log (ln) likelihood of each *K* value from the four best-scoring runs. Following the procedure outlined by Evanno et al. (2005), we calculated the modal value (ΔK) based on the second order rate of change of the likelihood function between successive *K* values. Because ΔK may favor smaller values of *K* representing basal levels of hierarchical structure (Evanno, Regnaut, and Goudet, 2005), we also examined subgroups created by the best individual assignments produced by STRUCTURE to identify sublevels of structuring (Evanno, Regnaut, and Goudet, 2005; Saisho and Purugganan, 2007; Groeneveld et al., 2009; Weisrock et al., 2010).

Morphological and biochemical comparisons—Considering recent studies (Arup and Grube, 2000; Ryan, 2001; Cansaran et al., 2006; Zhou et al., 2006; Zheng, Sheng, and An, 2007), a total of 14 morphological characters were quantified in an attempt to potentially identify diagnostic characters for candidate species identified in this study, including: point of attachment (distinctly umbilicate/squamulose), thallus form (polyphyllous/monophyllous), lobe morphology (distinct/intermediate/indistinct), upper surface (dull/shiny), upper surface texture (smooth/cracked), upper surface color (light to moderately greenish yellow/olive), lower surface (smooth/rough), lower surface edges (black near edges/not blackened edges), lower surface color (tan/brown), apothecia (sessile/basally constricted), apothecia pruinosity (heavily pruinose/moderately pruinose/not pruinose), thallus margin (entire/crenate), spores (ellipsoid/subglobose), spore size (continuous character).

Lichen compounds were extracted from 0.02g liquid nitrogen-ground specimens overnight in acetone at 4^{o} C. The supernatant was removed, dried, reconstituted in methanol, and analyzed using HPLC. Retention index values (RI) were calculated from benzoic acid and solorinic acid controls (Feige et al., 1993; Lumbsch, 2002). For HPLC, we used an Agilent Technologies 1200 series integrated system with a Zorbax Eclipse XDB8-CB column (4.6 × 150mm, 5μm) regulated at 30^{o} C, spectrometric detectors operating at 210, 254, 280, 310nm, and a flow rate of 0.7ml/min. Following established protocols (Feige et al., 1993; Lumbsch, 2002), two mobile phases, A and B, were used: 1% aqueous orthophosphoric acid (A) and methanol (B). The run started with 30% B for 1min and was raised to 70% B within 15min of the start time, then to 100% B during an additional 15min, followed by isocratic elution in 100% B for the final 20min. Mobile phase B was decreased to 30% within 1min and the column was flushed with 30% B for 15min following each run. UV spectra of each peak were recorded

and computer-matched against a library of ultraviolet spectra from authentic metabolites derived under identical conditions using Agilent Chemstation software. The correlation of UV spectra with the standards in the library was greater than 99.9 % for each substance identified. When multiple library entries matched with this level of identity, calculated R/I values were used to discriminate between compounds.

Results

For this study 635 new sequences were generated, including 150 ITS, 139 IGS, 75 group 1 intron, 137 β-tubulin, and 134 *MCM7* sequences. The data matrix of 2639 aligned nucleotide position characters in the combined analysis is summarized in Table 2. Missing data were generally limited to the outgroup taxa *R. chrysolueca* and *R. subdiscrepans*. However, we were unable to generate group I intron sequences from all accessions recovered in clade IVd from the combined analyses (defined below). All representative haplotypes of the five gene fragments have been deposited in GenBank under Accession Nos. HM576889-HM577515, and are summarized in Supplementary Table S2.

Polymorphism statistics and estimates of gene flow—Polymorphism statistics are reported in Table 3. The greatest nucleotide diversity for candidate species was generally recovered for ribosomal loci. High levels of genetic differentiation between all pairs of candidate species were calculated from the combined data set, as measured by F_{ST} (Table 4). Fixed differences between candidate species defined in this study were identified from ribosomal markers for all pairwise comparisons, and fixed differences were identified in at least one of the protein-coding fragments for 40 of 45 pairwise comparisons (Table 4). The ribosomal data matrix showed the greatest number of fixed character differences between each candidate species

compared to all remaining lineages; while the protein-coding matrixes generally did not reveal fixed character differences (Table 4). However, the β-tubulin fragment revealed 9 fixed nucleotide positions in clade I and 1 fixed locus in clade IVb, and the *MCM7* data revealed 2 fixed nucleotide positions in clade I and 5 fixed characters in *R. idahoensis* (clade IV). Group I intron sequences were missing for all individuals assigned to clade IVd and a single individual from *R. haydenii* ssp. *arbuscula* (092f), *R. idahoensis* (093) and clade II (693f).

Phylogenetic reconstructions—The ribosomal topology recovered multiple well-supported lineages within the *R. melanophthalma* species-complex. In contrast, weak phylogenetic signal was generally indentified in both protein-coding matrixes. However, using the $\geq$ 70% bootstrap method to identify conflict, we detected limited discordance between the ribosomal, β-tubulin and *MCM7* topologies restricted to clades with relatively shallow evolutionary histories. Conflicting terminals are shown in individual gene trees (Supplementary data 3). This conflict likely results from retained ancestral polymorphisms in the β-tubulin dataset relative to the more-rapidly evolving ribosomal markers, and given the overall congruence, the ribosomal, β-tubulin , and *MCM7* gene regions were combined to maximize the total number of characters for phylogenetic analyses and branch length estimation (Wiens, 1998; Rokas et al., 2003).

The partitioned Bayesian analyses, summed from four independent runs, yielded a consensus tree with a negative harmonic mean of 11,092.49. All parameters converged within the first 25% of sampled generations, leaving a posterior distribution estimated from 15,000 trees per run (60,000 total post-burn-in sampled trees). The partitioned ML analysis yielded a single best scoring tree -lnL = 10,755.758. As the recovered trees were similar across methods and the topologies did not show any strongly supported conflict; we present here the results of the ML

analysis with ML bootstrap (BS) and posterior probability (PP) values in Figure 2. The *R. melanophthalma* group is strongly supported as monophyletic and several other well-supported groups can be identified in the tree.

The ITS topology (Fig. 3) recovered most lineages identified in the combined analyses. GenBank accessions representing individuals collected in Austria (AF159935), China (AY509791, EF095286, and EF095297), and the United States (AF159929-Arizona and AF159935-Arizona) were recovered in a well-supported clade (91/1.0) corresponding to clade II identified in the combined analyses. Six accessions collected in China (EF095278, EF095280, EF095283, EF095285, EF095287, and EF095290) were recovered within a well-supported clade (81/0.98) corresponding to clade IVb from the combined analyses, and two accession representing *R. cerebriformis* (AF159942, Idaho, USA) and *R. subidahoensis* (AF159944, Idaho, USA) were recovered within a well-supported clade (90/1.0) corresponding to clade IVa from the combined analyses. A single accession representing *R. cylindrical* (AF159941, Idaho, USA) was recovered in a clade with high ML bootstrap support (82) and weak PP support (0.79) corresponding to clade IVd in the combined analyses. Two vagrant accessions representing *R. idahoensis* (AF159943-Idaho, USA) and *R. haydenii* (AF159937-Idaho, USA) were recovered in a well-supported clade (85/1.0) containing individuals all assigned to clades clades IVb, IVc, *R. haydenii*, *R. haydenii* ssp. *arbuscula*, and *R. idahoensis* in the combined analyses. *L. novomexicana* was recovered as polyphyletic in two well-supported lineages; one containing specimens collected in northeastern Utah, and the second (clade V, Fig. 3) in two GenBank accessions, one from Arizona (AF159923) and the other from New Mexico (AF159923). However, the relationship between the *L. novomexicana* lineages lacked strong statistical support.

Candidate Species—We defined 10 candidate species based on the results from our phylogenetic reconstructions and current taxonomic boundaries for additional empirical testing of species boundaries. Sampled *L. novomexicana* (clade I, Fig. 2) were recovered in a well-supported lineage (BS=100/PP=1.0), and is recovered as sister to the remaining *R. melanophthalma* taxa with weak nodal support. Clade II was recovered with high nodal support (95/1.0), and corresponds to a genetically and morphologically diverse assemblage of umbilicate saxicolous specimens collected throughout the intermountain western United States, all containing usnic and psoromic acids. However, the relationship of clade II to other well-supported sister lineages lacks strong nodal support (43/0.89). Clade III was also recovered with strong support (100/1.0), and is represented by umbilicate saxicolous individuals with little morphological or genetic variation collected from two plots (BM-3 and TLM-9) on the Aquarius Plateau in south central Utah, U.S.A. Clade III was recovered with strong nodal support (94/0.98) as sister to a fourth well-supported clade (99/1.0) containing a chemically diverse assemblage of umbilicate and vagrant specimens (clade IV). Seven additional candidate species were defined within clade IV to accommodate currently described vagrant taxa and an exhaustive subdivision of the remaining accessions.

All sampled vagrant taxa were recovered within a single monophyletic clade with weak nodal support (BS and PP < 50/0.50). *R. idahoensis, R. haydenii,* and *R. haydenii* spp. *arbuscula* were treated as independent lineages based on current taxonomic circumspection. Both *R. idahoensis* and *R. haydenii* spp. *arbuscula* were recovered as well-supported monophyletic lineages (94/1.0 and 81/1.0, respectively), while *R. haydenii* was found in two well-supported clades. A single saxicolous specimen with unique lobe morphology (715f) was recovered within the *R. haydenii* clade. In addition to the currently described vagrant taxa, four candidate species

were defined to accommodate exhaustive subdivision within the larger clade. Clade IVa (Fig. 2) was recovered with strong nodal support (100/1.0) and contains three morphologically and geographically diverse individuals. All specimens containing lecanoric or orscellinic acids were recovered within clade IVb with moderate to strong nodal support (BS = 83; PP = 0.93). Clade IVc (Fig. 2) was also recovered with strong support (82/1.0), and included five individuals; and clade IVd included the remaining 55 individuals. Although this lineage was recovered as monophyletic, it lacked strong support in the combined phylogenetic reconstructions.

Geographic distributions of candidate species and the distribution of these species along the altitudinal transect on Thousand Lakes Mountain and Boulder Mountain, Utah is summarized in Figure 4.

Haplotype networks—We recovered a total of five independent haplotype networks for the combined ribosomal data set, and two networks for both the β-tubulin and *MCM7* datasets (Fig. 5A). The ribosomal network haplotypes separated by up to 15 mutational steps had greater than 95% probability of being parsimoniously connected. In the β-tubulin and *MCM7* distinct networks were connected by up to 11 or 10 mutational steps, respectively. For all markers clade I (*L. novomexicana*) formed an independent network. In addition, clades II, III, and IVa formed independent networks constructed from the ribosomal dataset, while clades IVc, IVb, IVd, *R. haydenii* spp. *arbuscula* (clade IV), *R. haydenii* (clade IV), and *R. idahoensis* (clade IV), were found on a single network. In both the β-tubulin and *MCM7* datasets clades II, III, IVa, IVb, IVc, IVd, *R. haydenii* spp. *arbuscula* (clade IV), *R. haydenii* (clade IV), and *R. idahoensis* (clade IV) were found on a single network.

Bayesian population structure—The median ML values of the Bayesian clustering analysis using STRUCTURE with estimates of K = 1-12 are shown in Figure 6A. These

analyses reveal a general pattern of a plateau with a decrease in median maximum likelihood values above a K=6 level. In contrast, the ΔK method indicates that a K = 2 model best fits the data (Fig. 6B; ΔK = 137.170 for K = 2; ΔK = < 25 for all other K values), most likely identifying a basal level of hierarchical structure in the data (Evanno, Regnaut, and Goudet, 2005). The K = 2 model identifies individuals recovered in clades I, II, and III from the combined phylogenetic analysis in one population cluster, and individuals recovered in the remaining clades were assigned to a second cluster. However, the plateau in likelihood values around K = 6 suggest a higher number of population clusters (Figure 6A). A plot of individual membership coefficients for K=6 reveals a high number of population clusters with average individual membership coefficients (i.e. posterior probabilities) greater than 0.9 (Figure 5B). Population clusters inferred for $K>6$ did not yield additional clusters with high membership coefficients. Therefore, we place our focus on K = 6 as an uppermost level of population structure. The K = 6 model is generally consistent with the defined candidate species. However, all vagrant species (*R. haydenii*, *R. haydenii* ssp. *arbuscular*, and *R. idahoensis*) were recovered within a single population cluster, along with all individuals assigned to clade IVc in the combined phylogenetic analysis. A total of three saxicolous accessions (554f, 556f, and 715F) and three erratic, or facultatively unattached, accessions (668f, 669f, 670f) were assigned to the cluster with vagrant taxa. Clades IVa and IVd were also recovered as a single population cluster; however, membership coefficients for individuals with posterior probabilities were < 0.71 for clade IVa and $\geq$ 0.87 for clade IVd.

Morphology and Chemistry—We adopted the approach of Wiens and Penkrot (2002), suggesting that in order for characters to diagnose a lineage they must be invariant for alternative character states or show no overlap in trait values. Both vegetative morphology and reproductive

characters, spore size and shape, were highly variable within some candidate species, and overall we were unable to identify morphological or reproductive characters corroborating candidate species following Wiens and Penkrot (2002).

Occurrence of the 11 most common compounds identified in HPLC analyses within each defined lineage is summarized in Table 5. The majority of specimens belonged to the usnic/psoromic acids chemotype (119 specimens, including all specimens of L. *novomexicana*), having a broad geographical and ecological distribution; 9 specimens contained usnic, psoromic, and lecanoric acid; and 5 specimens contained usnic, psoromic, and orscellinic acid. All sampled vagrant specimens expressed usnic acid only. In addition to the previously reported psoromic acid, we found 2'-*O*-demethylsubpsoromic acid, 2'-*O*-demethylpsoromic acid, and the recently described β-orcinol depsidone, subpsoromic acid (Elix 2000). The dibenzofuran-derivative, usnic acid, was present in all samples, and some combination of the aliphatic acids, dehydroprotoconstipatic acid, and constipatic acid, were present in all individuals, except the sampled vagrant taxa. We found gyrophoric (triorsellininc) acid and also the monocyclic-depside precursor, orsellinic acid, restricted to specimens assigned to clade IVb (defined in 3.3) in the combined molecular analyses, in addition to previous reports for lecanoric (diorsellinic) acid (McCune, 1987; Arup and Grube, 2000).

Discussion

Taxonomic decisions are usually made on the basis of recognizable morphological characters. However, inferring species boundaries in lichenized fungi is not straightforward, as often interspecific boundaries based on traditional morphological and chemical characters misrepresent fungal diversity (Crespo and Pérez-Ortega, 2009; Printzen, 2009). In this study, we

assembled multiple lines of evidence to identify and delimit candidate species within the *Rhizoplaca melanophthalma* species-complex. Based on all of the available evidence, we identified ten candidate species within this complex. Many of these lineages fall within a nominal taxon currently recognized as a single cosmopolitan species, *R. melanophthalma*. Genetic patterns, generated by population-level processes operating within divergent lineages, provide an informative perspective about the process of speciation in the *R. melanophthalma* species-complex.

Generally, relationships estimated from the combined ribosomal dataset (ITS, IGS, and group I intron) recovered a highly structured topology with multiple well-supported clades, while the protein coding gene trees generally showed less resolution and fewer well-supported clades. Given the small $N_e s$ for haploid genomes, monophyly may be attained from rapidly evolving markers, even within recently derived lineages (Moore, 1995). As a result, most lineages that were well-supported in the ribosomal phylogeny were unresolved in both protein-coding phylogenies. Furthermore, a large proportion of ribosomal characters showed fixed, alternative character states between putative lineages identified in this study, protein-coding markers provided less resolution. Despite a lack of monophyly in the protein-coding phylogenies for most of the candidate species, gene networks generally supported the groupings, and the STRUCTURE analysis of the combined data set corroborated most groups recovered in the phylogenetic reconstruction. Results of the empirical tests delimiting species are summarized in Table 6.

Although our results provide a compelling case of diversification within the *R. melanophthalma* species-complex using molecular data and multiple analytical tools, most candidate species were not supported unambiguously by independent datasets. Besides the

placodiod crustose taxon, *Lecanora novomexicana*, we found that the greatest morphological and chemical variation was restricted to closely related lineages (sampled vagrant taxa and clades IVb and IVc), while morphological and chemical characters supporting more divergent groups were not identified. Ecological interactions are expected to drive phenotypic divergence during the early stages of lineage diversification when species richness is low and available niches are "open" (Schluter, 2000). The ecological transition from a saxicolous attached form to morphologically distinct vagrant forms appears to follow the ecological theory of adaptation (Funk, Nosil, and Etges, 2006). The STRUCTURE analysis assigned all vagrant forms to a single population cluster, suggesting a recent divergence of morphologically diverse vagrant taxa. However, the inclusion of saxicolous attached taxa within this cluster suggests a recent divergence from saxicolous attached forms or an underlying genetic predisposition to vagrancy in at least some saxicolous lineages. (Leavitt, Johnson, and St. Clair, submitted) indentified multiple independent origins of vagrancy within the lichen genus *Xanthoparmelia* (Parmeliaceae), but our data suggest that that vagrancy in the *R. melanophthalma* species-complex is limited to a single closely related lineage, even among morphologically distinct vagrant forms. However, a broader sample of vagrant individuals is essential to adequately addressing this question, particularly *R. haydenii* recently described in China (Zheng, Sheng, and An, 2007) .

Phylogenetic analyses of both the combined dataset and the ITS marker alone recovered clade IVa with strong support. However, the STRUCTURE analysis assigned all individuals from clade IVa (membership coefficient values between 0.65 and 0.70) to the same population cluster containing accessions recovered in clade IVd. Although nuclear ribosomal DNA (rDNA) repeats generally evolve together through concerted evolution, it has been documented that some

genomes contain a considerable diversity of paralogous rDNA (Buckler-IV, Ippolito, and Holtsford, 1997), and the lack of concordance between the ribosomal DNA with other nuclear markers suggests that the observed divergence in phylogenetic reconstructions may be a result of divergent ITS paralogs within the nuclear ribosomal repeat, rather than representing distinct lineages. The overall impact of paragolous rDNA markers in studies of lichenized ascomycetes remains uncertain, and these results highlights the importance of using multiple independent genetic markers to effectively assess evolutionary relationships.

Previous studies have used thin-layer-chromatography (TLC) to characterize lichen secondary metabolic products within *Rhizoplaca*. In this study HPLC provided a more sensitive approach to determine secondary metabolite diversity within the *R. melanophthalma* group, as many newly reported compounds here would be masked by other compounds, or likely found at levels undetectable by TLC. While data have supported the taxonomic use of some secondary metabolic characters for delimiting lichen taxa (Tehler and Källersjö, 2001; Schmitt and Lumbsch, 2004), other studies found no correlation between chemotypes and lineages identified using molecular phylogenetic reconstructions (Articus et al., 2002; Buschbom and Mueller, 2006; Nelsen and Gargas, 2009; Velmala et al., 2009). We have identified chemical characters corroborating some lineages identified within the *R. melanopthalma* group, including: clade IVb containing a combination of orsellinic, lecanoric, and gyrophoric acids; and *R. haydenii*, *R. haydenii*, ssp. *arbuscula*, and *R. idahoensis* all lack aliphatic acids related to constipatic acid. However, we were unable to identify secondary metabolic characters supporting most identified putative lineages, including the most genetically divergent groups.

McCune (1987) suggested three hypotheses to explain chemical diversity in the genus *Rhizoplaca*: (1) chemotypes are sibling species that cannot or seldom hybridize assuming there

are no reproductive barriers, (2) factors favoring polymorphism in chemistry do not differ markedly between regions, or (3) the polymorphism is neutral to natural selection. Although the present study was not designed to explicitly test these hypotheses, our results indicate within the usnic/psoromic acid race multiple lineages co-occur. The usnic/psoromic/lecanoric acid race appears to be a distinct lineage also containing specimens lacking lecanoric acid but expressing the lecanoric acid precursor, orsellinic acid. Additional studies will be needed to fully elucidate the relationship between *R. melanophthalma* s.l. containing lecanoric or orsellinic acids. Our sampling of the usnic acid chemical race in the *R. melanophthalma* species-complex was limited to a single saxicolous attached individual (715f) and all vagrant taxa. The saxicolous *R. melanophthalma* chemical race containing placodiolic acid was not sampled and its relationship to sampled taxa remains in question.

Porter (1999) reported a correlation between some secondary metabolites and elevation in *R. melanophthalma* populations along an altitudinal gradient on Thousand Lakes Mountain, Utah. Besides the strict correlation of lecanoric and orsellinic acid with clade IVb, the present study did not identify any specific correlations between lineages identified from molecular data and expressed secondary metabolites on Thousand Lake Mountain, suggesting that the production of most minor compounds may be environmentally induced. A combination of species diversity in lichen-forming symbionts (alga and fungus) and ecological factors may explain secondary metabolite variation among the Thousand Lake Mountain populations (Brunauer et al., 2007).

These results offer interesting insights into potential mechanisms driving speciation in lichenized ascomycetes. Cohesive sets of populations yielding distinct patterns in allele frequencies and gene trees often co-occur, suggesting the possibility of sympatric speciation in

the *R. melanophthalma* species-complex. Although our understanding of the relative importance of sympatric speciation is incomplete, recent studies suggest that sympatric speciation and parallel diversification may be more important than previously realized (Barluenga et al., 2006; Baloch and Grube, 2009; Kozak, Mendyk, and Wiens, 2009; Crow, Munehara, and Bernardi, 2010). Pre-conditions for sympatric speciation include: 1) sympatric distribution of the most closely related sister species; 2) genetic evidence for reproductive isolation among the lineages; 3) monophyly; and 4) an ecological setting in which allopatric divergence is unlikely (Coyne and Orr, 2004; Barluenga et al., 2006). Although our data appear to fit the first three criteria for sympatric speciation, they do not preclude the possibility that current distributions of the candidate species are an artifact of allopatric diversification followed by secondary sympatry.

The current study was generally limited to the Intermountain region of western North America, and robust data from a broader geographic sampling will be essential to understand the general geographic distribution of the candidate species identified in this study. We anticipate that with improved sampling, additional lineages may be identified within the *R. melanophthalma* species-complex, particularly within *L. novomexicana* s.l. However, with the exception of *L. novomexicana*, the ITS topology recovered GenBank accessions within the candidate species defined from our combined dataset set from samples in western North America, suggesting our candidate species may represent some lineages with cosmopolitan distributions. While most candidate species identified in this study appear to demonstrate early stages of species divergence, the occurrence of cohesive cosmopolitan lineages found sympatrically with closely related divergent populations poses challenging questions about the processes that yield and maintain cohesive lineages within widespread lichenized ascomycetes.

Clade-specific ecological or microhabitat differences considered alone do not appear to offer a plausible explanation of how sympatric diversification may occur in the candidate species. Some lineages exhibit extensive microsympatry (i.e., divergent lineages occurring within a single sampled plot), as well as the production of abundant perennial apothecia (sexual fruiting bodies) without detectable gene flow or hybridization between microsympatric individuals. This pattern suggests that candidate species may have achieved a significant level of reproductive isolation. However, the role of spatio-temporal isolation in lichenized fungal reproduction is relatively unexplored. It has been proposed that competition for symbiotic partners may be a major driver of diversity in mutualistic relationships (Bruns, 1995; O'Brien, Miadlikowska, and Lutzoni, 2009) and investigating competition for symbionts may provide insights into mechanisms that possibly drive sympatric speciation.

Within lichenized fungi, gene trees have often been used to infer species boundaries, and the over-reliance on a single locus has been problematic in delimiting species because gene duplication, horizontal gene transfer, and deep coalescence may create conflict between the sampled gene tree and the true species tree (de Queiroz and Donoghue, 1990; Maddison, 1997). In some cases, rapidly evolving molecular characters may reach fixation in ephemerally isolated demes, with the potential to reticulate with other conspecific lineages at some point in the future (O'Hara, 1993). Additionally, phylogenetic structure can extend below the level of the species, particularly within asexual and haploid genomes (Birky, Maruyama, and Fuerst, 1983; Birky-Jr, Fuerst, and Maruyama, 1989; de Queiroz and Donoghue, 1990; Davis, 1996) making species limits based on molecular data within lichenized fungi particularly susceptible to excessive subdivision.

In spite of the limitations in delimiting taxa using molecular data, most of the candidate species indentified in this study, were not supported by diagnostic morphological or chemical characters, and the effective use of molecular data appears to be an essential approach to appropriately identify natural groups in many fungal lineages (Crespo and Pérez-Ortega, 2009). The authors plan a detailed taxonomic revision for the *Rhizoplaca melanophthalma* species-complex in the near future, including additional taxonomic and morphological sampling to more fully characterize boundaries between candidate species. Results from this study suggest that robust taxon and molecular data sampling, using appropriate empirical operational criteria to delimit species, may provide an improved perspective on the diversification of lichenized fungi (Zwickl and Hillis, 2002), compared to traditional morphological and chemical characters. However, we are not advocating the use of genetic data to the exclusion of other evidence for delimiting species; due to the fact that corroboration of species boundaries via independent lines of evidence is important to the establishment of robust hypotheses of species diversity.

Conclusions

Analysis of the *R. melanophthalma* species-complex comprises the larger focus of this study, which is using robust species delimitation in morphologically cryptic and recently diverged lichenized fungi. *Rhizoplaca,* as traditionally circumscribed, is a small morphologically diverse lichen genus represented by 9 species (Arup and Grube, 2000; Zhou et al., 2006). This study indicates overall diversity within umbilicate *Rhizoplaca* species may be vastly underestimated, as multiple previously unrecognized lineages were identified within the *R. melanophthalma* group. Previous studies have identified well-supported lineages within *R. chrysoleuca* corresponding to two phenotypic groups (Zhou et al., 2006), and well-supported and

highly structured relationships within the outgroup taxon *R. chrysoleuca* were also recovered in this study, suggesting an additional nominal *Rhizoplaca* taxon may contained previously unrecognized lineages. Extending the present sampling of the *R. melanophthalma* species-complex to include a broader geographic context and robust sampling of underrepresented lineages will be critical to improve the understanding of the mechanisms driving speciation in lichenized fungi. Furthermore, an extension of the present sampling to other closely related cosmopolitan *Rhizoplaca* and *Lecanora* species-complexes will provide a potential opportunity for developing a comprehensive classification system for other closely related taxa. Additionally, continued investigation of independent characters supporting candidate lineages will be essential for generating robust hypotheses of species boundaries.

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Literature Cited

ARTICUS, K., J. E. MATTSSON, L. TIBELL, M. GRUBE, and M. WEDIN. 2002. Ribosomal DNA and beta-tubulin data do not support the separation of the lichens *Usnea florida* and *U. subfloridana* as distinct species. *Mycological Research* 106: 412-418.

ARUP, U., and M. GRUBE. 2000. Is *Rhizoplaca* (Lecanorales, lichenized Ascomycota) a monophyletic genus? *Canadian Journal of Botany* 78: 318-327.

ASLAN, A., G. BUDAK, and A. KARABULUT. 2004. The amounts Fe, Ba, Sr, K, Ca and Ti in some lichens growing in Erzurum province (Turkey). *Journal of Quantitative Spectroscopy and Radiative Transfer* 88: 423-431.

AVISE, J. C., and A. S. BALL. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. *In* D. Futuyma AND Antonovics [eds.], Oxford Surveys in Evolutionary Biology. Oxford University Press, Oxford, UK.

BALOCH, E., and M. GRUBE. 2009. Pronounced genetic diversity in tropical epiphyllous lichen fungi. *Molecular Ecology* 18: 2185-2197.

BARLUENGA, M., K. N. STÖLTING, W. SALZBURGER, M. MUSCHICK, and A. MEYER. 2006. Sympatric speciation in Nicaraguan crater lake cichlid fish. *Nature* 439: 719-723.

BAUM, D. A., and K. L. SHAW. 1995. Genealogical perspectives on the species problem. *In* P. C. Hoch AND A. G. Stephenson [eds.], Experimental and Molecular Approaches to Plant Biosystematics, 289-303. Missouri Botanical Garden, St. Louis, Missouri, USA.

BAURAIN, D., H. BRINKMANN, and H. PHILIPPE. 2007. Lack of Resolution in the Animal Phylogeny: Closely Spaced Cladogeneses or Undetected Systematic Errors? *Molecular Biology and Evolution* 24: 6-9.

BIRKY, C. W., P. FUERST, and T. MARUYAMA. 1989. Organelle Gene Diversity Under Migration, Mutation, and Drift: Equilibrium Expectations, Approach to Equilibrium, Effects of Heteroplasmic Cells, and Comparison to Nuclear Genes. *Genetics* 121: 613-627.

BIRKY, C. W., JR., T. MARUYAMA, and P. FUERST. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. *Genetics* 103: 513-527.

BLANCO, O., A. CRESPO, J. A. ELIX, D. L. HAWKSWORTH, and H. T. LUMBSCH. 2004a. A Molecular Phylogeny and a New Classification of Parmelioid Lichens Containing *Xanthoparmelia*-Type Lichenan (Ascomycota: Lecanorales). *Taxon* 53: 959-975.

BLANCO, O., A. CRESPO, P. K. DIVAKAR, T. L. ESSLINGER, D. L. HAWKSWORTH, and H. THORSTEN LUMBSCH. 2004b. *Melanelixia* and *Melanohalea*, two new genera segregated

from *Melanelia* (Parmeliaceae) based on molecular and morphological data. *Mycological Research* 108: 873-884.

BRUNAUER, G., A. HAGER, M. GRUBE, R. TÜRK, and E. STOCKER-WÖRGÖTTER. 2007. Alterations in secondary metabolism of aposymbiotically grown mycobionts of *Xanthoria elegans* and cultured resynthesis stages. *Plant Physiology and Biochemistry* 45: 146-151.

BRUNS, T. D. 1995. Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi. *Plant Soil* 170: 63-73.

BUCKLER-IV, E. S., A. IPPOLITO, and T. P. HOLTSFORD. 1997. The Evolution of Ribosomal DNA: Divergent Paralogues and Phylogenetic Implications. *Genetics* 145: 821-832.

BUSCHBOM, J., and G. M. MUELLER. 2006. Testing "species pair" hypotheses: Evolutionary processes in the lichen-forming species complex *Porpidia flavocoerulescens* and *Porpidia melinodes*. *Molecular Biology and Evolution* 23: 574-586.

CANSARAN, D., S. ARAS, I. KANDEMIR, and M. G. HALICI. 2006. Phylogenetic Relations of *Rhizoplaca* Zopf. from Anatolia Inferred from ITS Sequence Data. *Zeitschrift fur Naturforschung Section C Journal of Biosciences* 61: 405-412.

CARDINALE, M., J. V. D. C. JR, H. MÜLLER, G. BERG, and M. GRUBE. 2008. *In situ* analysis of the bacterial community associated with the reindeer lichen *Cladonia arbuscula* reveals predominance of *Alphaproteobacteria*. *FEMS Microbiology Ecology* 66: 63-71.

CARSTENS, B. C., and T. A. DEWEY. 2010. Species Delimitation Using a Combined Coalescent and Information-Theoretic Approach: An Example from North American Myotis Bats. *Systematic Biology*: in press.

CLEMENT, M., D. POSADA, and K. A. CRANDALL. 2000. TCS: A computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1659.

COYNE, J. A., and H. A. ORR. 2004. Speciation. Sinauer Associates, Sunderland, Massachusetts, USA.

CRESPO, A., and S. PÉREZ-ORTEGA. 2009. Cryptic species and species pairs in lichens: A discussion on the relationship between molecular phylogenies and morphological characters. *Anales del Jardin Botanico de Madrid* 66: 71-81.

CRESPO, A., M. C. MOLINA, O. BLANCO, B. SCHROETER, L. G. SANCHO, and D. L. HAWKSWORTH. 2002. rDNA ITS and β-tubulin gene sequence analyses reveal two monophyletic groups within the cosmopolitan lichen *Parmelia saxatilis*. *Mycological Research* 106: 788-795.

CRESPO, A., H. T. LUMBSCH, J.-E. MATTSSON, O. BLANCO, P. K. DIVAKAR, K. ARTICUS, E. WIKLUND, et al. 2007. Testing morphology-based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear RPB1 gene. *Molecular Phylogenetics and Evolution* 44: 812-824.

CROW, K. D., H. MUNEHARA, and G. BERNARDI. 2010. Sympatric speciation in a genus of marine reef fishes. *Molecular Ecology* 19: 2089-2105.

CULBERSON, C. F. 1972. Improved conditions and new data for identification of lichen products by standardized thin-layer chromatographic method. *Journal of Chromatography A* 72: 113-125.

DAVIS, J. I. 1996. Phylogenetics, Molecular Variation, and Species Concepts. *Bioscience* 46: 502-511.

DAYRAT, B. 2005. Towards integrative taxonomy. *Biological Journal of the Linnean Society* 85: 407-415.

DE QUEIROZ, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations, 57-75. Oxford University Press, Oxford, UK.

_____. 1999. The general lineage concept of species and the defining properties of the species category. *Species, New Interdisciplinary Essays*: 49-89.

_____. 2007. Species Concepts and Species Delimitation. *Systematic Biology* 56: 879-886.

DE QUEIROZ, K., and M. J. DONOGHUE. 1990. Phylogenetic systematics and species revisited. *Cladistics* 6: 83-90.

DEGNAN, J. H., and N. A. ROSENBERG. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution* 24: 332-340.

DETTMAN, J. R., D. J. JACOBSON, and J. W. TAYLOR. 2003. A multilocus genealogical approach to phylogenetic species recognition in the model eukaryote Neurospora. *Evolution* 57: 2703 - 2720.

DILLMAN, K. L. 1996. Use of the lichen Rhizoplaca melanophthalma as a biomonitor in relation to phosphate refineries near Pocatello, Idaho. *Environmental Pollution* 92: 91-96.

DIVAKAR, P. K., O. BLANCO, D. L. HAWKSWORTH, and A. CRESPO. 2005. Molecular phylogenetic studies on the *Parmotrema reticulatum* (syn. *Rimelia reticulata*) complex, including the confirmation of *P. pseudoreticulatum* as a distinct species. *The Lichenologist* 37: 55-65.

DUMINIL, J., H. CARON, I. SCOTTI, S.-O. CAZAL, and R. PETIT. 2006a. Blind population genetics survey of tropical rainforest trees. *Molecular Ecology* 15: 3505-3513.

DUMINIL, J., H. CARON, I. SCOTTII, S.-O. CAZAL, and R. J. PETIT. 2006b. Blind population genetics survey of tropical rainforest trees. *Molecular Ecology* 15: 3505-3513.

EDGAR, R. C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: 1-19.

EDWARDS, S. V. 2009. Is a new and general theory of molecular systematics emerging? *Evolution* 63: 1-19.

EDWARDS, S. V., L. LIU, and D. K. PEARL. 2007. High-Resolution Species Trees without Concatenation. *Proceedings of the National Academy of Sciences of the United States of America* 104: 5936-5941.

EVANNO, G., S. REGNAUT, and J. GOUDET. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611-2620.

FALUSH, D., M. STEPHENS, and J. K. PRITCHARD. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 164: 1567-1587.

FEIGE, G. B., H. T. LUMBSCH, S. HUNECK, and J. A. ELIX. 1993. Identification of lichen substances by a standardized high-performance liquid chromatographic method. *Journal of Chromatography A* 646: 417-427.

FUNK, D. J., P. NOSIL, and W. J. ETGES. 2006. Ecological divergence exhibits consistently positive associations with reproductive isolation across disparate taxa. *Proceedings of the National Academy of Sciences of the United States of America* 103: 3209-3213.

GANLEY, A. R. D., and T. KOBAYASHI. 2007. Highly efficient concerted evolution in the ribosomal DNA repeats: Total rDNA repeat variation revealed by whole-genome shotgun sequence data. *Genome Research* 17: 184-191.

GARDES, M., and T. D. BRUNS. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology Notes* 2: 113-118.

GROENEVELD, L., D. WEISROCK, R. RASOLOARISON, A. YODER, and P. KAPPELER. 2009. Species delimitation in lemurs: multiple genetic loci reveal low levels of species diversity in the genus Cheirogaleus. *BMC Evolutionary Biology* 9: 30.

GRUBE, M., M. CARDINALE, J. V. DE CASTRO, JR., H. MULLER, and G. BERG. 2009. Species-specific structural and functional diversity of bacterial communities in lichen symbioses. *ISME J* 3: 1105-1115.

GUTIÉRREZ, G., O. BLANCO, P. DIVAKAR, H. LUMBSCH, and A. CRESPO. 2007. Patterns of Group I Intron Presence in Nuclear SSU rDNA of the Lichen Family Parmeliaceae. *Journal of Molecular Evolution* 64: 181-195.

HALE, M. E. 1990. A synopsis of the lichen genus *Xanthoparmelia* (Vainio) Hale (Ascomycotina, Parmeliaceae), vol. Book, Whole. Smithsonian Institution Press, Washington D.C., USA.

HALL, B. G. 2007. Phylogenetic Trees Made Easy: A How-To Manual. Third ed. Sinauer Associates, Sunderland, Massachusetts, USA.

HAWKSWORTH, D. L. 2001. The magnitude of fungal diversity: the 1.5 million species estimate revisited. *Mycological Research* 105: 1422-1432.

HEATH, L., E. VAN DER WALT, A. VARSANI, and D. P. MARTIN. 2006. Recombination Patterns in Aphthoviruses Mirror Those Found in Other Picornaviruses. *Journal of Virology* 80: 11827-11832.

HEY, J., R. S. WAPLES, M. L. ARNOLD, R. K. BUTLIN, and R. G. HARRISON. 2003. Understanding and confronting species uncertainty in biology and conservation. *Trends in Ecology & Evolution* 18: 597-603.

HODKINSON, B., and F. LUTZONI. 2009. A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. *Symbiosis* 49: 163-180.

HUDSON, R. R., and J. A. COYNE. 2002. Mathematical Consequences of the Genealogical Species Concept. *Evolution* 56: 1557-1565.

HUELSENBECK, J. P., and F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754 - 755.

HUELSENBECK, J. P., F. RONQUIST, R. NIELSEN, and J. P. BOLLBACK. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science (Washington D C)* 294: 2310-2314.

HUNECK, S. 1999. The Significance of Lichens and Their Metabolites. *Naturwissenschaften* 86: 559-570.

HUNECK, S., and I. YOSHIMURA. 1996. Identification of Lichen Substances. Springer, New York, New York, USA.

KNOWLES, L. L., and B. C. CARSTENS. 2007. Delimiting Species without Monophyletic Gene Trees. *Systematic Biology* 56: 887-895.

KOLACZKOWSKI, B., and J. W. THORNTON. 2004. Performance of maximum parsimony and likelihood phylogenetics when evolution is heterogeneous. *Nature* 431: 980-984.

_____. 2007. Effects of Branch Length Uncertainty on Bayesian Posterior Probabilities for Phylogenetic Hypotheses. *Molecular Biology and Evolution* 24: 2108-2118.

_____. 2008. A Mixed Branch Length Model of Heterotachy Improves Phylogenetic Accuracy. *Molecular Biology and Evolution* 25: 1054-1066.

KOZAK, K. H., R. W. MENDYK, and J. J. WIENS. 2009. Can Parallel Diversification Occur in Sympatry? Repeated Patterns of Body-Size Evolution in Coexisting Clades of North American Salamanders. *Evolution* 63: 1769-1784.

KROKEN, S., and J. W. TAYLOR. 2001. A Gene Genealogical Approach to Recognize Phylogenetic Species Boundaries in the Lichenized Fungus Letharia. *Mycologia* 93: 38-53.

KUBATKO, L. S., and J. H. DEGNAN. 2007. Inconsistency of Phylogenetic Estimates from Concatenated Data under Coalescence. *Systematic Biology* 56: 17-24.

LAGRECA, S., and H. T. LUMBSCH. 2001. Three Species of Lecanora New to North America, with Notes on Other Poorly Known Lecanoroid Lichens. *The Bryologist* 104: 204-211.

LATCH, E., G. DHARMARAJAN, J. GLAUBITZ, and O. RHODES. 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics* 7: 295-302.

LEACHE, A. D. 2009. Species Tree Discordance Traces to Phylogeographic Clade Boundaries in North American Fence Lizards (*Sceloporus*). *Systematic Biology* 58: 547-559.

LEAVITT, S. D., L. A. JOHNSON, and L. L. ST. CLAIR. submitted. New insights into phylogenetic relationships and character evolution in the species-rich lichen-forming genus *Xanthoparmelia* (Parmeliaceae) in western North America.

LEUCKERT, C., J. POELT, and G. HAHNEL. 1977. Zur Chemotaxonomie der eurasischen Arten der Flechtengattung rhizoplaca. *Nova Hedwigia* 28: 71-129.

LIBRADO, P., and J. ROZAS. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451-1452.

LINDBLOM, L., and S. EKMAN. 2006. Genetic variation and population differentiation in the lichen-forming ascomycete *Xanthoria parietina* on the island Storfosna, central Norway. *Molecular Ecology* 15: 1545-1559.

LIU, L., L. YU, L. KUBATKO, D. K. PEARL, and S. V. EDWARDS. 2009. Coalescent methods for estimating phylogenetic trees. *Molecular Phylogenetics and Evolution* 53: 320-328.

LUMBSCH, H. T. 2002. Analysis of phenolic products in lichens. *In* I. Kranner, R. P. Beckett, AND A. Varma [eds.], Protocols in Lichenology, 281-295. Springer, Berlin.

LUMBSCH, H. T., and I. SCHMITT. 2001. Molecular Data Suggest that the Lichen Genus *Pertusaria* is not Monophyletic. *The Lichenologist* 33: 161-170.

LUTZONI, F., M. PAGEL, and V. REEB. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* 411: 937-940.

LUTZONI, F., F. KAUFF, C. J. COX, D. MCLAUGHLIN, G. CELIO, B. DENTINGER, M. PADAMSEE, et al. 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* 91: 1446-1480.

Maddison, W. P. 1997. Gene Trees in Species Trees. Systematic Biology 46: 523-536.

MARTIN, D. P., C. WILLIAMSON, and D. POSADA. 2005. RDP2: recombination detection and analysis from sequence alignments. *Bioinformatics* 21: 260-262.

MATSEN, F. A., and M. STEEL. 2007. Phylogenetic Mixtures on a Single Tree Can Mimic a Tree of Another Topology. *Systematic Biology* 56: 767-775.

MAYDEN, R. L. 1999. Consilience and a hierarchy of species concepts: Advances towards closure on the species puzzle. *The Journal of Nematology* 31: 95-116.

MCCUNE, B. 1987. Distribution of Chemotypes of Rhizoplaca in North America. *The Bryologist* 90: 6-14.

MOLINA, M. D. C., A. CRESPO, O. BLANCO, N. S. HLADUN, and D. L. HAWKSWORTH. 2002. Molecular phylogeny and status of Diploicia and Diplotomma, with observations on Diploicia subcanescens and Diplotomma rivas-martinezii. *The Lichenologist* 34: 509-519.

MOORE, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial genes versus nuclear-gene trees. *Evolution* 49: 718.

MURTAGH, G. J., P. S. DYER, P. A. FURNEAUX, and P. D. CRITTENDEN. 2002. Molecular and physiological diversity in the bipolar lichen-forming fungus *Xanthoria elegans*. *Mycological Research* 106: 1277-1286.

NEI, M. 1987. Molecular evolutionary genetics. New York : Columbia University Press, New York, New York, USA.

NELSEN, M. P., and A. GARGAS. 2009. Assessing clonality and chemotype monophyly in *Thamnolia* (Icmadophilaceae). *Bryologist* 112: 42-53.

NYLANDER, J. A. A., F. RONQUIST, J. P. HUELSENBECK, and J. NIEVES-ALDREY. 2004. Bayesian Phylogenetic Analysis of Combined Data. *Systematic Biology* 53: 47-67.

O'BRIEN, H. E., J. MIADLIKOWSKA, and F. LUTZONI. 2009. Assessing reproductive isolation in highly diverse communities of the lichen-forming funal genus *Peltigera*. *Evolution* 63: 2076-2086.

O'HARA, R. J. 1993. Systematic Generalization, Historical Fate, and the Species Problem. *Systematic Biology* 42: 231-246.

O'MEARA, B. C. 2009. New Heuristic Methods for Joint Species Delimitation and Species Tree Inference. *Systematic Biology* 59: 59-73.

OTT, S., M. BRINKMANN, N. WIRTZ, and H. T. LUMBSCH. 2004. Mitochondrial and nuclear ribosomal DNA data do not support the separation of the Antarctic lichens *Umbilicaria kappenii* and *Umbilicaria antarctica* as distinct species. *The Lichenologist* 36: 227-234.

PORTER, L. D. 1999. Chemical and metabolic differences in *Rhizoplaca melanophthalma* along an elevational gradient. Master's, Brigham Young University, Provo, Utah, USA.

POSADA, D., and K. A. CRANDALL. 2001. Selecting the Best-Fit Model of Nucleotide Substitution. *Systematic Biology* 50: 580-601.

POSADA, D., K. A. CRANDALL, and E. C. HOLMES. 2002. Recombination in evolutionary genomics. *Annual Review of Genetics* 36: 75-97.

PRINGLE, A., D. M. BAKER, J. L. PLATT, J. P. WARES, J. P. LATGÉ, and J. W. TAYLOR. 2005. Cryptic Speciation in the Cosmopolitan and Clonal Human Pathogenic Fungus *Aspergillus fumigatus*. *Evolution* 59: 1886-1899.

PRINTZEN, C. 2009. Lichen Systematics: The Role of Morphological and Molecular Data to Reconstruct Phylogenetic Relationships, Progress in Botany 71, vol. 71, 233-275. Springer Berlin Heidelberg, Berlin, Germany.

PRITCHARD, J. K., M. STEPHENS, and P. DONNELLY. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

RAMBAULT, A. 1996. Sequence Alignment Editor *Available from: <http://tree.bio.ed.ac.uk/software/seal/>*.

ROE, A. D., and F. A. H. SPERLING. 2007. Population structure and species boundary delimitation of cryptic *Dioryctria* moths: an integrative approach. *Molecular Ecology* 16: 3617-3633.

ROKAS, A., B. L. WILLIAMS, N. KING, and S. B. CARROLL. 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. *Nature* 425: 798-804.

ROSENTRETER, R. 1993. Vagrant Lichens in North America. *The Bryologist* 96: 333-338.

ROWE, G., and T. J. C. BEEBEE. 2007. Defining population boundaries: use of three Bayesian approaches with microsatellite data from British natterjack toads (*Bufo calamita*). *Molecular Ecology* 16: 785-796.

RUIZ-SANCHEZ, E., and V. SOSA. 2010. Delimiting species boundaries within the Neotropical bamboo Otatea (Poaceae: Bambusoideae) using molecular, morphological and ecological data. *Molecular Phylogenetics and Evolution* 54: 344-356.

RYAN, B. D. 2001. *Rhizoplaca*. *In* T. H. Nash III, B. D. Ryan, C. Gries, AND F. Bungartz [eds.], Lichen Flora of the Greater Sonoran Desert Region, vol. I (the pyrenolichens and most of the squamulose and macrolichens). Lichens Unlimited, Tempe, Arizona, USA.

SAISHO, D., and M. D. PURUGGANAN. 2007. Molecular Phylogeography of Domesticated Barley Traces Expansion of Agriculture in the Old World. *Genetics* 177: 1765-1776.

SCHLUTER, D. 2000. The ecology of adaptive radiations. Oxford University Press, Oxford, USA.

SCHMITT, I., and H. T. LUMBSCH. 2004. Molecular phylogeny of the Pertusariaceae supports secondary chemistry as an important systematic character set in lichen-forming ascomycetes. *Molecular Phylogenetics and Evolution* 33: 43-55.

SCHMITT, I., A. CRESPO, P. K. DIVAKAR, J. D. FANKHAUSER, E. HERMAN-SACKETT, K. KALB, M. P. NELSEN, et al. 2009. New primers for promising single-copy genes in fungal phylogenies and systematics. *Persoonia* 23: 35-40.

SELBMANN, L., L. ZUCCONI, S. RUISI, M. GRUBE, M. CARDINALE, and S. ONOFRI. 2010. Culturable bacteria associated with Antarctic lichens: affiliation and psychrotolerance. *Polar Biology* 33: 71-83.

SHAFFER, H. B., and R. THOMSON. 2007. Delimiting Species in Recent Radiations. *Systematic Biology* 56: 896-906.

SITES, J. W., and J. C. MARSHALL. 2004. Operational criteria for delimiting species. *Annual Review of Ecology, Evolution, and Systematics* 35: 199-227.

STAMATAKIS, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

STAMATAKIS, A., P. HOOVER, and J. ROUGEMONT. 2008. A Rapid Bootstrap Algorithm for the RAxML Web Servers. *Systematic Biology* 57: 758-771.

TEHLER, A., and M. KÄLLERSJÖ. 2001. *Parmeliopsis ambigua* and *P. hyperopta* (Parmeliaceae): species or chemotypes? *The Lichenologist* 33: 403-408.

TEMPLETON, A. R., K. A. CRANDALL, and C. F. SING. 1992. A Cladistic Analysis of Phenotypic Associations With Haplotypes Inferred From Restriction Endonuclease Mapping and DNA Sequence Data. III. Cladogram Estimation. *Genetics* 132: 619-633.

THELL, A. 1999. Group I Intron Versus its Sequences in Phylogeny of Cetrarioid Lichens. *The Lichenologist* 31: 441-449.

UGUR, A., B. ÖZDEN, M. SAÇ, G. YENER, Ü. ALTINBAŞ, Y. KURUCU, and M. BOLCA. 2004. Lichens and mosses for correlation between trace elements and 210Po in the areas near coal-fired power plant at Yatağan, Turkey. *Journal of Radioanalytical and Nuclear Chemistry* 259: 87-92.

VELMALA, S., L. MYLLYS, P. HALONEN, T. GOWARD, and T. AHTI. 2009. Molecular data show that *Bryoria fremontii* and *B. tortuosa* (Parmeliaceae) are conspecific. *The Lichenologist* 41: 231-242.

VIEITES, D. R., K. C. WOLLENBERG, F. ANDREONE, J. KÖHLER, F. GLAW, and M. VENCES. 2009. Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences* 106: 8267-8272.

WEDIN, M., M. WESTBERG, A. T. CREWE, A. TEHLER, and O. W. PURVIS. 2009. Species delimitation and evolution of metal bioaccumulation in the lichenized *Acarospora smaragdula* (Ascomycota, Fungi) complex. *Cladistics* 25: 161-172.

WEISROCK, D. W., R. M. RASOLOARISON, I. FIORENTINO, J. M. RALISON, S. M. GOODMAN, P. M. KAPPELER, and A. D. YODER. 2010. Delimiting Species without Nuclear Monophyly in Madagascar's Mouse Lemurs. *PLoS ONE* 5: e9883.

WHEELER, D. L., T. BARRETT, D. A. BENSON, S. H. BRYANT, K. CANESE, V. CHETVERNIN, D. M. CHURCH, et al. 2006. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*: gkl1031.

WHITE, T. J., T. D. BRUNS, S. LEE, and J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press, San Diego, California, USA.

WIENS, J. J. 1998. Combining Data Sets with Different Phylogenetic Histories. *Systematic Biology* 47: 568-581.

WIENS, JOHN J. 2004. What Is Speciation and How Should We Study It? *The American Naturalist* 163: 914-923.

WILL, K. W., B. D. MISHLER, and Q. D. WHEELER. 2005. The Perils of DNA Barcoding and the Need for Integrative Taxonomy. *Systematic Biology* 54: 844-851.

WIRTZ, N., C. PRINTZEN, and H. T. LUMBSCH. 2008. The delimitation of Antarctic and bipolar species of neuropogonoid *Usnea* (Ascomycota, Lecanorales): a cohesion approach of species recognition for the *Usnea perpusilla* complex. *Mycological Research* 112: 472-484.

YANG, Z., and B. RANNALA. 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences* 107: 9264-9269.

ZHENG, X.-L., H.-M. SHENG, and L.-Z. AN. 2007. Phylogenetic Analysis of Lichen-Forming Fungi *Rhizoplaca* Zopf from China Based on ITS Data and Morphology *Zeitschrift fur Naturforschung Section C Journal of Biosciences* 62c: 757-764.

ZHOU, Q.-M., S.-Y. GUO, M.-R. HUANG, and J.-C. WEI. 2006. A study of the genetic variability of *Rhizoplaca chrysoleuca* using DNA sequences and secondary metabolic substances. *Mycologia* 98: 57-67.

ZWICKL, D. J., and D. M. HILLIS. 2002. Increased Taxon Sampling Greatly Reduces Phylogenetic Error. *Systematic Biology* 51: 588-598.

Table 1.1. Primers used for PCR amplification and sequencing of the nuclear ribosomal IGS, ITS, and group I intron markers and nuclear markers β-tubulin and MCM7.

Marker	Primer name	Forward primer sequence	Annealing temperature (°C)	Reference
IGS	IGS12	5'-AGTCTGTGGATTAGTGGCCG-3'	66- 56 (touchdown)	Carbone & Kohn 1999
	NS1R	5'-GAGACAAGCATATGACTAC-3'		Carbone & Kohn 1999
ITS/group I intron	ITS1F	5'-CTT GGT CAT TTA GAG GAA GTA A-3'	55-60	Gardes and Bruns 1993
	ITS4	5'- TCC TCC GCT TAT TGA TAT GC-3'		White et al. 1990
β-tubulin	Bt3-LM	5'-GAACGTCTACTTCAACGAG-3'	55-60	Myllys et al. 2001
	Bt10-LM	5'-TCGGAAGCAGCCATCATGTTCTT-3'		Myllys et al. 2001
	Bt_rhizo_F	5'-GCA ACA AGT ATG TTC CTC GTG C-3'	66- 56 (touchdown)	this study
	Bt_rhizo_R	5'-GTAAGAGGTGCGAAGCCAACC-3'		this study
MCM7	Mcm7-709for	5'-ACI MGI GTI TCV GAY GTH AARCC-3'	56	Schmitt et al. 2009
	Mcm7-1348rev	5'-GAY TTD GCI ACI CCI GGR TCW CCC AT-3'		Schmitt et al. 2009
	Lec*MCM7*f	5'-TAC CAN TGT GAT CGA TGY GG-3'	66- 56 (touchdown)	this study
	Lec*MCM7*r	5'-GTC TCC RCG TAT TCG CAT NCC-3'		this study

Table 1.2. Genetic variability of sampled markers used in this study, including alignment length (number of basepairs); variable and parsimony-informative (PI) sites for each sampled locus; and locus-specific model of evolution identified using the Akaike information criterion in MrModeltest. Numbers in parentheses indicate the number of variable and parsimony-informative sites for the Rhizoplaca melanophthalma species-complex only.

Locus	Length	# variable sites	# PI sites	Model Selected
ITS	561	163 (91)	127 (57)	GTR+G
IGS	374	138 (84)	103 (54)	GTR+I
group I intron	269	98 (44)	84(30)	SYM+G
β-tubulin	819	165 (90)	132(55)	HKY+I+G
MCM7	616	158 (123)	123 (42)	GTR+G
total	2639	722 (432)	569 (238)	-

Table 1.3. Polymorphism statistics for candidate species within the R. melanophthalma species-complex. N, number of individuals sampled, Npoly, number of polymorphics sites; h, number of unique haplotypes; π, estimate of 4 Nμ per base pair using the average pairwise differences.

	ITS		**IGS**		**intron**		**β-tubulin**		***MCM7***	
	N/ N_{poly} /h	**π**	***N/ N_{poly} /h***	**II**	***N/ N_{poly} /h***	**II**	***N/ N_{poly} /h***	**π**	***N/ N_{poly} /h***	**π**
clade I (*L. novomexicana*)	3/0/1	0	4/0/1	0	3/0/1	0	4/2/3	0.00146	2/11/2002	0.02041
clade II	24/35/17	0.00930	21/37/18	0.01776	23/19/17	0.1089	24/34/17	0.01430	23/10/8	0.00278
clade III	13/5/5	0.00188	13/1/2	0.0014	13/0/1	0	13/3/2	0.00067	13/4/4	0.00157
clade IV (*R. haydenii*)	5/6/4	0.00475	4/4/4	0.00318	5/4/3	0.00723	5/2/2	0.00117	5/7/2	0.00779
clade IV (*R. h.* spp. *arbuscula*)	2/1/2	0.00182	2/1/2	0.00272	1/0/1	na	1/0/1	na	2/0/1	0
clade IV (*R. idahoensis*)	3/3/2	0.00367	3/1/2	0.00272	2/0/1	0	3/4/2	0.0039	37316	0.00124
clade IVa	3/3/3	0.00427	3/2/3	0.00363	3/0/1	0	3/0/1	0	3/0/1	0
clade IVb	14/9/7	0.00235	13/3/4	0.00265	14/3/4	0.00327	13/9/9	0.00285	13/19/6	0.01308
clade IVc	5/1/2	0.00088	5/3/3	0.00381	5/0/1	0	5/5/3	0.00439	5/2/2	0.00148
clade IVd	55/11/10	0.00162	55/19/18	0.01191	0/na/na	na	55/32/8	0.00266	55/5/6	0.00040
Total	127/91/52	0.02221	122/84/54	0.02494	69/43/27	0.03521	127/71/40	0.01309	126/112/33	0.01486

Table 1.4. Fixed differences and fixation indices (FST) for all pairwise comparisons of candidate species identified within R. melanophthalma species-complex. Numbers across the top row correspond to candidate species numbers in the first column. Numbers of fixed differences (ribosomal /β-tubulin/MCM7 characters) are represented for all comparisons below the diagonal and FST values are represented above the diagonal. The last column indicates total number of fixed nucleotides identified between each candidate species and the remaining data matrix. Numbers within parentheses represent fixed ribosomal characters/fixed protein-coding characters. Accessions representing R. haydenii subspecies arbuscula were not included in FST calculations because of the small sample sizes and pairwise comparisons are not represented.

Candidate species	1	2	3	4	5	6	7	8	9	10	fixed characters
1. clade I (*L. novomexicana*)	-	0.77102	0.89534	0.86359	na	0.85763	0.88863	0.85574	0.88172	0.88085	**32(21/11)**
2. clade II	49 (31/13/5)	-	0.75792	0.732	na	0.69564	0.76148	0.72461	0.75139	0.7426	**3(3/0)**
3. clade III	77(55/18/4)	32(28/0/4)	-	0.90524	na	0.89291	0.9382	0.88716	0.9273	0.92874	**15(15/0)**
4. clade IV (*R. haydenii*)	77(51/20/6)	32(26/0/6)	55(36/11/8)	-	na	0.58915	0.82339	0.67851	0.66667	0.71894	**1(0/1)**
5. clade IV (*R. h.* spp. *arbuscula*)	82(54/19/9)	36(28/1/7)	56(39/9/8)	7(2/4/1)	-	na	na	na	na	na	**0 (0/0)**
6. clade IV (*R. idahoensis*)	71(53/8/10)	33(28/0/5)	55(38/8/9)	12(1/0/11)	15(2/0/13)	-	0.84298	0.6808	0.71146	0.75427	**7(1/6)**
7. clade IVa	65(38/20/7)	36(29/0/7)	54(36/10/8)	27(21/5/1)	27(24/3/0)	38(23/2/13)	-	0.82136	0.83333	0.80228	**7(7/0)**
8. clade IVb	76(54/19/3)	31(29/2/0)	48(39/7/2)	13(4/8/1)	11(5/6/0)	15(5/5/5)	30(23/7/0)	-	0.67031	0.72953	**3(2/1)**
9. clade IVc	76(51/18/7)	35(28/0/7)	55(39/8/8)	18(14/3/1)	6(6/0/0)	18(5/0/13)	24(24/0/0)	9(4/5/0)	-	0.66841	**1(1/0)**
10. clade IVd	61(36/18/7)	22(16/0/6)	45(29/8/8)	10(6/3/1)	8(8/0/0)	14(6/0/12)	14(14/0/0)	13(8/5/0)	7(7/0/0)	-	**1(1/0)**

Table 1.5. Chemotypic variation by candidate species in the R. melanophthalma species-complex based on HPLC analysis. Superscript number following acid nominal indicate acid occurrence: 1, major or minor; 2, major or not present; 3, minor or not present; 4, minor or trace; and 5, trace or not present.

Acid	clade I	clade II	clade III	*R. haydenii* (clade IV)	*R. h.* ssp. *arbuscula* (clade IV)	*R. idahonesis* (clade IV)	Clade IVa	Clade IVb	Clade IVc	Clade IVd
Usnic1	1	1	1	1	1	1	1	1	1	1
Psoromic2	1	0.91	1	0	0	0	0.66	1	0.40	0.95
Lecanoric2	0	0	0	0	0	0	0	0.57	0	0
Orsellinic3	0	0	0	0	0	0	0	0.64	0	0
Gyrophoric5	0	0	0	0	0	0	0	0.43	0	0
Constipatic3	0	0.91	0.64	0	0	0	1	0.93	1	0.91
Dehydroconstipatic3	0.25	0.91	0.36	0	0	0	1	0.93	1	0.95
Dehydroprotoconstipatic3	0.25	0.7	0.36	0	0	0	0.33	0.86	1	0.55
subpsoromic acid3	0.25	0.43	1	0	0	0	0	0.57	1	0.78
2'-*O*-demethylsubpsoromic4	0.75	0.52	1	0	0	0	1	0.29	1	0.87
2'-*O*-demethylpsoromic3	0.75	0.39	0.82	0	0	0	1	0.5	0.5	0.73

Table 1.6. Summary of data supporting candidate species within the R. melanophthalma species-complex. Fixed characters, the total number of fixed nucleotide characters relative to the remaining data matrix; genealogical exclusivity, candidate species recovered as an exclusive lineage in gene haplotype networks, '*' indicate support from individual ribosomal, β-tubulin, and MCM7 network reconstructions. STRUCTURE, indicates if the candidate species was recovered as a unique population cluster in the Bayesian clustering analysis, supported from population aggregation analysis; independent characters support, support from independent morphological or chemical data.

Candidate species	**Fixed characters**	**Genealogical exclusivity**	**STRUCTURE**	**Independent character support**
clade I (*L. novomexicana*)	Yes (21-9-2)	Yes***	Yes	Lobate, placodioid thallus morphology
clade II	Yes (3-0-0)	Yes*-*	Yes	Not identified
clade III	Yes (15-0-0)	Yes***	Yes	Not identified
clade IV (*R. haydenii*)	Yes (0-0-1)	No	= vagrant taxa & clade IVc	Vagrant thallus morphology and usnic acid only
clade IV (*R. h.* ssp. *arbuscula*)	No	No	= vagrant taxa & clade IVc	Vagrant thallus morphology and usnic acid only
clade IV (*R. idahonesis*)	Yes (1-0-5)	No	= vagrant taxa & clade IVc	Vagrant thallus morphology and usnic acid only
clade IVa	Yes (7-0-0)	Yes*--	= clade IVa & IVd	Not identified
clade IVb	Yes (2-1-0)	Yes**-	Yes	Lecanoric/ orsellinic acid are exclusive to this lineage
clade IVc	Yes (1-0-0)	Yes*--	= vagrant taxa & clade IVc	Not identified
clade IVd	Yes (1-0-0)	Yes*-*	= clade IVa & IVd	Not identified

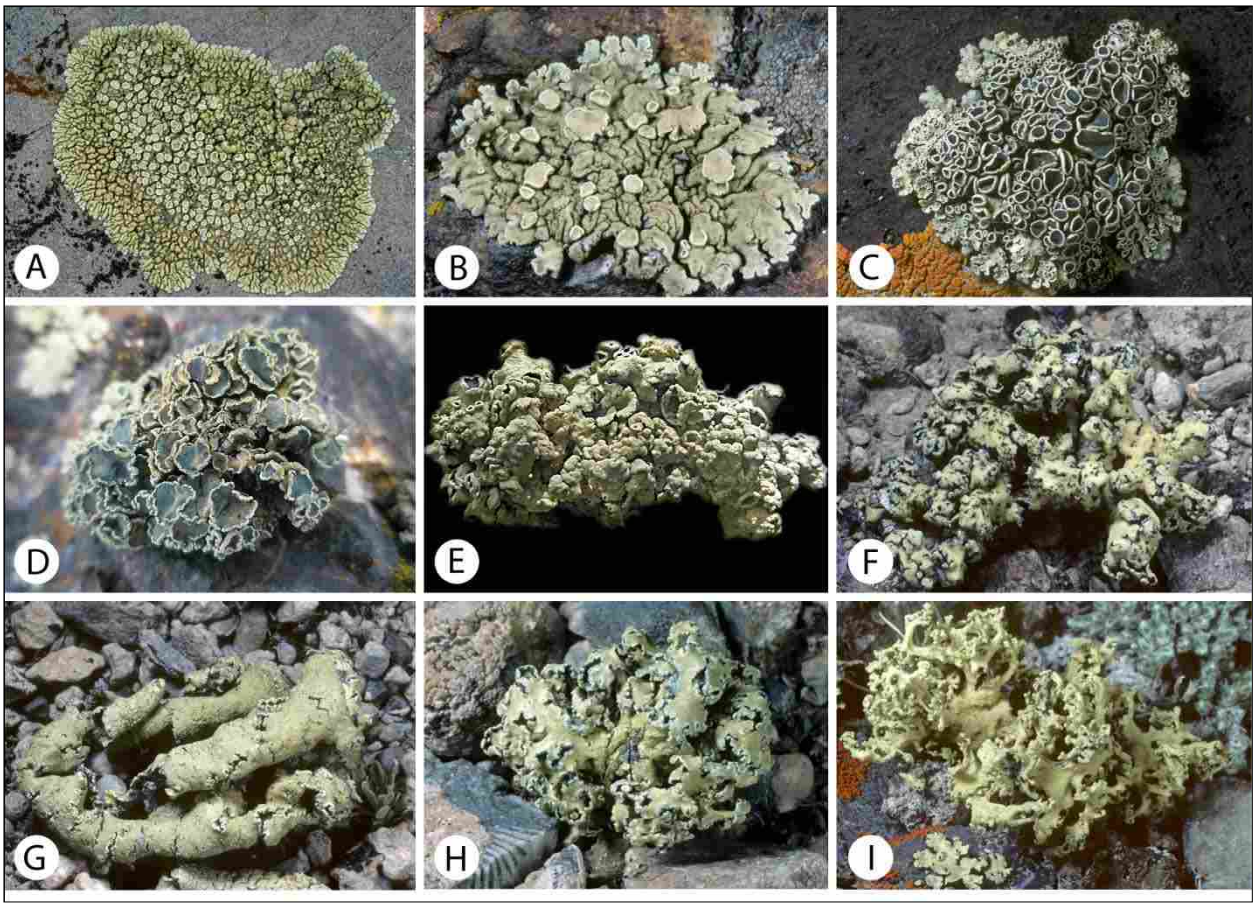

Figure 1.1. Variation in morphology and habit within the *Rhizoplaca melanophthalma* species-complex (Lecanoraceae) in western North America: (A) the lobate, placodioid taxon *Lecanora novomexicana*; (B) *Rhizoplaca melanophthalma* sensu lato (s.l.), with distinct light colored, pruinose apothecia discs; (C) *Rhizoplaca melanophthalma* sensu lato (s.l.), umblicate form with distinct lobes and dark apothecia; (D) *R. melanophthalma* s.l., umblicate form lacking lobes with pruinose apothecia (E) *R. melanophthalma* s.l., erratic form completely lacking umbilicus growing free on soil from western Idaho, with apothecia. Images F-I vagrant taxa endemic to the high plains and mountains of the northern Rocky Mountains: (F) *R. melanophthalma* ssp. crispa; (G) *R. idahoensis*; (H) *R. haydenii*; (I) *R. haydenii* ssp. *arbuscula.*

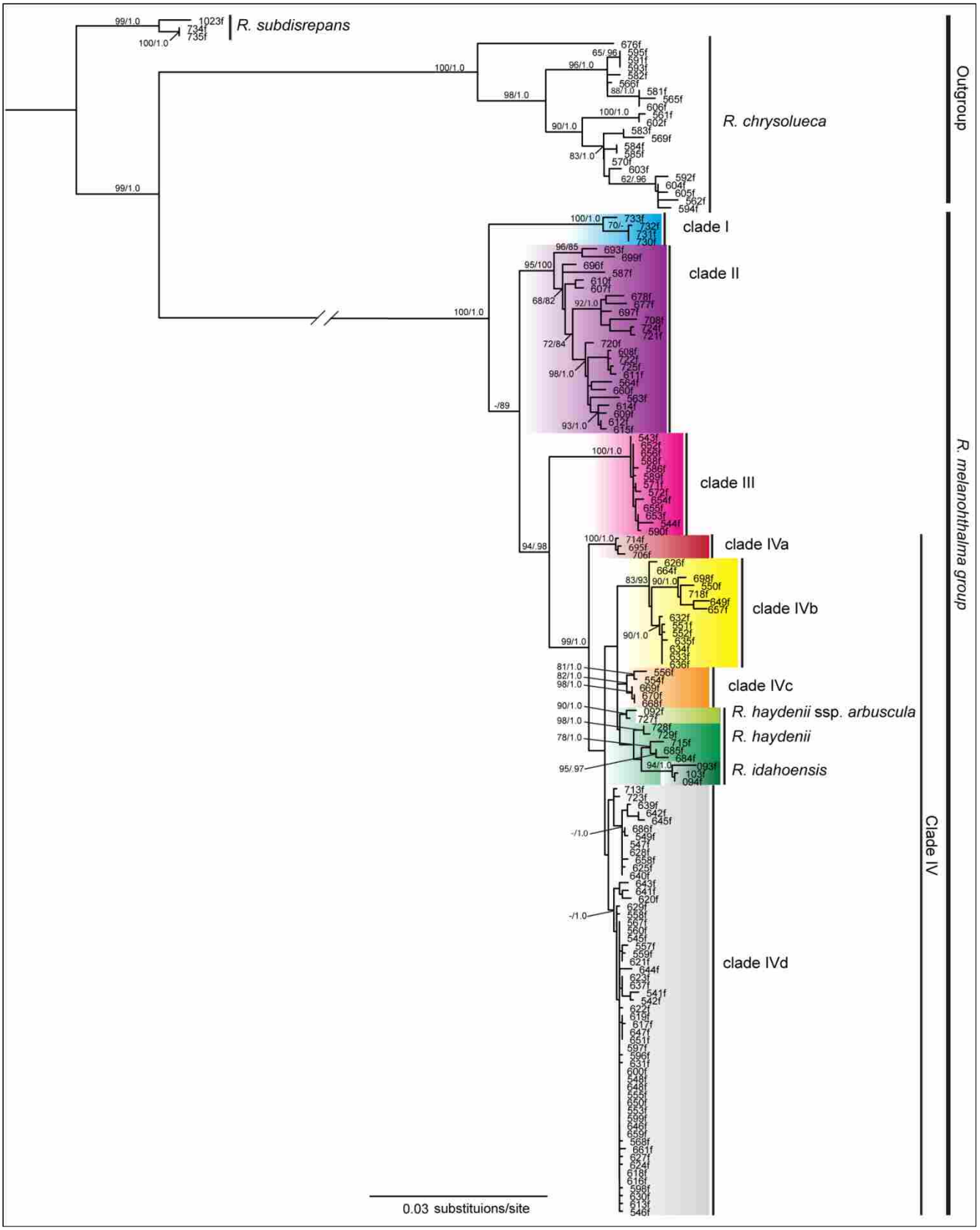
R. subdisrepans
R. chrysolueca
clade I
clade II
clade III
clade IVa
clade IVb
clade IVc
R. haydenii ssp. arbuscula
R. haydenii
R. idahoensis
clade IVd
Clade IV
R. melanophthalma group
Outgroup
0.03 substituions/site

Figure 1.2 (on previous page). Relationships among sampled specimens collected from the *Rhizoplaca melanophthalma* group inferred from a maximum likelihood analysis of ribosomal and nuclear DNA sequence data (~2600 bp, ITS, IGS, intron, β-tubulin, and *MCM7*). Values at each node indicate non-parametric-bootstrap support/posterior probability. Only support indices ≥ 50/0.50 are indicated. Clade numbers plotted to the right of the tree indicate candidate species. GenBank accessions represented solely by ITS sequences were not included.

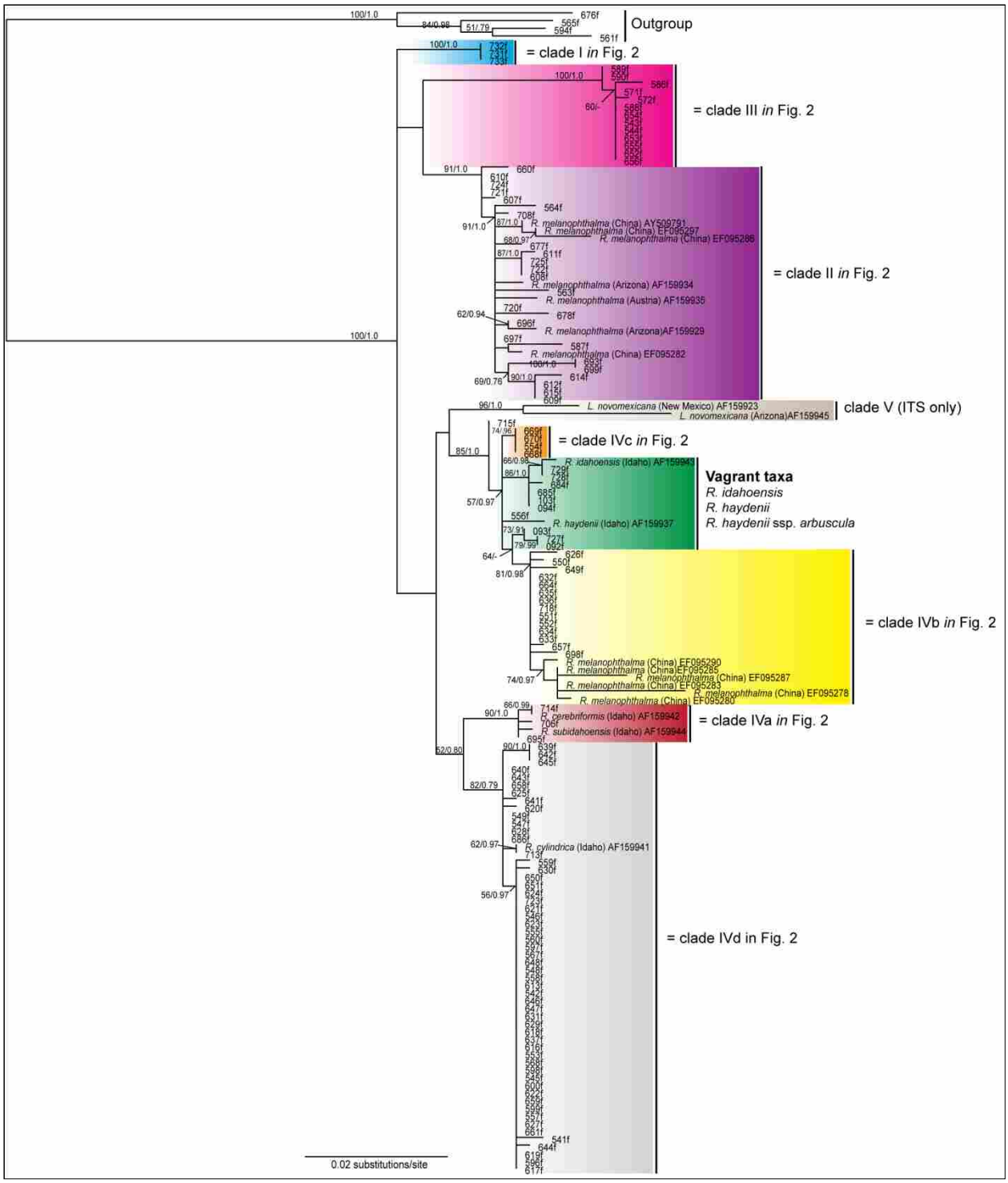

Figure 1.3. The maximum likelihood ITS topology obtained from all sampled specimens and available GenBank accessions collected from the *Rhizoplaca melanophthalma* species-complex. Values at each node indicate non-parametric-bootstrap support/posterior probability. Only support indices ≥ 50/0.50 are indicated. Clade numbers plotted to the right of the tree indicate lineages corresponding to candidate species shown in Figure 2.

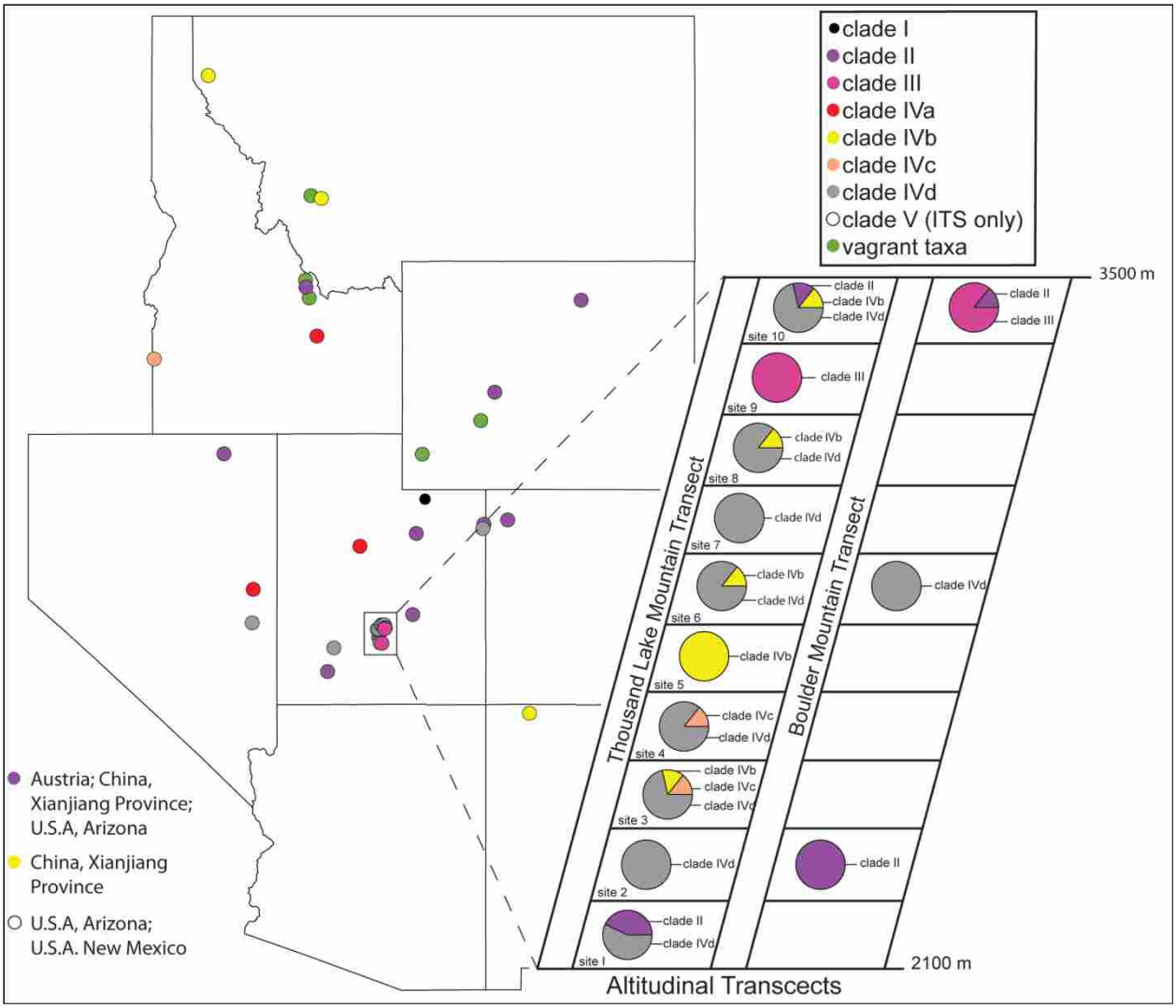

Figure 1.4. Geographical distributions of candidate *Rhizoplaca* species in the Intermountain western USA. Colors refer to different lineages, indicated in key. Insert shows distributions of putative lineages along two altitudinal gradients in southern Utah, U.S.A. A total of 7 individual were included from each plot and the proportion of candidate species recovered at each plot is represented.

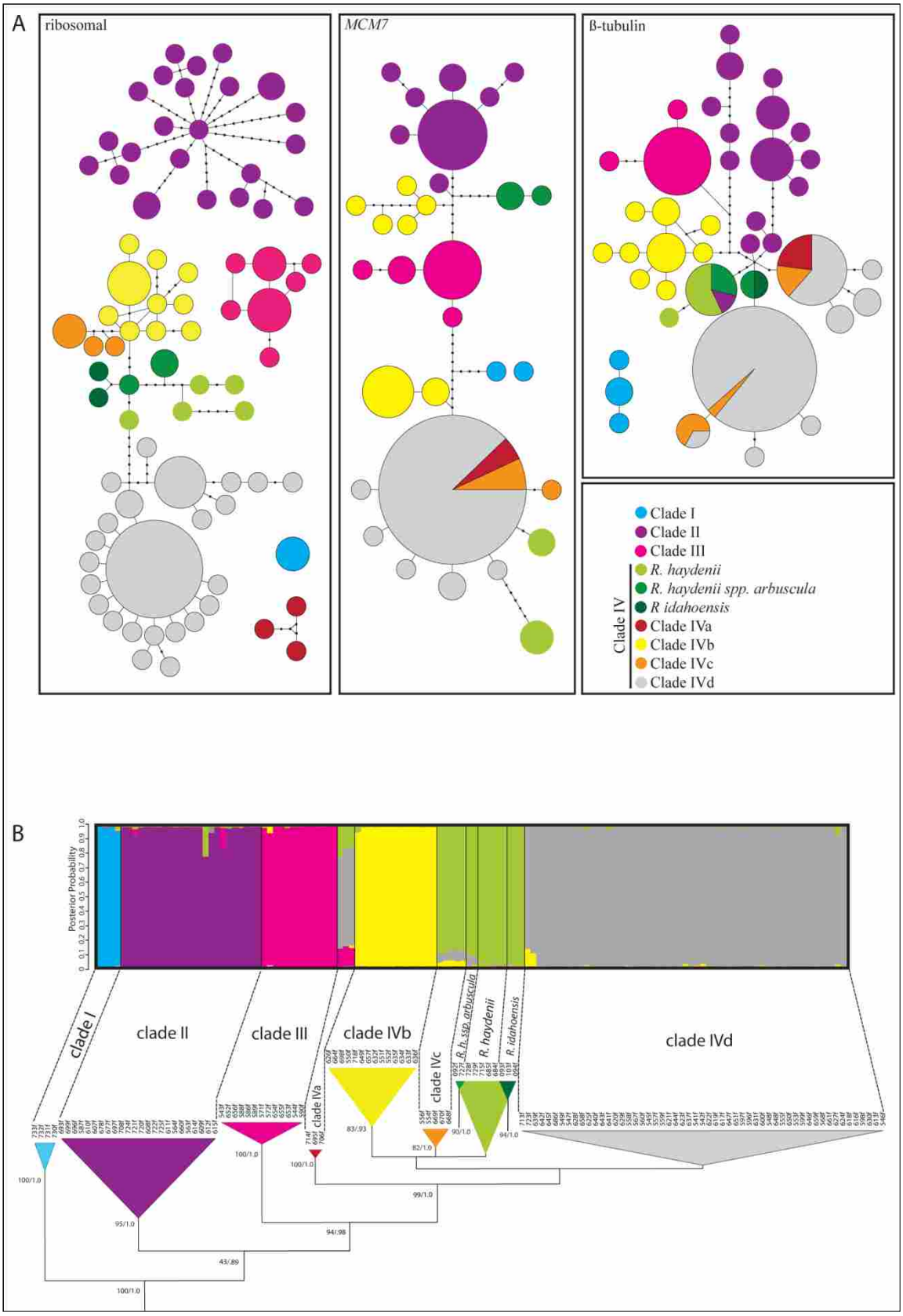
A
ribosomal
MCM7
ß-tubulin
Clade I
Clade II
Clade III
R. haydenii
R. haydenii spp. arbuscula
R idahoensis
Clade IVa
Clade IVb
Clade IVc
Clade IVd
Clade IV
B
Posterior Probability
clade I
clade II
clade III
clade IVb
clade IVa
clade IVc
R. h. spp. arbuscula
R. haydenii
R. idahoensis
clade IVd
100/1.0
95/1.0
100/1.0
100/1.0
83/.93
82/1.0
95/1.0
94/1.0
99/1.0
94/.98
43/.89
100/1.0

Figure 1.5 (on previous page). Figure 5A) Unrooted statistical parsimony haplotype networks at 95% probability of the ribosomal, *MCM7*, and β-tubulin loci representing relationship within the *R. melanophthalma* species-complex. Each candidate species is designated by a different color. Size of circles is proportional to the number of individuals of a given haplotype, and black dots represent inferred haplotypes not sampled. Figure 5B) Correspondence between candidate species identified from the combined maximum likelihood analysis and the population clusters identified using STRUCTURE. Numbers at nodes represent maximum likelihood bootstrap values and posterior probabilities, and relationships within candidate species are collapsed for ease of presentation (see Fig. 2 for detailed relationships). Candidate species are mapped to corresponding clusters in the STRUCTURE plot. Each population cluster is represented by a different color, and vertical bars within each cluster represent individuals and the proportion of a bar assigned to a single color represents the posterior probability that an individual is assigned to that cluster. The colors in the topology and STRUCTURE plot correspond to candidate species colors shown in Figure 5A and phylogenetic hypothesis of relationships in the *Rhizoplaca melanophthalma* species-complex in western North America.

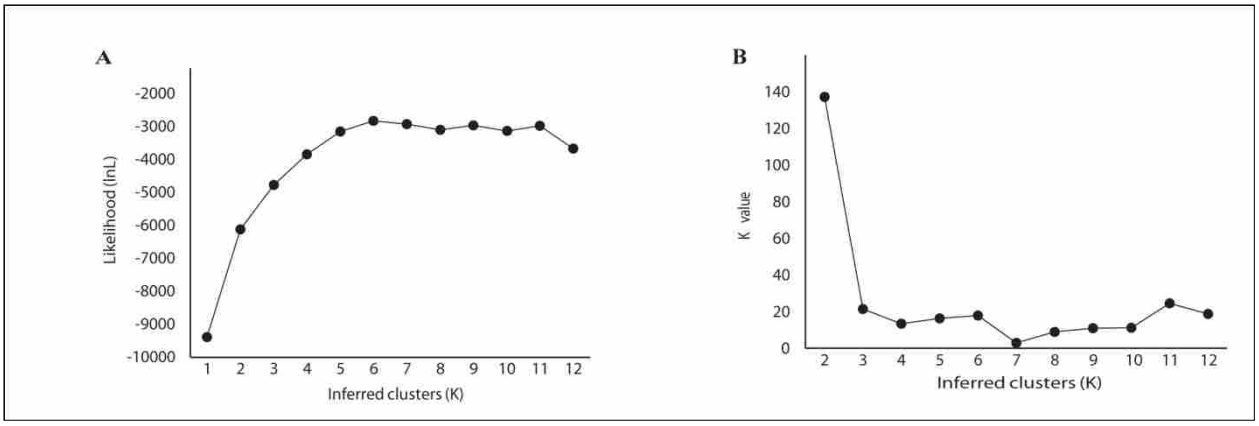

Figure 1.6. Plots of calculations for *K* values 1-12 in STRUCTURE analysis of the combined dataset. (A) The mean log probability of the data for K = 1 to12, calculated from the four best scoring runs for each *K* value. (B) Δ*K* values for K=2 to 12.

Supplementary data 1.1. All specimens included in the present study: ID, specimen identification and DNA collection number; voucher, herbarium collection number; plot, specific to sampling plots along altitudinal gradients on Thousand Lakes and Boulder Mountains in southern Utah, USA; Lat, latitude; Lon, longitude; Ele., altitude in m. a. s. l.; Collector(s); and source of specimen. Collectors include: MD, M. Devito; KBK, K. Knight; G. Leavitt; HCL, H. Leavitt; JHL, J. Leavitt; SDL, S. Leavitt; LDP, L. Porter; PAR, P. Ririe; GS, G. Shrestha; LLS, L. St. Clair; and EA indicates specimens sampled from the Elemental Analysis collection at the Herbarium of Nonvascular Cryptogams (BRY), Brigham Young University, Provo, Utah, USA.

ID	Voucher	Plot	Location	Lat.	Lon.	Ele.	Collector(s)	Source
Outgroup taxa								
R. chrysolueca								
561f	BRY-55000	-	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
562f	BRY-55001	-	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
565f	BRY-55002	-	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5794	2809 m	SDL, HCL, JHL, PAR	this study
566f	BRY-55003	-	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5794	2809 m	SDL, HCL, JHL, PAR	this study
569f	BRY-55004	-	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
570f	BRY-55005	-	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
581f	BRY-55570	-	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
582f	BRY-55006	-	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
583f	BRY-55007	-	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
584f	BRY-55008	-	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
585f	BRY-55009	-	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
591f	BRY-55010	-	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5795	2809 m	SDL, HCL, JHL, PAR	this study
592f	BRY-55011		USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5795	2809 m	SDL, HCL, JHL, PAR	this study
593f	BRY-55012	-	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5795	2809 m	SDL, HCL, JHL, PAR	this study
594f	BRY-55571	-	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5795	2809 m	SDL, HCL, JHL, PAR	this study
595f	BRY-	-	USA, Wayne Co.: Boulder Mountain (BM-	38.17228	-111.5795	2809 m	SDL, HCL, JHL, PAR	this study

	55013		2)					
602f	BRY-55014	-	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
603f	BRY-55015	-	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
604f	BRY-55016	-	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
605f	BRY-55017	-	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
606f	BRY-55018	-	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
676f	BRY-55019	-	USA, UT, Summit County; High Uinta Wilderness Area	40.82699	-110.5004	3500 m	SDL, LLS, MD	this study
R. subdiscrepans								
1023f	BRY-55020	-	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5795	2809 m	SDL, HCL, JHL, PAR	this study
734f	BRY-55021	-	USA, UT, Uintah Co.: Snake John Reef	40.29259	-109.1214	1631 m	SDL, LLS, GS	this study
735f	BRY-55022	-	USA, UT, Uintah Co.: Snake John Reef	40.29259	-109.1214	1631 m	SDL, LLS, GS	this study
<u>*R. melanophthalma* species-complex</u>								
clade I – *Lecanora novomexicana*								
730f	BRY-55023	-	USA, UT, Summit Co.: Ashley National Forest	40.8551	-110.8747	2793 m	SDL, LLS, MD	this study
731f	BRY-55024	-	USA, UT, Summit Co.: Ashley National Forest	40.5976	-109.8406	2606 m	SDL, LLS, GS	this study
732f	BRY-55025	-	USA, UT, Summit Co.: Ashley National Forest	40.5976	-109.8406	2606 m	SDL, LLS, GS	this study
733f	BRY-55026	-	USA, UT, Uintah Co.: Snake John Reef	40.29259	-109.1208	1631 m	SDL, LLS, GS	this study
clade V – *Lecanora novomexicana* (from ITS gene tree)								
-	AF159923	-	USA, New Mexico	-	-	-	-	Arup and Grub 2000
-	AF159945	-	USA, Arizona	-	-	-	-	Arup and Grub 2000
clade II – *R. melanophthalma* sensu lato								
563f	BRY-55037	BM-1	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
564f	BRY-55038	BM-1	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
587f	BRY-	BM-3	USA, UT, Wayne Co.: Boulder Mountain	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study

	55039		(BM-3)					
607f	BRY-55040	BM-1	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
608f	BRY-55041	BM-1	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
609f	BRY-55042	BM-1	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
610f	BRY-55043	BM-1	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
611f	BRY-55044	BM-1	USA, UT, Wayne Co.: northwest of Boulder Mountain (BM-1)	38.27364	-111.6106	2344 m	SDL, HCL, JHL, PAR	this study
612f	BRY-55045	TLM-1	USA, Utah, Wayne Co.: Thousand Lake Mountain (1)	38.4243	-111.6446	2220 m	LDP	this study
614f	BRY-55046	TLM-1	USA, Utah, Wayne Co.: Thousand Lake Mountain (1)	38.4243	-111.6446	2220 m	LDP	this study
615f	BRY-55047	TLM-1	USA, Utah, Wayne Co.: Thousand Lake Mountain (1)	38.4243	-111.6446	2220 m	LDP	this study
660f	BRY-55048	TLM-10	USA, Utah, Wayne Co.: Thousand Lake Mountain (10)	38.44317	-111.4703	3400 m	LDP	this study
677f	BRY-55049	-	USA, UT, Emery Co.: San Rafael Swell	38.70424	-110.7964	1967 m	SDL	this study
678f	BRY-55050	-	USA, UT, Emery Co.: San Rafael Swell	38.70424	-110.7964	1967 m	SDL	this study
693f	BRY-55051	-	USA, NV, Elko Co.: Humboldt National Forest	41.64676	-115.3130	2023 m	EA 15-123A	this study
696f	BRY-55052	-	USA, UT, Uintah Co.: Dinosaur National Monument	40.37167	-109.0930	2447 m	EA 18-143	this study
697f	BRY-55053	-	USA, CO, Moffat Co.: Dinosaur National Monument	40.44957	-108.5234	1721 m	EA 18-145	this study
699f	BRY-55054	-	USA, UT, Iron Co.: Cedar Breaks National Monument	37.63043	-112.8317	3186 m	EA 22-177	this study
708f	BRY-55055	-	USA, ID, Lemhi Co.: Salmon Challis National Forest	44.56022	-113.3507	1194 m	EA 41-403	this study
720f	BRY-55056	-	USA, WY, Johnson Co.: west of Buffalo	44.33849	-106.7656	1581 m	SDL	this study
721f	BRY-55057	-	USA, WY, Fremont Co.: Wind River Mountains	42.73869	-108.8352	2122 m	SDL	this study
722f	BRY-	-	USA, UT, Uintah Co.: Snake John Reef	40.29259	-109.1208	1631 m	SDL, LLS, GS	this study

	55058							
724f	BRY-55059	-	USA, UT, Uintah Co.: Snake John Reef	40.29259	-109.1208	1631 m	SDL, LLS, GS	this study
725f	BRY-55060	-	USA, UT, Duchesne Co.: Pinyon Ridge Rest Area	40.20385	-110.7108	2055 m	SDL, LLS, GS	this study
-	AF159929 (ITS only)	-	USA, Arizona	-	-	-	-	Arup and Grub 2000
-	AF159934 (ITS only)	-	USA, Arizona	-	-	-	-	Arup and Grub 2000
-	AF159935 (ITS only)	-	Austria	-	-	-	-	Arup and Grub 2000
-	AY509791 (ITS only)	-	China, Xianjiang Province	-	-	-	-	Zhou et al. 2006
-	EF095282 (ITS only)	-	China, Xianjiang ProvinceTianshan Mountains	-	-	-	-	Zheng et al. 2007
-	EF095286 (ITS only)	-	China, Xianjiang ProvinceTianshan Mountains	-	-	-	-	Zheng et al. 2007
-	EF095297 (ITS only)	-	China, Xianjiang ProvinceTianshan Mountains	-	-	-	-	Zheng et al. 2007
clade III – *R. melanophthalma* sensu lato								
543f	BRY-55061	TLM-9	USA, Utah, Wayne Co.: Thousand Lake Mountain (9)	38.4366	-111.4677	3270 m	LDP	this study
544f	BRY-55062	TLM-9	USA, Utah, Wayne Co.: Thousand Lake Mountain (9)	38.4366	-111.4677	3270 m	LDP	this study
571f	BRY-55063	BM-3	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
572f	BRY-55064	BM-3	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
586f	BRY-55065	BM-3	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
588f	BRY-55066	BM-3	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
589f	BRY-55067	BM-3	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
590f	BRY-55068	BM-3	USA, UT, Wayne Co.: Boulder Mountain (BM-3)	38.16257	-111.5351	3360 m	SDL, HCL, JHL, PAR	this study
652f	BRY-55069	TLM-9	USA, Utah, Wayne Co.: Thousand Lake Mountain (9)	38.4366	-111.4677	3270 m	LDP	this study
653f	BRY-55070	TLM-9	USA, Utah, Wayne Co.: Thousand Lake Mountain (9)	38.4366	-111.4677	3270 m	LDP	this study

654f	BRY-55071	TLM-9	USA, Utah, Wayne Co.: Thousand Lake Mountain (9)	38.4366	-111.4677	3270 m	LDP	this study
655f	BRY-55072	TLM-9	USA, Utah, Wayne Co.: Thousand Lake Mountain (9)	38.4366	-111.4677	3270 m	LDP	this study
656f	BRY-55073	TLM-9	USA, Utah, Wayne Co.: Thousand Lake Mountain (9)	38.4366	-111.4677	3270 m	LDP	this study
clade IVa – *R. melanophthalma* sensu lato								
695f	BRY-55074	-	USA, Utah, Juab Co.: West of Goshen	39.9697	-112.0601	1840 m	EA 18-140	this study
706f	BRY-55075	-	USA, ID, Butte Co.: Salmon Challis National Forest	43.7197	-113.0891	2432 m	EA 37-356	this study
714f	BRY-55076	-	USA, NV, White Pine Co.: Humboldt-Toiyabe N.F.	39.1734	-114.6130	3166 m	SDL, LLS	this study
clade IVb – *R. melanophthalma* sensu lato								
550f	BRY-55077	TLM-6	USA, Utah, Wayne Co.: Thousand Lake Mountain (6)	38.5111	-111.4732	2875 m	LDP	this study
551f	BRY-55078	TLM-5	USA, Utah, Wayne Co.: Thousand Lake Mountain (5)	38.5076	-111.4904	2725 m	LDP	this study
552f	BRY-55079	TLM-5	USA, Utah, Wayne Co.: Thousand Lake Mountain (5)	38.5076	-111.4904	2725 m	LDP	this study
626f	BRY-55080	TLM-3	USA, Utah, Wayne Co.: Thousand Lake Mountain (3)	38.5079	-111.5505	2400 m	LDP	this study
632f	BRY-55081	TLM-5	USA, Utah, Wayne Co.: Thousand Lake Mountain (5)	38.5076	-111.4904	2725 m	LDP	this study
633f	BRY-55082	TLM-5	USA, Utah, Wayne Co.: Thousand Lake Mountain (5)	38.5076	-111.4904	2725 m	LDP	this study
634f	BRY-55083	TLM-5	USA, Utah, Wayne Co.: Thousand Lake Mountain (5)	38.5076	-111.4904	2725 m	LDP	this study
635f	BRY-55084	TLM-5	USA, Utah, Wayne Co.: Thousand Lake Mountain (5)	38.5076	-111.4904	2725 m	LDP	this study
636f	BRY-55085	TLM-5	USA, Utah, Wayne Co.: Thousand Lake Mountain (5)	38.5076	-111.4904	2725 m	LDP	this study
649f	BRY-55086	TLM-8	USA, Utah, Wayne Co.: Thousand Lake Mountain (8)	38.4557	-111.4581	3175 m	LDP	this study
657f	BRY-55087	TLM-10	USA, Utah, Wayne Co.: Thousand Lake Mountain (10)	38.44317	-111.4703	3400 m	LDP	this study
664f	BRY-55088	-	USA, NM, San Juan Co.: vicinity of Aztec Ruins National Monument	36.83479	-108.0002	1721 m	SDL, HCL	this study

698f	BRY-55089	-	MT, Deer Lodge Co.: southwest of Anaconda Copper Smelter	46.05645	-112.9820	1890 m	EA 21-166	this study
718f	BRY-55090	-	MT, Sanders Co.: Cabinet Mountains	48.06068	-115.6894	1939 m	SDL, LLS, GS	this study
-	EF095278 ((ITS only))	-	China, Xianjiang ProvinceTianshan Mountains	-	-	-	-	Zheng et al. 2007
-	EF095280 (ITS only)	-	China, Xianjiang ProvinceTianshan Mountains	-	-	-	-	Zheng et al. 2007
-	EF095283 (ITS only)	-	China, Xianjiang ProvinceTianshan Mountains	-	-	-	-	Zheng et al. 2007
-	EF095285 (ITS only)	-	China, Xianjiang ProvinceTianshan Mountains	-	-	-	-	Zheng et al. 2007
-	EF095287 (ITS only)	-	China, Xianjiang ProvinceTianshan Mountains	-	-	-	-	Zheng et al. 2007
-	EF095290 (ITS only)	-	China, Xianjiang ProvinceTianshan Mountains	-	-	-	-	Arup and Grub 2000
clade IVc – *R. melanophthalma* sensu lato								
554f	BRY-55091	TLM-4	USA, Utah, Wayne Co.: Thousand Lake Mountain (4)	38.5079	-111.5161	2550 m	LDP	this study
556f	BRY-55092	TLM-3	USA, Utah, Wayne Co.: Thousand Lake Mountain (3)	38.5079	-111.5505	2400 m	LDP	this study
668f	BRY-55093	-	USA, ID, Owynee Co.: McBride Creeks Badlands	43.32021	-116.9795	1291 m	SDL, HCL, JHL	this study
669f	BRY-55094	-	USA, ID, Owynee Co.: McBride Creeks Badlands	43.32021	-116.9795	1291 m	SDL, HCL, JHL	this study
670f	BRY-55095	-	USA, ID, Owynee Co.: McBride Creeks Badlands	43.32021	-116.9795	1291 m	SDL, HCL, JHL	this study
clade IVd – *R. melanophthalma* sensu lato								
541f	BRY-55096	TLM-10	USA, Utah, Wayne Co.: Thousand Lake Mountain (10)	38.44317	-111.4703	3400 m	LDP	this study
542f	BRY-55097	TLM-10	USA, Utah, Wayne Co.: Thousand Lake Mountain (10)	38.44317	-111.4703	3400 m	LDP	this study
545f	BRY-55098	TLM-8	USA, Utah, Wayne Co.: Thousand Lake Mountain (8)	38.4557	-111.4581	3175 m	LDP	this study
546f	BRY-55099	TLM-8	USA, Utah, Wayne Co.: Thousand Lake Mountain (8)	38.4557	-111.4581	3175 m	LDP	this study
547f	BRY-55100	TLM-7	USA, Utah, Wayne Co.: Thousand Lake Mountain (7)	38.4557	-111.4497	3000 m	LDP	this study

548f	BRY-55101	TLM-7	USA, Utah, Wayne Co.: Thousand Lake Mountain (7)	38.4557	-111.4497	3000 m	LDP	this study
549f	BRY-55102	TLM-6	USA, Utah, Wayne Co.: Thousand Lake Mountain (6)	38.5111	-111.4732	2875 m	LDP	this study
553f	BRY-55103	TLM-4	USA, Utah, Wayne Co.: Thousand Lake Mountain (4)	38.5079	-111.5161	2550 m	LDP	this study
555f	BRY-55104	TLM-3	USA, Utah, Wayne Co.: Thousand Lake Mountain (3)	38.5079	-111.5505	2400 m	LDP	this study
557f	BRY-55105	TLM-2	USA, Utah, Wayne Co.: Thousand Lake Mountain (2)	38.431	-111.6119	2285 m	LDP	this study
558f	BRY-55106	TLM-2	USA, Utah, Wayne Co.: Thousand Lake Mountain (2)	38.431	-111.6119	2285 m	LDP	this study
559f	BRY-55107	TLM-1	USA, Utah, Wayne Co.: Thousand Lake Mountain (1)	38.4243	-111.6446	2220 m	LDP	this study
560f	BRY-55108	TLM-1	USA, Utah, Wayne Co.: Thousand Lake Mountain (1)	38.4243	-111.6446	2220 m	LDP	this study
567f	BRY-55109	BM-2	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5785	2809 m	SDL, HCL, JHL, PAR	this study
568f	BRY-55110	BM-2	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5785	2809 m	SDL, HCL, JHL, PAR	this study
596f	BRY-55111	BM-2	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5785	2809 m	SDL, HCL, JHL, PAR	this study
597f	BRY-55112	BM-2	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5785	2809 m	SDL, HCL, JHL, PAR	this study
598f	BRY-55113	BM-2	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5785	2809 m	SDL, HCL, JHL, PAR	this study
599f	BRY-55114	BM-2	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5785	2809 m	SDL, HCL, JHL, PAR	this study
600f	BRY-55115	BM-2	USA, Wayne Co.: Boulder Mountain (BM-2)	38.17228	-111.5785	2809 m	SDL, HCL, JHL, PAR	this study
613f	BRY-55116	TLM-1	USA, Utah, Wayne Co.: Thousand Lake Mountain (1)	38.4243	-111.6446	2220 m	LDP	this study
616f	BRY-55117	TLM-1	USA, Utah, Wayne Co.: Thousand Lake Mountain (1)	38.4243	-111.6446	2220 m	LDP	this study
617f	BRY-55118	TLM-2	USA, Utah, Wayne Co.: Thousand Lake Mountain (1)	38.4243	-111.6446	2220 m	LDP	this study
618f	BRY-55119	TLM-2	USA, Utah, Wayne Co.: Thousand Lake Mountain (2)	38.431	-111.6119	2285 m	LDP	this study

619f	BRY-55120	TLM-2	USA, Utah, Wayne Co.: Thousand Lake Mountain (2)	38.431	-111.6119	2285 m	LDP	this study
620f	BRY-55121	TLM-2	USA, Utah, Wayne Co.: Thousand Lake Mountain (2)	38.431	-111.6119	2285 m	LDP	this study
621f	BRY-55122	TLM-2	USA, Utah, Wayne Co.: Thousand Lake Mountain (2)	38.431	-111.6119	2285 m	LDP	this study
622f	BRY-55123	TLM-3	USA, Utah, Wayne Co.: Thousand Lake Mountain (3)	38.5079	-111.5505	2400 m	LDP	this study
623f	BRY-55124	TLM-3	USA, Utah, Wayne Co.: Thousand Lake Mountain (3)	38.5079	-111.5505	2400 m	LDP	this study
624f	BRY-55125	TLM-3	USA, Utah, Wayne Co.: Thousand Lake Mountain (3)	38.5079	-111.5505	2400 m	LDP	this study
625f	BRY-55126	TLM-3	USA, Utah, Wayne Co.: Thousand Lake Mountain (3)	38.5079	-111.5505	2400 m	LDP	this study
627f	BRY-55127	TLM-4	USA, Utah, Wayne Co.: Thousand Lake Mountain (4)	38.5079	-111.5161	2550 m	LDP	this study
628f	BRY-55128	TLM-4	USA, Utah, Wayne Co.: Thousand Lake Mountain (4)	38.5079	-111.5161	2550 m	LDP	this study
629f	BRY-55129	TLM-4	USA, Utah, Wayne Co.: Thousand Lake Mountain (4)	38.5079	-111.5161	2550 m	LDP	this study
630f	BRY-55130	TLM-4	USA, Utah, Wayne Co.: Thousand Lake Mountain (4)	38.5079	-111.5161	2550 m	LDP	this study
631f	BRY-55131	TLM-4	USA, Utah, Wayne Co.: Thousand Lake Mountain (4)	38.5079	-111.5161	2550 m	LDP	this study
637f	BRY-55132	TLM-6	USA, Utah, Wayne Co.: Thousand Lake Mountain (6)	38.5111	-111.4732	2875 m	LDP	this study
639f	BRY-55133	TLM-6	USA, Utah, Wayne Co.: Thousand Lake Mountain (6)	38.5111	-111.4732	2875 m	LDP	this study
640f	BRY-55134	TLM-6	USA, Utah, Wayne Co.: Thousand Lake Mountain (6)	38.5111	-111.4732	2875 m	LDP	this study
641f	BRY-55135	TLM-6	USA, Utah, Wayne Co.: Thousand Lake Mountain (6)	38.5111	-111.4732	2875 m	LDP	this study
642f	BRY-55136	TLM-7	USA, Utah, Wayne Co.: Thousand Lake Mountain (7)	38.4557	-111.4497	3000 m	LDP	this study
643f	BRY-55137	TLM-7	USA, Utah, Wayne Co.: Thousand Lake Mountain (7)	38.4557	-111.4497	3000 m	LDP	this study
644f	BRY-55138	TLM-7	USA, Utah, Wayne Co.: Thousand Lake Mountain (7)	38.4557	-111.4497	3000 m	LDP	this study

645f	BRY-55139	TLM-7	USA, Utah, Wayne Co.: Thousand Lake Mountain (7)	38.4557	-111.4497	3000 m	LDP	this study
646f	BRY-55140	TLM-7	USA, Utah, Wayne Co.: Thousand Lake Mountain (7)	38.4557	-111.4497	3000 m	LDP	this study
647f	BRY-55141	TLM-8	USA, Utah, Wayne Co.: Thousand Lake Mountain (8)	38.4557	-111.4581	3175 m	LDP	this study
648f	BRY-55142	TLM-8	USA, Utah, Wayne Co.: Thousand Lake Mountain (8)	38.4557	-111.4581	3175 m	LDP	this study
650f	BRY-55143	TLM-8	USA, Utah, Wayne Co.: Thousand Lake Mountain (8)	38.4557	-111.4581	3175 m	LDP	this study
651f	BRY-55144	TLM-8	USA, Utah, Wayne Co.: Thousand Lake Mountain (8)	38.4557	-111.4581	3175 m	LDP	this study
658f	BRY-55570	TLM-10	USA, Utah, Wayne Co.: Thousand Lake Mountain (10)	38.44317	-111.4703	3400 m	LDP	this study
659f	BRY-55146	TLM-10	USA, Utah, Wayne Co.: Thousand Lake Mountain (10)	38.44317	-111.4703	3400 m	LDP	this study
661f	BRY-55147	TLM-10	USA, Utah, Wayne Co.: Thousand Lake Mountain (10)	38.44317	-111.4703	3400 m	LDP	this study
686f	BRY-55148	-	USA, Utah, Iron County	38.07714	-112.6841	1813 m	SDL, HCL, JHL, GDL	this study
713f	BRY-55149	-	USA, NV, White Pine Co.: Humboldt-Toiyabe National Forest	38.54642	-114.6385	2744 m	SLD, LLS	this study
723f	BRY-55150	-	USA, UT, Uintah Co.: Snake John Reef	40.29259	-109.1208	1631 m	SDL, LLS, GS	this study
Vagrant taxa in the *R. melanophthalma* species complex (clade IV)								
R. cerebriformis (clade Iva)								
-	AF159942 (ITS only)	-	USA, Idaho	-	-	-	-	Arup and Grub 2000
R. cylindrica - (clade IVd)								
	AF159941 (ITS only)	-	USA, Idaho	-	-	-	-	Arup and Grub 2000
R. haydenii								
	AF159937 (ITS only)	-	USA, Idaho	-	-	-	-	Arup and Grub 2000
684f	BRY-55029	-	USA, WY, Lincoln County	41.63877	-110.5699	2018 m	SDL, JHL	this study
685f	BRY-55030	-	USA, WY, Lincoln County	41.63877	-110.5699	2018 m	SDL, JHL	this study

728f	BRY-55032	-	USA, WY, Sweetwater County	42.23702	-109.1712	2112 m	SDL	this study
729f	BRY-55033	-	USA, WY, Sweetwater County	42.23702	-109.1712	2112 m	SDL	this study
715f*	BRY-55031	-	USA, MT, Deerlodge Co.: Beaverhead/Deerlodge National Forest	46.10273	-113.2326	2382 m	SDL, LLS, GS	this study
R. haydenii spp. a*rbuscula*								
092f	BRY-55027	-	USA, ID, Lemhi Co.: city of Leadore	44.68116	-113.3623	1819 m	SDL, LLS, KBK	this study
727f	BRY-55028	-	ID, Lemhi Co.: Salmon Challis National Forest	44.37694	-113.2719	2987 m	LLS, KBK	this study
R. idahoensis								
-	AF159943 (ITS only)	-	USA, Idaho	-	-	-	-	Arup and Grub 2000
093f	BRY-55034	-	USA, ID, Lemhi Co.: city of Leadore	44.68116	-113.3623	1819 m	SDL, LLS, KBK	this study
094f	BRY-55035	-	USA, ID, Lemhi Co.: city of Leadore	44.68116	-113.3623	1819 m	SDL, LLS, KBK	this study
103f	BRY-55036	-	USA, ID, Lemhi Co.: city of Leadore	44.68116	-113.3623	1819 m	SDL, LLS, KBK	this study
R. subidahoensis (clade IVa)								
-	AF159944 (ITS only)	-	USA, Idaho	-	-	-	-	Arup and Grub 2000

Supplementary data 1.2. GenBank accession numbers for all sequence include in the present study. Specimen ID, lineage and identification number (*L. no.*, *Lecanora novomexicana*; *R. ce.*, *Rhizoplaca cerebriformis*; *R. cy.*, *R. cylindrical*; *R. h.* spp. *ar.*, *R. haydeni*i ssp. *arbuscula*; *R. ha.*, *R. haydenii*; *R. id.*, *R. idahoensis*; *R. me*, *R. melanophthalma*; and *R. su*, *R. subidahoensis*), Herbarium Acc. No., location and number of deposited voucher specimen; GenBank Accession numbers.

Specimen ID	Herbarium Acc. No.	ITS	IGS	intron	Mcm7	β-tubulin
R. chrysoleuca 561f	BRY-55000	HM577233	-	HM577158	HM577385	HM576891
R. chrysoleuca 562f	BRY-55001	HM577234	HM577027	-	-	HM576892
R. chrysoleuca 565f	BRY-55002	HM577235	HM577028	-	HM577386	-
R. chrysoleuca 566f	BRY-55003	HM577236	HM577029	-	HM577387	-
R. chrysoleuca 569f	BRY-55004	HM577237	HM577030	-	-	HM576893
R. chrysoleuca 570f	BRY-55005	HM577238	-	-	-	-
R. chrysoleuca 581f	BRY-55570	HM577239	-	-	-	-
R. chrysoleuca 582f	BRY-55006	HM577240	-	-	-	-
R. chrysoleuca 583f	BRY-55007	HM577241	-	-	-	-
R. chrysoleuca 584f	BRY-55008	HM577242	-	-	-	-
R. chrysoleuca 585f	BRY-55009	HM577243	-	-	-	-
R. chrysoleuca 591f	BRY-55010	HM577244	-	-	-	-
R. chrysoleuca 592f	BRY-55011	HM577245	HM577031	-	HM577388	HM576894
R. chrysoleuca 593f	BRY-55012	HM577246	-	-	-	-
R. chrysoleuca 594f	BRY-55571	HM577247	-	-	-	-
R. chrysoleuca 595f	BRY-55013	HM577248	-	-	-	-
R. chrysoleuca 602f	BRY-55014	HM577249	-	HM577159	-	-
R. chrysoleuca 603f	BRY-55015	HM577250	-	-	-	HM576895
R. chrysoleuca 604f	BRY-55016	HM577251	-	-	-	HM576896
R. chrysoleuca 605f	BRY-55017	HM577252	HM577032	-	HM577389	HM576897
R. chrysoleuca 606f	BRY-55018	HM577253	-	-	-	-
R. chrysoleuca 676f	BRY-55019	HM577254	-	HM577160	HM577390	HM576898
R. subdiscrepans 1023f	BRY-55020	HM577232	-	HM577157	HM577384	-
R. subdiscrepans 734f	BRY-55021	HM577230	HM577026	HM577155	HM577382	HM576889
R. subdiscrepans 735f	BRY-55022	HM577231	-	HM577156	HM577383	HM576890
L. no. clade I 730f	BRY-55023	-	HM577033	-	-	HM576899
L. no. clade I 731f	BRY-55024	HM577255	HM577034	HM577161	HM577391	HM576900
L. no. clade I 732f	BRY-55025	HM577256	HM577035	HM577162	-	HM576901
L. no. clade I 733f	BRY-55026	HM577257	HM577036	HM577163	HM577392	HM576902
L. no. clade V AF159923	NA	AF159923	-	-	-	-
L. no. clade V AF159945	NA	AF159945	-	-	-	-
R. ce. clade IVa AF159942	NA	AF159942	-	-	-	-
R. cy. clade IVd AF159941	NA	AF159941	-	-	-	-
R. h. spp. *ar.* clade IV 092f	BRY-55027	HM577303	HM577077	-	HM577437	HM576948
R. h. spp. *ar.* clade IV 727f	BRY-55028	HM577304	HM577078	HM577207	HM577438	HM576949
R. ha. clade IV 684f	BRY-55029	HM577298	HM577073	HM577202	HM577432	HM576943
R. ha. clade IV 685f	BRY-55030	HM577299	HM577074	HM577203	HM577433	HM576944
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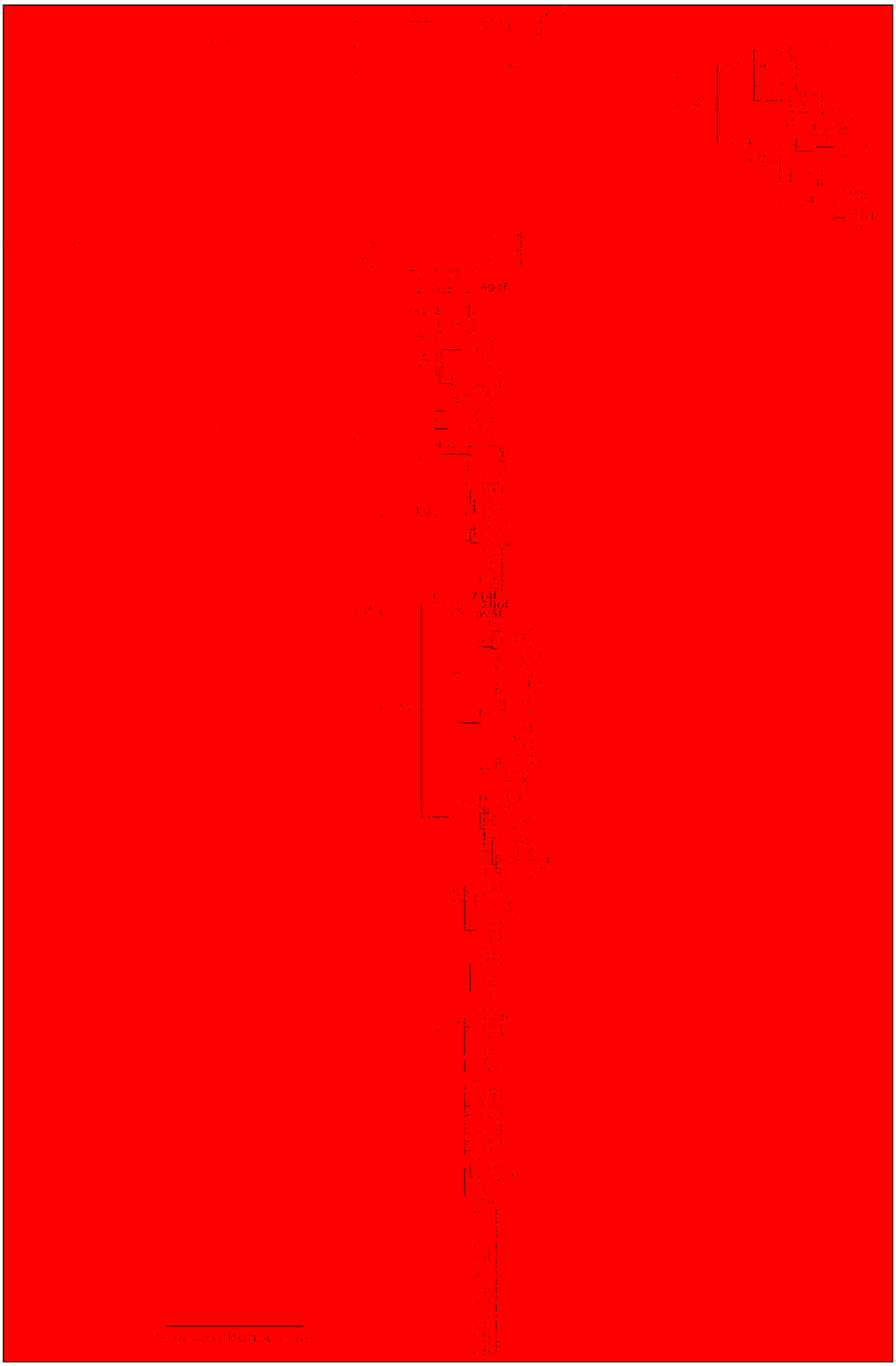
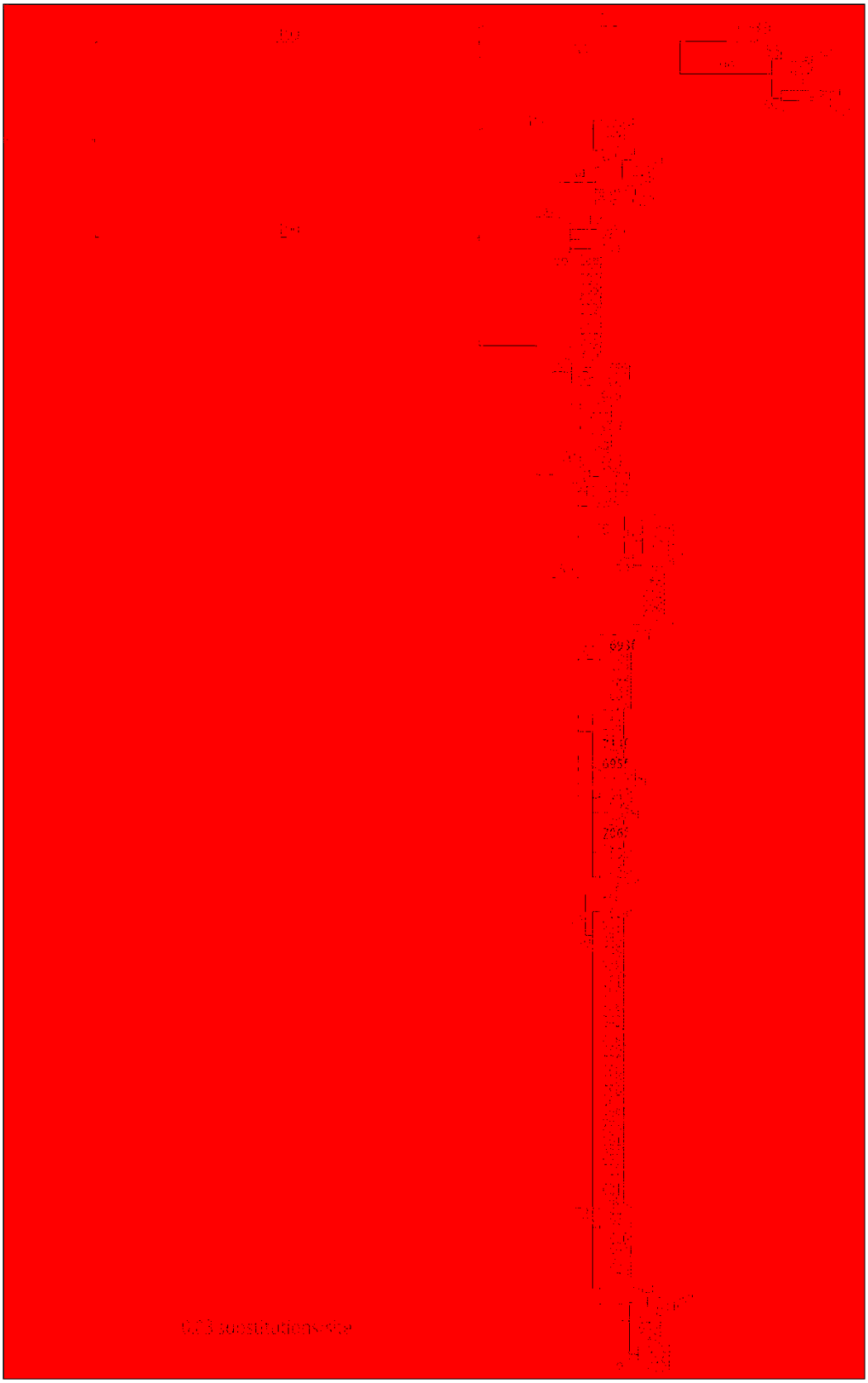
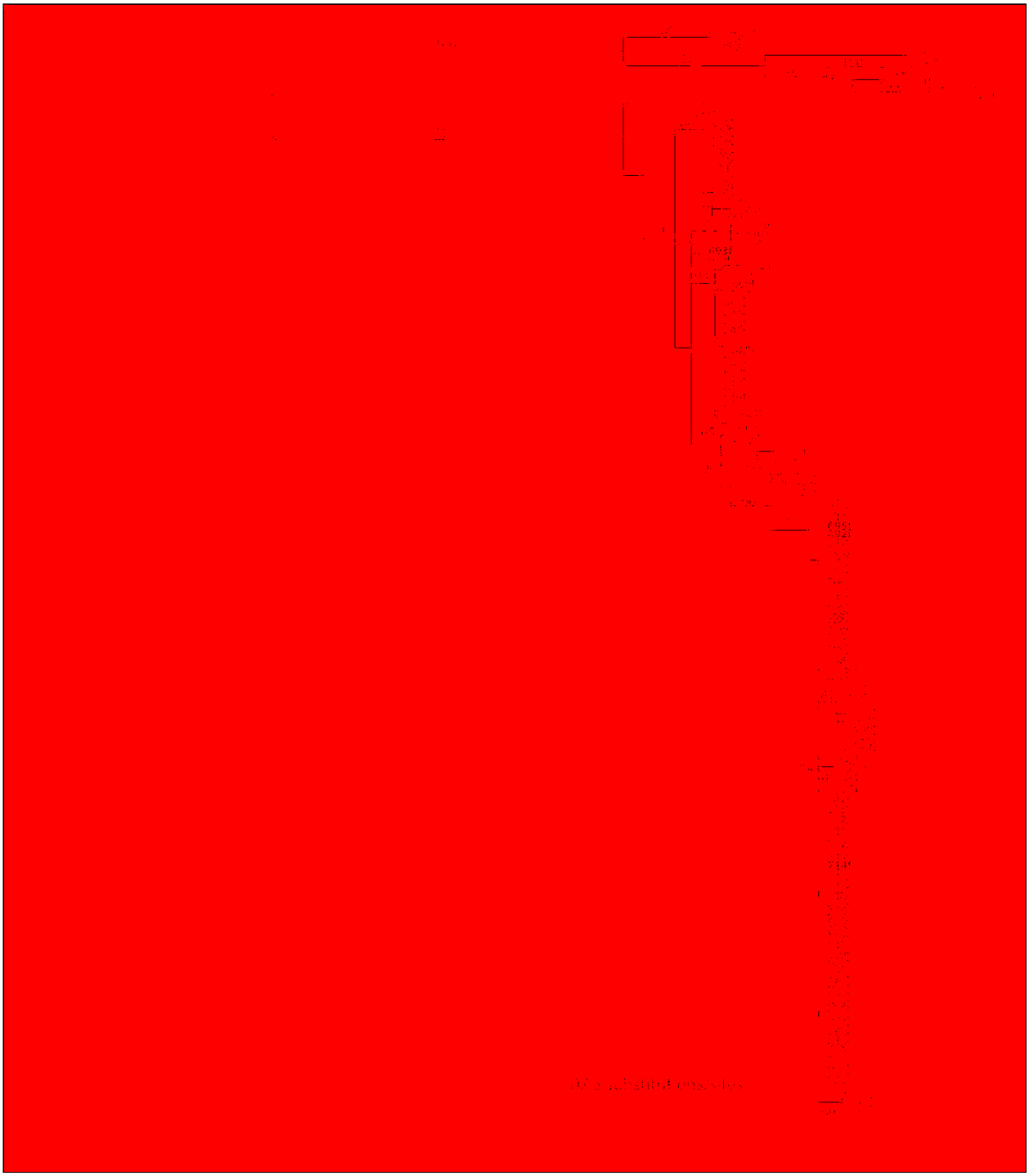
R. me. clade IVd 723f	BRY-55150	HM577381	HM577154	-	HM577515	HM577025
R. su. clade IVa AF159944	NA	AF159944	-	-	-	-



Supplementary data 1.3a (on previous page). Maximum likelihood topology of concatenated ribosomal loci (IGS, ITS, and group I intron), with bootstrap support indicated at nodes. Accessions found to be in conflict with other markers are **bolded**.



Supplementary data 1.3b (on previous page). Maximum likelihood topology of the β-tubulin fragment, with bootstrap support indicated at nodes. Accessions found to be in conflict with other markers are **bolded.**



Supplementary data 1.3c. Maximum likelihood topology of the *MCM7* fragment, with bootstrap support indicated at nodes. Accessions found to be in conflict with other markers are **bolded.**

CHAPTER TWO

New insights into phylogenetic relationships and character evolution in the species-rich lichen-forming fungal genus *Xanthoparmelia* (Parmeliaceae) in western North America

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Abstract

The lichen-forming ascomycete genus *Xanthoparmelia* includes over 800 described species displaying a considerable range of morphological and chemical variation. Traditionally, species delimitations have been based on morphological characters, medullary chemistry, and various reproductive features. However, the evolution of these characters has remained unclear, and many traditional classifications have been shown to be highly artificial. Using sequence data from four nuclear ribosomal markers, IGS, ITS, LSU and a group I intron, and fragments from two nuclear loci, β-tubulin, and *MCM7*, we reconstructed a phylogenetic hypothesis from 422 individuals representing 20 putative species to assess the evolution of taxonomically important characters. Most sampled species as currently circumscribed were recovered as polyphyletic and major diagnostic characters have evolved in a highly homoplasious manner. The vagrant growth form, distinct medullary chemistries, and production of vegetative diaspores appear to have evolved independently multiple times. These results are consistent with other studies of lichenized fungi indicating that traditional morphological and chemistry-based species delimitations fail to accurately represent fungal diversity.

Keywords: Character evolution, convergence, lichens, Parmeliaceae, secondary metabolites, speciation, vagrant lichens, *Xanthoparmelia*

Introduction

Lichens are obligate symbiotic associations consisting of a fungus (the mycobiont), a green alga and/or cyanobacterium (the photobiont), and, at least in some cases, non-photosynthetic bacteria (Cardinale, Puglia, and Grube, 2006; Cardinale et al., 2008; Hodkinson and Lutzoni, 2009; Selbmann et al., 2010). Lichen systems have been very successful from an evolutionary perspective and include approximately one-fifth of all known extant fungal species (Lutzoni, Pagel, and Reeb, 2001). The co-evolution of lichen symbionts has resulted in a wide array of morphological and metabolic adaptations unique to lichen systems, termed symbiotic phenotypes (Honegger, 2001), which promote the overall success of the symbionts (Rikkinen, 1995; Clark et al., 2001; Sanders, 2001). Traditionally anatomical, morphological and chemical characters of the complete lichen association have been employed to characterize taxonomy of the mycobiont (the taxonomy of the other symbionts, e.g. algae and cyanobacteria, has no official nomenclatural status relative to the intact lichen). However, key taxonomic characters within lichenized ascomycetes appear to have evolved independently or changed character states frequently over the course of lichen evolution (Printzen, 2009), and the value of these characters for defining taxonomic boundaries appears to be overestimated in many groups (Arup and Grube, 2000; Blanco et al., 2004a; Reeb, Lutzoni, and Roux, 2004; Buschbom and Mueller, 2006; Lumbsch et al., 2007; Reese Næsborg, Ekman, and Tibell, 2007; Nelsen et al., 2009; Schmitt et al., 2009a).

The ascomycete family Parmeliaceae represents the largest and best studied family of lichenized-fungi within the Lecanorales (Ascomycota), and includes approximately 2000 species in 90 genera (Crespo et al., 2007). In some cases, morphological and chemical characters used to define species within the Parmeliaceae are not useful taxonomic discriminators at an intrageneric

level (Louwhoff and Crisp, 2000; Velmala et al., 2009), and cryptic phylogenetic lineages have been identified within several species defined by morphological characters (Kroken and Taylor, 2001; Crespo et al., 2002; Blanco et al., 2004b; Molina et al., 2004; Argüello et al., 2007; Wirtz, Printzen, and Lumbsch, 2008). On the other hand, both chemistry and morphology based taxonomic boundaries may appropriately represent species diversity in some groups within the Parmeliaceae (Tehler and Källersjö, 2001; McCune and Schoch, 2009; Truong, Naciri, and Clerc, 2009). However, the utility of traditional characters used to define species within most genera in the Parmeliaceae has not been evaluated in a molecular context.

Within the Parmeliaceae, *Xanthoparmelia* (Vainio) Hale is the largest genus, including more than 800 species characterized by the presence of usnic or iosusnic acid and the polysaccharide *Xanthoparmelia*-type lichenan in the hyphal cell walls (Elix, 1993; Blanco et al., 2004a; Crespo et al., 2007). The use of molecular data has revised the generic circumspection of *Xanthoparmelia* and suggests chemical and morphological characters previously used to define taxonomic groups within the genus have been overemphasized (Crespo, Blanco, and Hawksworth, 2001; Blanco et al., 2004a; Blanco et al., 2006; Thell et al., 2006; Arup et al., 2007; Crespo et al., 2007; Del Prado et al., 2007; Gutiérrez et al., 2007; Hodkinson and Lendemer, in press). Congeners in *Xanthoparmelia* display great morphological and chemical diversity, which traditionally have been used to differentiate species. The current classification has been problematic and many of the current groupings are disputed (Esslinger, 1977, 1978; Elix, 1986; Hawksworth and Crespo, 2002; Blanco et al., 2004a; Ahti and Hawksworth, 2005; Crespo et al., 2007; Thell, Elix, and Søchting, 2009). Contrasting reproductive modes have also been important characters for diagnosing species within *Xanthoparmelia* (Hale, 1990). Sexual reproduction occurs through the production of ascospores produced through meiosis in sexual

fruiting bodies (the apothecia), and these are dispersed independent of the photobiont partner and require de novo acquisition of the appropriate photobiont partner. In contrast, specialized vegetative reproductive propagules (the isidia or soredia) contain both symbionts, eliminating the requirement of acquiring the appropriate photobiont partner de novo.

In spite of the recognized importance of molecular data for effectively investigating deeper phylogenetic relationships in the Parmeliaceae, relatively little has been done to investigate α-level patterns of morphological and chemical diversity within and between *Xanthoparmelia* species in a framework incorporating molecular data (Thell, Elix, and Søchting, 2009; Hodkinson and Lendemer, in press; Leavitt, Johnson, and St. Clair, submitted). Recent studies of some *Xanthoparmelia* species suggest that several distinct lineages may be hidden within nominal species defined on chemical and morphological grounds (Del-Prado et al., 2010).

Xanthoparmelia contains the greatest number of vagrant species with the greatest geographic distributions (Rosentreter, 1993). Vagrant forms of lichenized fungi represent an interesting phenomenon seen in diverse lichen clades, including *Aspicilia* (Megasporaceae), *Masonhalea* (Parmeliaceae), *Rhizoplaca* (Lecanoraceae), and *Xanthoparmelia*. The term "vagrant" is used for obligatory unattached taxa that grow, persist, and reproduce without attachment to a substrate (Rosentreter, 1993). These are generally conspicuous lichens found growing unattached on soils in many deserts, steppes, and high plain areas of North America, Eastern Europe, Russia, Mongolia, Australia, and South Africa. The occurrence of vagrant lichens in multiple lineages leads to questions concerning the evolutionary advantages and ecological factors that have given rise to vagrancy.

A high degree of morphological variation in most vagrant forms of *Xanthoparmelia* has resulted in species boundaries often based on variation in the expression of signature secondary

metabolites (Hale, 1990; Rosentreter, 1993). Unspecialized vegetative fragments are generally the only method of reproduction for vagrant *Xanthoparmelia* species, limiting dispersal and genetic exchange between populations (Bailey, 1976; Rosentreter, 1993), although it has been proposed that some long distance dispersal may be mediated by migrating pronghorn antelope and other ungulates which transport unspecialized thallus fragments (Thomas and Rosentreter, 1992; Rosentreter, 1993; St. Clair et al., 2007). Although sexual reproductive structures (apothecia) are extremely rare in vagrant *Xanthoparmelia* species, they have occasionally been found on *X. chlorochroa* and *X. camtschadalis* (Hale, 1990), and methods of dispersal and the role of sexual reproduction in vagrant growth-forms have not been explicitly tested.

The lichen genus *Xanthoparmelia* provides a model system for assessing problems caused by homoplasy of morphological and chemical characters in lichenized fungi (Del-Prado et al., 2010). Furthermore, morphologically and chemically diverse vagrant *Xanthoparmelia* taxa in North America offer an excellent opportunity to evaluate patterns of vagrancy, identify divergent vagrant lineages, and assess the evolution of taxonomically important secondary metabolites and reproductive modes within a comprehensive molecular phylogenetic context. Blanco et al. (2004b) recovered some taxa included in the present study within a single well-supported monophyletic lineages, sister to *X. brachinaensis,* and other recent studies suggest that most North American taxa belong to this lineage (Thell, Elix, and Søchting, 2009; Hodkinson and Lendemer, in press). The objectives of this research are to: 1) estimate a robust phylogenetic hypothesis concerning the relationship of vagrant growth-forms to attached saxicolous forms of *Xanthoparmelia* in North America; 2) identify divergent lineages of vagrant forms within their North American distribution; and 3) assess the evolution of morphological, chemical, and reproductive characters, with an emphasis on those important for the effective and consistent

treatment of this group. To this end, we obtained samples of *Xanthoparmelia* specimens representing morphologically and chemically diverse taxa, including all described North American vagrant *Xanthoparmelia* species, throughout their known distributions in western North America, and accessions of other divergent *Xanthoparmelia* lineages to assess monophyly of the focal group. We used sequence data from 4 nuclear ribosomal markers (ITS, IGS, LSU, group I intron) and two low-copy nuclear protein-coding fragments (β-tubulin and *MCM7*) to recover a well-supported phylogenetic hypothesis for this group.

Materials and Methods

Taxon sampling—Over 4000 *Xanthoparmelia* specimens were collected between 2005 and 2009 from locations throughout western North America for initial analyses of morphological and chemical variation. Sampling emphasized: 1) described vagrant *Xanthoparmelia* taxa, 2) the known distribution of *X. chlorochroa* sensu lato (s. l.), 3) any co-occuring saxicolous attached species of *Xanthoparmelia*; and 4) included all specimens presented in Leavitt et al. (submitted-b). Additionally, limited sampling was included to assess relationships within a broader taxonomic and phylogeographic context to confirm the monophyly of the focal group. Specimens were selected to represent the ecological range of these taxa, with effective sampling across the morphological and biochemical variation of the collection, including both vagrant and saxicolous attached species. Material from the Herbarium of Nonvascular Cryptogams, Brigham Young University (BRY), Snake River Plains Herbarium, Boise State University (SRP), Oregon State University Herbarium (OSC), University of Nebraska at Omaha Herbarium (OMA), and Theodore Esslinger's personal collection (North Dakota) was included to improve taxonomic sampling and represent unsampled localities. Although *Xanthoparmelia* has been relatively well

studied from a generic perspective, uncertainty in the outgroup relationships between species within the genus is potentially problematic in determining basal relationships within the ingroup. Major lineages identified in Blanco et al. (2004) were represented by ITS and LSU sequence data from18 individuals to identify the phylogenetic position within the genus and assess monophyly, and *Karoowia saxeti* was selected as the outgroup (Blanco et al., 2004a; Crespo et al., 2007). The geographical distribution of a total 414 specimens representing 20 species (focal group) is shown in Figure 1. Collection information for all material used in this study is summarized in Supplementary Data S1, and all new voucher material generated from this study is maintained at the Brigham Young University Herbarium of Nonvascular Cryptogams, Provo, Utah, U.S.A.

Morphology and chemistry—We evaluated all taxonomically important characters, with emphasis on the vagrant growth-form, the production of distinct secondary metabolites, and reproductive mode. Secondary metabolite data were generated for all vouchers using thin layer chromatography (TLC). Lichen compounds were extracted in acetone using 0.02 grams of thallus material; the acetone wash was subsequently used for chromatography in solvents C and G following the methods of Orange, James, and White (2001). Taxonomic assignments were based on morphological and chemical data following Hale (1990) and Nash and Elix (2004). However, confusion surrounding the *diagnosability* and significance of most vegetative morphological characters has been documented (Blanco et al., 2004a; Thell, Elix, and Søchting, 2009; Del-Prado et al., 2010; Leavitt, 2010; Hodkinson and Lendemer, in press), and we chose to represent all taxonomic assignments sensu lato. Some of the morphological variation typical of sampled taxa is shown in Figure 2.

DNA isolation, PCR and sequencing—Total genomic DNA was extracted using either the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) according to manufacturer's instructions, or

the Prepease DNA Isolation Kit (USB, Cleveland, OH), following the plant leaf extraction protocol. Fungal specific primers were used to amplify six fungal nuclear markers, including four nuclear ribosomal loci: the entire internal transcribed spacer (ITS: ITS1, 5.8S, ITS2), a fragment of the intergenic spacer (IGS), a fragment of the large subunit (LSU), and a group I intron located in the small subunit (Gutiérrez et al., 2007); and fragments from two low-copy protein coding loci, *β*-tubulin and *MCM7*. The nuRNA gene tandem repeat exists in large copy numbers (100-200 copies) facilitating the amplification of the selected markers from herbarium specimens. Although low levels of intragenomic variation in fungal rDNA repeats suggests convergent evolution in which homogenization effectively maintains highly similar repeat arrays (Ganley and Kobayashi, 2007), previous studies have confirmed the utility of the sampled ribosomal loci for species and population-level studies in lichenized ascomycetes (Thell, 1999; Kroken and Taylor, 2001; Blanco et al., 2004a; Blanco O and et al., 2004; Buschbom and Mueller, 2006; Lindblom and Ekman, 2006; Brunauer et al., 2007; Gutiérrez et al., 2007; Wirtz, Printzen, and Lumbsch, 2008; O'Brien, Miadlikowska, and Lutzoni, 2009; Wedin et al., 2009). Although a duplication of the β-tubulin gene has occurred within Ascomycota, the paralogs are easily distinguishable within the analyzed group and the marker has been successfully employed to investigate α-level relationships in other lichenized ascoymycetes (Buschbom and Mueller, 2006; O'Brien, Miadlikowska, and Lutzoni, 2009; Wedin et al., 2009).

Standard polymerase chain reactions (PCR) were used to amplify targeted loci. Fungal-specific primers used in PCR amplifications and in the cycle sequencing reactions are shown in Table 1. PCR cycling parameters used for amplifying the ITS, group I intron, LSU, and β-tubulin loci followed the methods of Blanco et al (2004); cycling parameters for amplifying the IGS followed the 66-56° touchdown reaction described in Lindblom and Ekman (2006); and

PCR cycling parameters for amplifying the *MCM7* fragment followed Schmitt et al. (2009b). PCR products were quantified on 1% agrose gel and stained with ethidium bromide. In cases where no PCR product was visualized for the β-tubulin, *MCM7*, and IGS fragments, internally nested PCR reactions were performed using 0.3 ul of the PCR product from the original reaction with recently developed internal primers 'BT-RhizoF' and 'BT-RhizoR'(Leavitt et al., submitted), for the β-tubulin fragment, 'X*MCM7*f' and 'X *MCM7*r' (Leavitt, 2010),for the *MCM7* fragment, and IGS rDNA: IGS12a-5' (Carbone and Kohn, 1999) and 'XIGSr' (Leavitt, 2010), for the IGS fragment, using the same touchdown PCR cycling parameters described above used to amplify the IGS marker. PCR fragments were cleaned using the PrepEase PCR Purification Kit, following the manufacturer's protocol (USB, Cleveland, OH), and complementary strands were sequenced using the same primers used for amplification. Sequencing reactions were performed using the Big Dye3 Termination Sequencing Kit (Applied Biosystems, Foster City, CA) at 1/8 the standard reaction volume. Products were run on an AB 3730xl automated sequencer at the DNA Sequencing Center at Brigham Young University, Provo, Utah, USA.

Sequence alignment—Sequences were assembled and edited using Sequencher version 4.2 (Gene Codes Corporation, Ann Arbor, MI) and Se-Al v2.0a11 (Rambault, 1996), and sequence identity was checked using the 'megablast' search option in GenBank (Wheeler et al., 2006). All sequences were aligned using defaults settings in Muscle version 3.7 because of the improved speed and alignment accuracy compared with currently available programs (Edgar, 2004; Edgar and Botzoglou, 2006), and minor manual adjustments were made to maximize sequence similarity at a single position in the IGS alignment.

Individual gene tree reconstruction—Preliminary phylogenetic reconstructions were performed independently for each sampled marker, and individual gene trees from all loci recovered generally weak phylogenetic signal. We preferred to concatenate all markers for phylogenetic reconstructions to improve topology and increase nodal support (Wiens, 1998). Although potential pitfalls of concatenating independent nuclear genes in phylogenetic analyses exist (Degnan and Rosenberg, 2009; Edwards, 2009), coalescent-based methods using multi-locus data to simultaneously indentify independently evolving lineages and infer relationships among these are limited (O'Meara, 2010), and coalescent-based phylogenetic methods are still very sensitive to deviations from assumptions, especially post-divergence introgression (Leache, 2009; Liu et al., 2009). Given that the ribosomal genome behaves as a single linked region the four ribosomal markers (ITS, IGS, LSU, and group I intron) were concatenated a priori; but before combining the ribosomal and protein-coding datasets we assessed heterogeneity in the phylogenetic signal between sampled markers (Lutzoni et al., 2004). Maximum likelihood (ML) analyses were performed for the concatenated ribosomal dataset, β-tubulin, and *MCM7* markers separately using the program RAxML 7.0.4 (Stamatakis, 2006; Stamatakis, Hoover, and Rougemont, 2008), and robustness of the gene trees were assessed using 1000 "fastbootstrap" replicates to evaluate support for each node as implemented in the CIPRES Web Portal. Although RAxML allows analyses of partitioned data, we chose to treat the entire fragment under a single model of evolution because exploratory analyses did not improve topologies or nodal support under more complex partitions (i.e. codon positions in protein-coding fragments). We implemented the GTRGAMMA model, which includes a parameter (Γ) for rate heterogeneity among sites, but chose not to include a parameter for estimating the proportion of invariable sites because Γ accounts for this source of rate heterogeneity by using 25 rate

categories (Stamatakis, 2006). Support values for the ribosomal, β-tubulin, and *MCM7* phylogenies were examined for well-supported (≥70%) conflicts between data sets (Lutzoni et al., 2004).

Tree reconstruction—Because of the large size of the combined dataset (432 individuals and ~ 3600 bp) we implemented RAxML to analyze the data due to a combination of speed, accuracy, and scalability across numerous processors (Stamatakis, 2006; Stamatakis, Hoover, and Rougemont, 2008; Arnold et al., 2009). We conducted a ML analysis of the combined dataset using locus-specific model partitions (Stamatakis, 2006; Stamatakis, Hoover, and Rougemont, 2008). Each ribosomal marker was treated as a separate partition, and for protein-coding gene fragments we compared two partition strategies. First, we treated the entire marker as a single partition. Second, we used a 3-partition approach using the first, second and third codon positions as separate model partitions for the *MCM7* marker, and a 4-partition strategy for the β-tubulin marker using the first, second and third codon positions and an 55 base pair (bp) non-coding intron located within the fragment as separate model partitions, assuming that partitions within genes had the same overall model as the entire gene, as simulations have shown that there may be frequent errors in supporting complex models from a sample of limited characters (Posada and Crandall, 2001). We used the GTRGAMMA model, which includes a parameter (Γ) for rate heterogeneity among sites. Following the recommendations of Stamatakis (2008) we did not include a parameter for the proportion of invariable sites. A search combining 200 separate ML searches (to find the optimal tree) and 1000 "fastbootstrap" replicates to evaluate support for each node was conducted on the complete dataset. Bootstrap values ≥ 70 % were assumed to indicate strong support (Felsenstein, 2004).

We also estimated phylogenetic relationships using mixed-model Bayesian inference (BI) as implemented in Mr.Bayes ver. 3.1.2 (Huelsenbeck and Ronquist, 2001). We used MrModeltest ver. 2.3 (Nylander et al., 2004) to identify the appropriate model of evolution for each marker using the Akaike Information Criterion (AIC) see (Posada and Buckley, 2004). We compared the two partition strategies described for the ML analyses (section 2.3.3). Four independent replicate searches were executed with eight Metropolis-coupled Markov chains (MCMC) for both partition strategies; each run started with randomly generated trees and involved sampling every 1000 generations for 20,000,000 generations. To evaluate stationarity and convergence between runs we evaluated log-likelihood scores and effective sample size statistics (ESS) using TRACER ver. 1.5 (Drummond et al., 2003), and assessed the average standard deviation in split frequencies. Under both partition strategies independent runs failed to converge, and we initiated four additional independent replicate searches, starting each with a randomly selected tree taken from the post-burnin sample of the previous run with the highest mean likelihood score, identical to those described above for both partition strategies. Post-burnin trees generated from runs executed from starting topologies from the original analyses were summarized with a 50% majority-rule consensus tree based (Huelsenbeck et al., 2001; Huelsenbeck and Rannala, 2004). Bayesian posterior probabilities (PP) were assessed at all nodes and clades with PP values ≥ 0.95 were considered strongly supported (Huelsenbeck and Rannala, 2004).

Topologies from the full dataset were compared to those from a reduced ML analysis consisting of 54 accessions, containing 5-8 divergent representatives for each recovered lineage, to assess the exploration of parameter space. The reduced dataset generally recovered the same lineages, but some relationships were ambiguous or lacked strong nodal support, suggesting the

robust taxon sampling is important for resolving relationships (Zwickl and Hillis, 2002), and analyses of the full dataset adequately explores parameter space.

Clade-specific analyses—Because of the large size of the complete data set and given the problem with convergence, we chose to assess relationships within monophyletic lineages identified in the ML analyses described in 2.3.3 individually to facilitate computation of parameters during ML and Bayesian analyses and incorporate tree reconstruction under maximum parsimony (MP) criterion. A total of six clades were identified in the ML topology for independent phylogenetic reconstructions (see below), and all individuals assigned membership to each given clade were realigned with a single outgroup taxon, *X. mougeotii* 907f, in Muscle version 3.7 using the identical parameters described in 2.4.1. Maximum likelihood and BI analyses were conducted for each individually defined clade as described in 2.4.3 under the less complex partitioning strategy. However, independent Bayesian analyses were sampled every 1000 generations for 10,000,000 generations, and independent runs converged from random starting trees. MP heuristic searches were performed in PAUP* v4.0b10 (Swofford, 2002) with tree bisection-reconnection (TBR) branch swapping and 1000 random-addition sequence replicates. All characters were equally weighted, and gaps were treated as missing data. Branch support was evaluated via fast bootstrapping with 10,000 replicates.

Ancestral character state reconstruction—The program Mesquite version 2.72 (Maddison and Maddison, 2007) was implement to reconstruct ancestral character states. Both ML and MP character states reconstruction methods were used with the complete ML phylogeny. Maximum likelihood optimization used the Markov k-state one-parameter model (Lewis, 2001). In parsimony calculations, character states were treated as unordered. Characters considered were growth-form (coded as 0 = saxicolous attached and 1 = vagrant), expressed

major secondary metabolites (coded as 0 = salazinic acid complex, 1 = stictic acid, 2 = norstictic acid, and 3 = psoromic acid), and production of vegetative reproductive structures (isidia) (coded as 0 = not observed and 1 = present).

Results

Sequence statistics—The resulting molecular dataset representing 432 operational taxonomic units (OTU) was comprised of 2,262 new sequences from a total of six loci consisting of 3583 aligned nucleotide positions. Table 2 summarizes patterns of variation in sampled loci and the resulting best-fit model of evolution selected using the AIC. All ribosomal markers showed length heterogeneity (IGS, 372-381 bp; ITS, 352-541; LSU, 781-842; and group I intron, 293-383), although in some cases trimmed ambiguous nucleotide positions at the 5' or 3' end of ribosomal markers exaggerated length heterogeneity. All representative haplotypes from the six gene fragments were submitted to GenBank under accession numbers HM577516-HM579777 (Supplementary data S2).

Phylogenetic analyses—Individual gene trees generally showed weak genetic structure (Supplementary data S3), and phylogenetic reconstructions of single genes were insufficient to resolve topological relationships with strong support. No incongruence was identified between datasets using method identifying conflict with ≥ 70 ML boostrap values (section 2.4.2), and all loci were combined for subsequent phylogenetic analyses. A comparison of partitioning strategies for the combined dataset indicated that the more complex strategy of the protein-coding fragments generally did not improve nodal support across the topology. Therefore, we opted to present results from the less complex partitioning strategy in order to minimize potential effects of over-parameterization on topological reconstruction and nodal support values (Sullivan

and Joyce, 2005). Partitioned ML analysis of the combined ribosomal and protein-coding genes yielded a single best-score tree (-ln = 24,596.17) presented in a simplified form shown in Figure 3. An expanded version of the same tree is presented in Supplementary data 4. The Bayesian analysis executed from starting topologies yielded a consensus tree with a negative harmonic mean likelihood = 26,024.594, which was summed from four convergent runs. Likelihood scores, ESS statistics, and standard deviation of split frequencies showed independent runs converged within the first 50% of sampled generations, leaving a posterior distribution estimated from 10,000 trees per run (40,000 total post-burn-in sampled trees). Both analyses produced essentially the same topology and no conflict between well-supported clades was identified. Nodal support values for major clades are presented in Figure 3 (support values at all nodes are presented in the expanded tree presented in Supplementary data 4). Focal group taxa from western North America formed a well-supported monophyletic lineage, with high ML bootstrap (BS) and Bayesian posterior probabilities (PP) (BS = 94 and PP =1.00). The focal group's relationship to major *Xanthoparmelia* lineages is presented in Figure 4. *X. brachinaensis* was recovered with high support (BS = 84; PP = 0.96) as sister to all focal group samples.

Our results do not support the monophyly of sampled vagrant and saxicolous attached species as defined by traditional taxonomic characters. Six major clades were identified within the focal group: *X*-I, *X*-II, *X*-III, *X*-IV, *X*-V, and *X*-VI (Fig. 3), although relationships between some strongly supported clades lack support. Table 3 summarizes patterns of variation in the concatenated dataset (IGS, ITS, LSU, group I intron, β-tubulin, and *MCM7*) across the six recovered major clades. All individuals assigned to clade *X*-VI were identified in previous work and are treated comprehensively in Leavitt, Johnson, and St. Clair (submitted). Two minor well-supported groups were recovered as sister to clades *X*-III, *X*-IV, *X*-V, and *X*-VI (BS $\leq$ 50; PP =

0.63), and were not included in the reduced clade-specific analyses. One minor clade (clade A, Fig. 3) represents *X. idahoensis* s. l. (318f and 319f) collected from the type locality in Lemhi County, Idaho, U.S.A., and the other clade (clade B, Fig. 3) contains two vagrant individuals representing *X. camtschadalis* s. l. (205f and 206f) collected from a single location in Saskatchewan, Canada.

Clade *X*-I was recovered as a monophyletic clade with strong nodal support (BS = 75 and PP = 0.98) in both ML and BI analyses estimated from the complete dataset. Partitioned ML analysis of the combined clade *X*-1 dataset yielded a single best-scoring tree (-ln = 5,430.461) shown in Figure 5A. The Bayesian analyses yielded a consensus tree with a negative harmonic mean of likelihood = 5,520.389, summed from four convergent runs, and simultaneous runs were met with an average standard deviation of split frequencies of 0.006678. All parameters converged within the first 25% of sampled generations, leaving a posterior distribution estimated from 7,500 trees per run (30,000 total post-burn-in sampled trees). The combined MP analysis resulted in the 30 most parsimonious trees (L = 201) with a consistency index (CI) of 0.90 and a retention index (RI) of 0.95. The overall topologies recovered from ML, BI, and MP analyses were identical at all well-supported nodes and generally similar across the topology. OTUs representing vagrant *X. camtschadalis* s. l. and *X. idahoensis* s. l., and attached saxicolous *X. stenophylla* s. l. were recovered within clade *X*-I. Morphologically, all vagrant individuals (*X. camtschadalsis* s. l.) with membership in this clade were characterized by a strongly white maculate upper cortex, light-colored spots on the upper surface caused by differences in thickness of the cortex or clumping of algae beneath the cortex; while the upper cortex of the saxicolous attached samples (*X. stenophylla* s. l.) were emaculate to weakly maculate. All individuals recovered in this lineage expressed the salazinic acid complex. Multiple well-

supported lineages representing *X. camtschadalis* s. l. and two well-supported lineages representing *X. stenophylla s. l.* were recovered. Although saxicolous *X. stenophylla* s. l. were recovered as monophyletic in the ML analysis (BS ≤ 50), both Bayesian and MP analyses recovered a well-supported clade (ML BS = 100; PP = 1.0; and MP BS = 99) containing *X. stenophylla* 934f, 940f, and 957f as sister to all *X. camtschadalis* s. l. specimens (excluding 334f and 335f) with weak nodal support (PP ≤ 0.50 and MP BS ≤ 50). *X. camtschadalis* s. l. was not recovered as monophyletic.

Clade *X*-II was recovered as a monophyletic lineage with strong nodal support in both ML and BI analyses estimated from the complete dataset (ML BS = 87 and PP = 1.00). Partitioned ML analysis of the clade *X*-II dataset yielded a single best-scoring tree (-ln = 6,717.653) presented in Figure 5B. The Bayesian analyses yielded a consensus tree with a negative harmonic mean of likelihood = 6,801.186, which was summed from four convergent runs. All parameters converged within the first 25% of sampled generations, leaving a posterior distribution estimated from 7,500 trees per run (30,000 total post-burn-in sampled trees), and simultaneous runs were met with an average standard deviation of split frequencies of 0.004095. The combined MP analysis resulted in 2 most parsimonious trees (L = 319) with CI = 0.87 and RI = 0.87. The overall topologies recovered from ML, BI, and MP analyses were identical at all well-supported nodes and nearly identical across the topology. OTUs representing *X. camtschadalis* s. l., *X. dierythra* s. l., *X. idahoensis* s. l., *X. mexicana* s. l., and *X. plittii* s. l. were recovered within clade *X*-II. Generally, individuals assigned membership in clade *X*-II were morphologically characterized by weakly to strongly maculate upper surfaces; both vagrant and saxicolous attached taxa; norstic, salazinic, and stictic acid complexes; and two distinct reproductive modes (unspecialized vegetative fragments or production of isidia) were recovered

within this clade. The vagrant taxa (*X. camtschadalis* s. l. and *X. idahoensis* s. l.) were all characterized by a strongly maculate upper cortex, while the isidiate saxicolous taxa (*X. dierythra* s. l., *X. mexicana* s. l., and *X. plittii* s. l.) were characterized by an emaculate to weakly maculate upper cortex. Although some topological relationships were recovered with strong nodal support, relationships between most well-supported lineages generally lacked support.

Clade *X*-III was also recovered as a monophyletic lineage with strong nodal support in both ML and BI analyses estimated from the complete dataset (BS = 99 and PP = 1.00). Partitioned ML analysis of the combined clade *X*-III dataset yielded a single best-scoring tree (-ln = 7,371.576) presented in Figure 5C. The Bayesian analyses yielded a consensus tree with a negative harmonic mean of likelihood = 7,444.990, which was summed from four convergent runs. Likelihood scores, ESS statistics, and standard deviations of split frequencies indicated that independent runs converged within the first 25% of sampled generations, leaving a posterior distribution estimated from 7,500 trees per run (30,000 total post-burn-in sampled trees). The combined MP analysis resulted in the 52 most parsimonious trees (L = 410) with CI = 0.72 and RI = 0.68. The overall topologies recovered from ML, BI, and MP analyses provided a generally unresolved view of relationships within this clade, although relationships for all well-supported nodes were identical across all methods. Both salazinic and stictic acid complexes were recovered within this group as polyphyletic. OTUs representing *X. chlorochroa* s. l., *X. dierythra* s. l., *X. lineola* s. l., *X. mexicana* s. l., *X. plittii* s. l., and *X. subplittii* s. l. were recovered within clade *X*-III. Saxicolous attached specimens with an emaculate to weakly maculate upper surface and the production of isidia generally characterized individuals assigned membership in clade *X*-III. However, four individuals (070f, 170f, 285f, and 509f) lacked isidia and produced sexual reproductive structures (apothecia); reproductive structures (apothecia or isidia) were not

observed in three individuals (442f, 580f, and 786f); and a single vagrant individual (*X. chlorochroa* s. l., 157f) was also assigned membership in this clade.

Clade *X*-IV was recovered as a monophyletic lineage with strong nodal support in both ML and BI analyses estimated from the complete dataset (BS = 88 and Pp = 1.00). Partitioned ML analysis of the clade *X*-IV dataset yielded a single best-scoring tree (-ln = 10,950.703) shown in Figure 6. The Bayesian analyses yielded a consensus tree with a negative harmonic mean of likelihood = 11,255.0624, which was summed from three convergent runs. A single run failed to converge and was not included. Likelihood scores, ESS statistics, and standard deviation of split frequencies indicated that independent runs converged within the first 25% of sampled generations, leaving a posterior distribution estimated from 7,500 trees per run (22,500 total post-burn-in sampled trees). The combined MP analysis resulted in 53,918 most parsimonious trees (L = 929) with CI = 0.52 and RI = 0.82. The overall topologies recovered from ML, BI, and MP analyses were identical at all well-supported nodes and generally similar across the topology. Clade *X*-IV is a large and diverse group represented by *X. angustiphylla* s. l., *X. chlorochroa* s. l., *X. dierythra* s. l., *X. lineola* s. l., *X. mexicana* s. l., *X. neochlorochroa* s. l., *X. norchlorochroa s. l.*, *X. plittii* s. l., *X. psoromifera* s. l., *X. subplittii* s. l., and *X. wyomingica* s.l. Individuals assigned membership in clade *X*-IV were morphologically and chemically diverse, but characterized by specimens with an emaculate to weakly maculate upper surface.

Clade *X*-V was recovered as a monophyletic lineage with moderate nodal support in both ML and BI analyses estimated from the combined ribosomal and protein-coding loci dataset (BS = 57 and Pp = 1.0). Partitioned ML analysis of the combined dataset yielded a single best-scoring tree (-ln=7,512.385) presented in Figure 5D. The Bayesian analyses yielded a consensus tree with a negative harmonic mean of likelihood = 7,627.553, which was summed from four

convergent runs. All parameters converged within the first 25% of sampled generations, leaving a posterior distribution estimated from 7,500 trees per run (30,000 total post-burn-in sampled trees). Simultaneous runs were met with an average standard deviation of split frequencies of 0.005652. The combined MP analysis resulted in 5,062 most parsimonious trees (L = 350) with CI = 0.79 and RI = 0.84. The overall topologies recovered from ML, BI, and MP analyses were identical at all well-supported nodes and nearly identical across the topology. Clade *X*-Va was recovered without support as a monophyletic lineage and with morphologically and chemically similar specimens representing *X. coloradoënsis* s. l. and *X. lineola* s. l. However, clade *X*-Vb was recovered with strong nodal support in both ML and BI analyses. Two specimens representing *X. coloradoënsis* were recovered with high support (ML BS = 74; PP = 0.99; and MP BS ≤ 50) as sister to a well-supported (ML BS = 97; PP = 1.0; Mp BS = 85) monophyletic lineage represented exclusively by *X. chlorochroa* s. l.

Ancestral state reconstruction—Parsimony-based ancestral state reconstruction results for major chemotypes are shown in Figure 7. Both parsimony and maximum likelihood ancestral character state reconstructions are similar and suggest multiple independent origins of vagrancy, major secondary metabolite complexes, and reproductive patterns.

Discussion

Species delimitations in the morphologically, bio-chemically, and reproductively diverse lichen genus *Xanthoparmelia* in western North America are notoriously challenging. Molecular data from the present study strongly suggest that the current classification system does not reflect natural lineages. Phylogenetic relationships estimated from the analysis of four nuclear ribosomal markers and two low-copy protein-coding fragments reveal a generally well-supported

hypothesis of relationships between *Xanthoparmelia* lineages in western North America (Fig. 3). However, relationships inferred from individual gene topologies generally lacked support or remained unresolved. The lack of a clear phylogenetic signal in individual datasets suggests a recent divergence of sampled lineages (incomplete lineage sorting) or historic or rare ongoing gene flow. Only with concatenation of six loci were we able to provide a robust hypothesis of relationships within the focal group. Repeated evolution of similar morphological and chemical traits and modes of reproduction in *Xanthoparmelia* inhabiting similar environments provides evidence of adaptation, suggesting that environmentally induced selection pressures may generate parallel patterns of diversification within the genus (Endler, 1986; Schluter, 2000). The results presented here, within a molecular phylogenetic framework, provide the most detailed evaluation to date of character evolution and α-level relationships in one of the largest genera of lichenized fungi.

Evolution of the vagrant form—Evolutionary relationships between saxicolous attached and vagrant growth-forms in lichenized ascomycetes have long been disputed. It has been proposed that vagrant forms represent self-perpetuating populations, genetically distinct from those growing on rocks (Mereschkowsky, 1918). Later thinking suggested that vagrant taxa were originally derived from attached forms but have since achieved some level of genetic divergence through reproductive isolation and now represent distinct species (Klement, 1950). However, some vagrant lichen species appear to represent ecomorphs with the same genetic composition as species generally attached to rock substrates (Weber, 1967, 1977; Rosentreter and McCune, 1992). The co-occurrence of vagrant and erratic taxa within higher level taxonomic groups (i.e. genera) provides some evidence for a mechanism which ultimately yields

vagrant taxa; a pattern where erratic individuals may reproduce through fragmentation, subsequently achieving some level of genetic isolation (Rosentreter and McCune, 1992).

Our results provide strong evidence for multiple independent origins of vagrancy in the *Xanthoparmelia* of western North America. Vagrant forms were identified in multiple well-supported monophyletic lineages, most with relatively broad geographic distributions. Specific morphological adaptations to ecological conditions common in habitats supporting vagrant *Xanthoparmelia* (Modenesi et al., 2000; Clark et al., 2001), suggest a similar genetic architecture exhibited within widespread *Xanthoparmelia* populations that could give rise to similar patterns of phenotypic evolution among local populations, thus resulting in parallel morphological evolution under common selective pressures.

Analytical expectations indicate that a substantial amount of time is required after the initial divergence of species before there will be a high probability of observing reciprocal monophyly at a sample of multiple loci (Hudson and Coyne, 2002; Hudson and Turelli, 2003). A direct consequence of clonal reproduction is that each new individual is essentially identical to its parent, and current theory suggests that exclusive asexuality is not viable in the long term. High haplotype diversity (relative to expected haplotype diversity in strictly clonal organisms) and well-supported monophyletic vagrant clades suggest that vagrant lineages in *Xanthoparmelia* may be relatively long lived. The occasional occurrence of sexual reproductive structures (apothecia) in some vagrant *Xanthoparmelia* species, generally characterized by unspecialized vegetative reproduction, suggests that cyclical parthenogenesis, the alternation between sexual and asexual reproduction, may provide an important mechanism for generating genotypic diversity essential for long-term viability. However, additional investigations are required to

explicitly assess the evolutionary significance of gene flow in typically clonal vagrant *Xanthoparmelia* species.

In spite of the wide distribution of most identified vagrant *Xanthoparmelia* lineages, others appear to be threatened with extinction (Rosentreter, 1993). Habitat fragmentation poses a significant threat to vagrant species adapted to relatively continuous open spaces. Agriculture, livestock overgrazing, altered fire frequencies, and invasive plant species have already reduced or extirpated many significant vagrant lichen populations in both North American and the Russian steppe (Rosentreter, 1993), including the type localities of *X. chlorochroa*, *X. neochlorochroa*, and *X. wyomingica* (personal observation).

Extensive homoplasy in morphological, chemical, and reproductive modes— Traditionally, species descriptions in *Xanthoparmelia* have relied heavily on chemical characters due to confusion surrounding the consistent *diagnosability* and significance of most morphological characters. These results indicate that extensive homoplasy in most characters traditionally used to delimit *Xanthoparmelia* species obscures recognition of natural lineages. Our data indicate that there is not a simple dichotomy between expressed biochemical complexes or reproductive modes in *Xanthoparmelia*. Our data suggest repeated evolution of both the stictic acid and the norstictic acid only (or loss of salazinic and stictic acids) complexes in *Xanthoparmelia*. Nearly all sampled individuals expressed norstictic acid regardless of other expressed major compounds (stictic or salazinic acid), but the expression of both salazinic and stictic acid chemotypes in a single individual was never identified. Our limited sampling of the psoromic acid complex is inadequate to assess the evolution of this compound within *Xanthoparmelia*. However, all diagnostic major secondary metabolites identified in this study are closely related β-orcinal depsidones, and genetic and biological mechanisms influencing the

expression of distinct compounds are uncertain (Asplund and Gauslaa, 2007; Asplund, Solhaug, and Gauslaa, 2009).

Although phylogenetic analyses recovered some well-supported monophyletic lineages exclusively containing individuals expressing the stictic acid complex, other individuals with identical chemotypes were recovered in well-supported lineages intermixed with individuals expressing the salazinic acid complex. Leavitt, Johnson, and St. Clair (submitted) found that although the stictic acid complex was not recovered as monophyletic, population-level analyses recovered most individuals containing stictic acid in a single inferred population cluster. These data suggest that incomplete lineage sorting or rare or historic recombination may obscure phylogenetic signal. Coupled with independent changes of chemical character states, the role of medullary chemistry in identifying natural groups within *Xanthoparmelia* is particularly challenging. Furthermore, the relationship of unsampled major secondary metabolites, including: atranorin, barbatic, dehydroconstipatic, diffractaic, fumaroprotocetraric, hypoprotocetraric, lecanoric, lichesteric, subdecipienic, succinprotocetraric, 3-*α*-hydroxybarbatic, 4-*O*-demethylnotatic and the evolution of minor and trace compounds also remains unclear (Nash III and Elix, 2004).

Morphological and chemical characters generally employed to infer taxonomic boundaries between vagrant forms appear to have been overemphasized, as multiple independent changes of most diagnostic characters are revealed across the topology. Vagrant samples expressing the salazinic acid complex with an emaculate to weakly maculate upper cortex, treated here as *X. chlorochroa* s. l., were recovered in four major clades identified in this study (*X*-III, *X* -IV, *X* -V, and *X* -VI); furthermore, evidence of multiple independent origins of vagrancy within some major clades was also identified. The discovery that *X. chlorochroa*

comprises multiple independent lineages in western North America suggests that the true number of vagrant species may be seriously underestimated. However, we were unable to identify fixed morphological or chemical characters corroborating independent *X. chlorochroa* s. l. lineages. In contrast, both the absence of rhizines (*X. norchlorochroa*) and the expression of norstictic acid only (*X. neochlorochroa*) in vagrant growth forms were found to be homoplasious, suggesting that the more conspicuous chemical and morphological characters currently used to differentiate vagrant species do not reflect natural groupings. Adding to the challenge of understanding the role of morphology in defining taxonomic boundaries, vagrant specimens with a strongly maculate upper cortex (*X. camtschadalis* s. l. and *X. idahoensis* s. l.) were restricted to the more basal clades *X* -I and *X* –II and the two minor clades A and B in our analyses, although the phylogenetic position of the two minor clades remains obscure. The absence of vagrant individuals with a maculate upper cortex in other lineages suggests that upper cortical features may provide limited taxonomic utility. The lack of congruence between molecular data and the current classification of vagrant *Xanthoparemlia* species suggest the need for significant taxonomic revision.

Although our sampling strategy emphasized vagrant growth forms, this study provides some insight into the evolution of reproductive patterns in saxicolous *Xanthoparmelia*. The reproductive pattern in nearly two thirds (129) of all sampled attached saxicolous individuals was not observed (sexual or asexual). Isidiate forms were represented by 46 OTUs overall, and the expression of sexual structures (apothecia) was observed in only 40 of the sampled accessions, including four vagrant specimens.

Our results suggest that transitions between reproductive modes within sampled *Xanthoparmelia* occurred several times independently of each other. Taylor et al. (1993)

reported that multigene systems underlie sexual and asexual reproduction in nonlichenized ascomycetes, and our data suggest that reproductive systems in lichenized ascomycetes may also be determined by similar complex genetic systems. The occurrence of perennial structures of multiple reproductive strategies were occasionally found on a single thallus (apothecia/isidia – and apothecia/unspecialized fragmentation)and indicate, that at least in some cases, the underlying genetic structure controlling the expression of reproductive modes is maintained across reproductively diverse groups. Other recent molecular studies also suggest that complex evolutionary patterns in reproductive modes exist across many lichenized ascomycete groups (Lohtander et al., 1998; Myllys et al., 1999; Kroken and Taylor, 2001; Myllys, Lohtander, and Tehler, 2001; Printzen and Ekman, 2003). It has been proposed that the sexual reproductive mode can be considered the baseline reproductive mode found in all species (Buschbom and Barker, 2006) but predominantly vegetative taxa appear to maintain the capacity to periodically reproduce sexually which may accommodate long-term viability. Isidia occur in nearly a third of *Xanthoparmelia* species (Hale, 1990), with significant variation in isidial structure (Kurokawa and Filson, 1975; Elix, 1981). Generally, isidiate specimens included in the present study had morphologically similar subglobose to cylindrical and irregularly branched isidia, although variation in isidial structure was only superficially evaluated in this study. A more detailed investigation of the evolutionary relationships and genetic structure controlling the expression of distinct reproductive modes in lichenized ascomycetes is clearly needed to better understand the underlying mechanisms controlling reproduction.

Conclusions

These results highlight some of the challenges with species delimitation in this notoriously difficult and variable group of lichens. The traditional use of morphological and chemical characters in *Xanthoparmelia*, in particular vagrancy, biochemical variation, and reproductive mode, are obscured by extensive homoplasy, rendering them of limited suitability for species delimitation, and clearly indicate that the interpretation of morphological and chemical diversity found within one of the most speciose genera of lichenized fungi has been too superficial. More detailed investigations of potential mechanisms driving the evolution of morphological, chemical, and reproductive patterns in *Xanthoparmelia* are needed to better understand the biological mechanisms influencing these characters.

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Literature Cited

AHTI, T., and D. L. HAWKSWORTH. 2005. *Xanthoparmelia stenophylla*, the correct name for *X. somloënsis*, one of the most widespread usnic acid containing species of the genus. *The Lichenologist* 37: 363-366.

ARGÜELLO, A., R. DEL PRADO, P. CUBAS, and A. CRESPO. 2007. *Parmelina quercina* (Parmeliaceae, Lecanorales) includes four phylogenetically supported morphospecies. *Biological Journal of the Linnean Society* 91: 455-467.

ARNOLD, A. E., J. MIADLIKOWSKA, K. L. HIGGINS, S. D. SARVATE, P. GUGGER, A. WAY, V. HOFSTETTER, et al. 2009. A Phylogenetic Estimation of Trophic Transition Networks for Ascomycetous Fungi: Are Lichens Cradles of Symbiotrophic Fungal Diversification? *Systematic Biology* 58: 283-297.

ARUP, U., and M. GRUBE. 2000. Is *Rhizoplaca* (Lecanorales, lichenized Ascomycota) a monophyletic genus? *Canadian Journal of Botany* 78: 318-327.

ARUP, U., S. EKMAN, M. GRUBE, J. E. MATTSSON, and M. WEDIN. 2007. The sister group relation of Parmeliaceae (Lecanorales, Ascomycota). *Mycologia* 99: 42-49.

ASPLUND, J., and Y. GAUSLAA. 2007. Content of secondary compounds depends on thallus size in the foliose lichen *Lobaria pulmonaria*. *The Lichenologist* 39: 273-278.

ASPLUND, J., K. A. SOLHAUG, and Y. GAUSLAA. 2009. Fungal depsidones - an inducible or constitutive defence against herbivores in the lichen *Lobaria pulmonaria*? *Basic and Applied Ecology* 10: 273-278.

BAILEY, R. H. 1976. Ecological aspects of dispersal and establishment in lichens. *In* D. H. Brown, D. L. Hawksworth, AND R. H. Bailey [eds.], Lichenology: Progress and Problems, 215-247. Academic Press, New York, New York, USA.

BLANCO, O., A. CRESPO, R. H. REE, and H. T. LUMBSCH. 2006. Major clades of parmelioid lichens (Parmeliaceae, Ascomycota) and the evolution of their morphological and chemical diversity. *Molecular Phylogenetics and Evolution* 39: 52-69.

BLANCO, O., A. CRESPO, J. A. ELIX, D. L. HAWKSWORTH, and H. T. LUMBSCH. 2004a. A Molecular Phylogeny and a New Classification of Parmelioid Lichens Containing *Xanthoparmelia*-Type Lichenan (Ascomycota: Lecanorales). *Taxon* 53: 959-975.

BLANCO, O., A. CRESPO, P. K. DIVAKAR, T. L. ESSLINGER, D. L. HAWKSWORTH, and H. THORSTEN LUMBSCH. 2004b. *Melanelixia* and *Melanohalea*, two new genera segregated from *Melanelia* (Parmeliaceae) based on molecular and morphological data. *Mycological Research* 108: 873-884.

BRUNAUER, G., A. HAGER, M. GRUBE, R. TÜRK, and E. STOCKER-WÖRGÖTTER. 2007. Alterations in secondary metabolism of aposymbiotically grown mycobionts of *Xanthoria elegans* and cultured resynthesis stages. *Plant Physiology and Biochemistry* 45: 146-151.

BUSCHBOM, J., and G. M. MUELLER. 2006. Testing "Species Pair" Hypotheses: Evolutionary Processes in the Lichen-Forming Species Complex *Porpidia flavocoerulescens* and *Porpidia melinodes*. *Molecular Biology and Evolution* 23: 574-586.

BUSCHBOM, J., and D. BARKER. 2006. Evolutionary History of Vegetative Reproduction in *Porpidia* s.l. (Lichen-Forming Ascomycota). *Systematic Biology* 55: 471-484.

CARBONE, I., and L. M. KOHN. 1999. A Method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. *Mycologia* 91: 553-556.

CARDINALE, M., A. M. PUGLIA, and M. GRUBE. 2006. Molecular analysis of lichen-associated bacterial communities. *FEMS Microbiology Ecology* 57: 484-495.

CARDINALE, M., J. V. D. C. JR, H. MÜLLER, G. BERG, and M. GRUBE. 2008. *In situ* analysis of the bacterial community associated with the reindeer lichen *Cladonia arbuscula* reveals predominance of *Alphaproteobacteria*. *FEMS Microbiology Ecology* 66: 63-71.

CLARK, B. M., L. L. ST. CLAIR, N. F. MANGELSON, L. B. REES, P. G. GRANT, and G. S. BENCH. 2001. Characterization of mycobiont adaptations in the foliose lichen *Xanthoparmelia chlorochroa* (Parmeliaceae). *American Journal of Botany* 88: 1742-1749.

CRESPO, A., O. BLANCO, and D. L. HAWKSWORTH. 2001. The Potential of Mitochondrial DNA for Establishing Phylogeny and Stabilising Generic Concepts in the Parmelioid Lichens. *Taxon* 50: 807-819.

CRESPO, A., M. C. MOLINA, O. BLANCO, B. SCHROETER, L. G. SANCHO, and D. L. HAWKSWORTH. 2002. rDNA ITS and β-tubulin gene sequence analyses reveal two monophyletic groups within the cosmopolitan lichen *Parmelia saxatilis*. *Mycological Research* 106: 788-795.

CRESPO, A., H. T. LUMBSCH, J.-E. MATTSSON, O. BLANCO, P. K. DIVAKAR, K. ARTICUS, E. WIKLUND, et al. 2007. Testing morphology-based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear RPB1 gene. *Molecular Phylogenetics and Evolution* 44: 812-824.

DEGNAN, J. H., and N. A. ROSENBERG. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution* 24: 332-340.

DEL-PRADO, R., P. CUBAS, H. T. LUMBSCH, P. K. DIVAKAR, O. BLANCO, G. A. DE PAZ, M. C. MOLINA, et al. Genetic distances within and among species in monophyletic lineages of

Parmeliaceae (Ascomycota) as a tool for taxon delimitation. *Molecular Phylogenetics and Evolution* 56: 125-133.

DEL PRADO, R., Z. FERENCOVÁ, V. ARMAS-CRESPO, G. AMO DE PAZ, P. CUBAS, and A. CRESPO. 2007. The arachiform vacuolar body: an overlooked shared character in the ascospores of a large monophyletic group within Parmeliaceae (*Xanthoparmelia* clade, Lecanorales). *Mycological Research* 111: 685-692.

DRUMMOND, A., O. PYBUS, A. RAMBAUT, R. FORSBERG, and A. RODRIGO. 2003. Measurably evolving populations. *Trends in Ecology & Evolution* 18: 481 - 488.

EDGAR, R. C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: 1-19.

EDGAR, R. C., and S. BOTZOGLOU. 2006. Multiple sequence alignment. *Current Opinion in Structural Biology* 16: 1-6.

EDWARDS, S. V. 2009. Is a new and general theory of molecular systematics emerging? *Evolution* 63: 1-19.

ELIX, J. A. 1981. New species of Parmelia Subgen. *Xanthoparmelia* (Lichens) from Australia and New Zealand. *Australian Journal of Botany* 29: 349-376.

_____. 1986. *Canoparmelia*, *Paraparmelia* and *Relicinopsis*, three new genera in the Parmeliaceae (lichenized Ascomycotina) *Mycotaxon* 27: 271-282.

_____. 1993. Progress in the Generic Delimitation of *Parmelia* Sensu Lato Lichens (Ascomycotina: Parmeliaceae) and a Synoptic Key to the Parmeliaceae. *The Bryologist* 96: 359-383.

ENDLER, J. A. 1986. Natural selection in the wild. Princeton University Press, Princeton.

ESSLINGER, T. L. 1977. A chemosytematic revision of the brown Parmeliae. *Journal of Hattori Botanical Laboratory* 42: 1-211.

_____. 1978. A new status for brown Parmeliae. *Mycotaxon* 7: 45-54.

FELSENSTEIN, J. 2004. Inferring phylogenies. Sunderland, Mass. : Sinauer Associates, Sunderland, Massachusetts, USA..

GANLEY, A. R. D., and T. KOBAYASHI. 2007. Highly efficient concerted evolution in the ribosomal DNA repeats: Total rDNA repeat variation revealed by whole-genome shotgun sequence data. *Genome Research* 17: 184-191.

GARDES, M., and T. D. BRUNS. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology Notes* 2: 113-118.

GUTIÉRREZ, G., O. BLANCO, P. DIVAKAR, H. LUMBSCH, and A. CRESPO. 2007. Patterns of Group I Intron Presence in Nuclear SSU rDNA of the Lichen Family Parmeliaceae. *Journal of Molecular Evolution* 64: 181-195.

HALE, M. E. 1990. A synopsis of the lichen genus *Xanthoparmelia* (Vainio) Hale (Ascomycotina, Parmeliaceae), vol. Book, Whole. Smithsonian Institution Press, Washington D.C., USA.

HAWKSWORTH, D. L., and A. CRESPO. 2002. Proposal to conserve the name *Xanthoparmelia* against *Chondropsis* nom. cons, (Parmeliaceae). *Taxon* 51: 437-466.

HODKINSON, B., and F. LUTZONI. 2009. A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. *Symbiosis* 49: 163-180.

HODKINSON, B. P., and J. C. LENDEMER. in press. Molecular analyses reveal semi-cryptic species in *Xanthoparmelia tasmanica*. *Bibliotheca Lichenologica*.

HONEGGER, R. 2001. The symbiotic phenotype of lichen-forming ascomyces. *In* B. Hock [ed.], The Mycota; IX Fungal Associations, 165-188. Springer Verlag, Berlin, Germany.

HUDSON, R. R., and J. A. COYNE. 2002. Mathematical Consequences of the Genealogical Species Concept. *Evolution* 56: 1557-1565.

HUDSON, R. R., and M. TURELLI. 2003. Stochasticity Overrules the "Three-Times Rule": Genetic Drift, Genetic Draft, and Coalescence Times for Nuclear Loci versus Mitochondrial DNA. *Evolution* 57: 182-190.

HUELSENBECK, J. P., and F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754 - 755.

HUELSENBECK, J. P., and B. RANNALA. 2004. Frequentist Properties of Bayesian Posterior Probabilities of Phylogenetic Trees Under Simple and Complex Substitution Models. *Systematic Biology* 53: 904 - 913.

HUELSENBECK, J. P., F. RONQUIST, R. NIELSEN, and J. P. BOLLBACK. 2001. Bayesian Inference of Phylogeny and Its Impact on Evolutionary Biology. *Science* 294: 2310-2314.

KLEMENT, O. 1950. Über die Artberechtigung einiger *Parmelien*. *Berichten der Deutschen Botanischen Gesellschaft, Jahrgang* 63: 47-52.

KROKEN, S., and J. W. TAYLOR. 2001. A Gene Genealogical Approach to Recognize Phylogenetic Species Boundaries in the Lichenized Fungus Letharia. *Mycologia* 93: 38-53.

KUROKAWA, S., and R. B. FILSON. 1975. New species of *Parmelia* from South Australia. *Bulletin of the National Science Museum (Tokyon)* series B: 35-48.

LEACHE, A. D. 2009. Species Tree Discordance Traces to Phylogeographic Clade Boundaries in North American Fence Lizards (*Sceloporus*). *Systematic Biology* 58: 547-559.

LEAVITT, S. D. 2010. Assessing traditional morphology- and chemistry-based species circumspections in lichenized ascomycetes: character evolution and species delimitation in common western North American lichens. PhD, Brigham Young University, Provo, Utah, USA.

LEAVITT, S. D., L. A. JOHNSON, and L. L. ST. CLAIR. submitted. Assessing species diversity and evolution in morphologically and chemically diverse communities of the lichen-forming genus *Xanthoparmelia* (Parmeliaceae, Ascomycota) in western North America.

LEAVITT, S. D., J. D. FANKHAUSER, D. H. LEAVITT, and L. D. PORTER. submitted. Complex patterns of speciation in cosmopolitan "rock posy" lichens - an integrative approach to discovering and delimiting fungal species in the lichen-forming *Rhizoplaca melanophthalma* species complex (Lecanoraceae, Lecanorales).

LEWIS, P. O. 2001. A Likelihood Approach to Estimating Phylogeny from Discrete Morphological Character Data. *Systematic Biology* 50: 913-925.

LINDBLOM, L., and S. EKMAN. 2006. Genetic variation and population differentiation in the lichen-forming ascomycete *Xanthoria parietina* on the island Storfosna, central Norway. *Molecular Ecology* 15: 1545-1559.

LIU, L., L. YU, L. KUBATKO, D. K. PEARL, and S. V. EDWARDS. 2009. Coalescent methods for estimating phylogenetic trees. *Molecular Phylogenetics and Evolution* 53: 320-328.

LOHTANDER, K., L. MYLLYS, R. SUNDIN, M. KÄLLERSJÖ, and A. TEHLER. 1998. The Species Pair Concept in the Lichen *Dendrographa leucophaea* (Arthoniales): Analyses Based on ITS Sequences. *The Bryologist* 101: 404-411.

LOUWHOFF, S. H. J. J., and M. D. CRISP. 2000. Phylogenetic Analysis of *Parmotrema* (Parmeliaceae: Lichenized Ascomycotina). *The Bryologist* 103: 541-554.

LUMBSCH, H. T., I. SCHMITT, A. MANGOLD, and M. WEDIN. 2007. Ascus types are phylogenetically misleading in Trapeliaceae and Agyriaceae (Ostropomycetidae, Ascomycota). *Mycological Research* 111: 1133-1141.

LUTZONI, F., M. PAGEL, and V. REEB. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* 411: 937-940.

LUTZONI, F., F. KAUFF, C. J. COX, D. MCLAUGHLIN, G. CELIO, B. DENTINGER, M. PADAMSEE, et al. 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* 91: 1446-1480.

MADDISON, W. P., and D. R. MADDISON. 2007. Mesquite: A modular system for evolutionary analysis, version 2.01. Website <http://mesquiteproject.org>.

MCCUNE, B., and C. SCHOCH. 2009. *Hypogymnia minilobata* (Parmeliaceae), a new lichen from coastal California. *The Bryologist* 112: 94-100.

MERESCHKOWSKY, C. 1918. Note sur une nouvelle forme de *Parmelia* vivant à l'état libre. *Bulletin de la Société Botanique de Genève, series 2* 10:26-34.

MODENESI, P., M. PIANA, P. GIORDANI, A. TAFANELLI, and A. BARTOLI. 2000. Calcium Oxalate and Medullary Architecture in *Xanthomaculina convoluta*. *The Lichenologist* 32: 505-512.

MOLINA, M. D. C., A. CRESPO, O. BLANCO, H. T. LUMBSCH, and D. L. HAWKSWORTH. 2004. Phylogenetic relationships and species concepts in Parmelia s. str. (Parmeliaceae) inferred from nuclear ITS rDNA and β-tubulin sequences. *The Lichenologist* 36: 37-54.

MYLLYS, L., K. LOHTANDER, and A. TEHLER. 2001. β-Tubulin, ITS and Group I Intron Sequences Challenge the Species Pair Concept in *Physcia aipolia* and *P. caesia*. *Mycologia* 93: 335-343.

MYLLYS, L., K. LOHTANDER, M. KÄLLERSJÖ, and A. TEHLER. 1999. Sequence Insertions and ITS Data Provide Congruent Information on Roccella canariensis and R. tuberculata (Arthoniales, Euascomycetes) Phylogeny. *Molecular Phylogenetics and Evolution* 12: 295-309.

NASH III, T. H., and J. A. ELIX. 2004. *Xanthoparmelia*. *In* T. H. Nash III, B. D. Ryan, P. Diederich, C. Gries, AND F. BUNGARTZ [eds.], Lichen Flora of the Greater Sonoran Desert Region, vol. 2. Lichens Unlilmited, Tempe, Arizona, USA.

NELSEN, M. P., R. LUCKING, M. GRUBE, J. S. MBATCHOU, L. MUGGIA, E. R. PLATA, and H. T. LUMBSCH. 2009. Unravelling the phylogenetic relationships of lichenised fungi in Dothideomyceta. *Studies in Mycology* 64: 135-1444.

NYLANDER, J. A. A., F. RONQUIST, J. P. HUELSENBECK, and J. NIEVES-ALDREY. 2004. Bayesian Phylogenetic Analysis of Combined Data. *Systematic Biology* 53: 47-67.

O'BRIEN, H. E., J. MIADLIKOWSKA, and F. LUTZONI. 2009. Assessing reproductive isolation in highly diverse communities of the lichen-forming funal genus *Peltigera*. *Evolution* 63: 2076-2086.

O'MEARA, B. C. 2010. New Heuristic Methods for Joint Species Delimitation and Species Tree Inference. *Systematic Biology* 59: 59-73.

ORANGE, A., P. W. JAMES, and F. J. WHITE. 2001. Microchemical methods for the identification of lichens, 1-101. British Lichen Society, London, UK.

POSADA, D., and K. A. CRANDALL. 2001. Selecting the Best-Fit Model of Nucleotide Substitution. *Systematic Biology* 50: 580-601.

POSADA, D., and T. R. BUCKLEY. 2004. Model Selection and Model Averaging in Phylogenetics: Advantages of Akaike Information Criterion and Bayesian Approaches Over Likelihood Ratio Tests. *Systematic Biology* 53: 793-808.

PRINTZEN, C. 2009. Lichen Systematics: The Role of Morphological and Molecular Data to Reconstruct Phylogenetic Relationships, Progress in Botany 71, vol. 71, 233-275. Springer Berlin Heidelberg, Berlin, Germany.

PRINTZEN, C., and S. EKMAN. 2003. Local population subdivision in the lichen *Cladonia subcervicornis* as revealed by mitochondrial cytochrome oxidase subunit 1 intron sequences. *Mycologia* 95: 399-406.

RAMBAULT, A. 1996. Sequence Alignment Editor *Available from: <http://tree.bio.ed.ac.uk/software/seal/>*.

REEB, V., F. LUTZONI, and C. ROUX. 2004. Contribution of RPB2 to multilocus phylogenetic studies of the euascomycetes (Pezizomycotina, Fungi) with special emphasis on the lichen-forming Acarosporaceae and evolution of polyspory. *Molecular Phylogenetics and Evolution* 32: 1036-1060.

REESE NÆSBORG, R., S. EKMAN, and L. TIBELL. 2007. Molecular phylogeny of the genus *Lecania* (Ramalinaceae, lichenized Ascomycota). *Mycological Research* 111: 581-591.

RIKKINEN, J. 1995. What's beind the pretty colours? A study of the photobiology of lichens. *Bryobrothera* 4: 1-239.

ROSENTRETER, R. 1993. Vagrant Lichens in North America. *The Bryologist* 96: 333-338.

ROSENTRETER, R., and B. MCCUNE. 1992. Vagrant *Dermatocarpon* in Western North America. *The Bryologist* 95: 15-19.

SANDERS, W. B. 2001. Lichens: The Interface between Mycology and Plant Morphology. *Bioscience* 51: 1025-1035.

SCHLUTER, D. 2000. The ecology of adaptive radiations. Oxford University Press, Oxford, UK.

SCHMITT, I., R. D. PRADO, M. GRUBE, and H. T. LUMBSCH. 2009a. Repeated evolution of closed fruiting bodies is linked to ascoma development in the largest group of lichenized fungi (Lecanoromycetes, Ascomycota). *Molecular Phylogenetics and Evolution* 52: 34-44.

SCHMITT, I., A. CRESPO, P. K. DIVAKAR, J. D. FANKHAUSER, E. HERMAN-SACKETT, K. KALB, M. P. NELSEN, et al. 2009b. New primers for promising single-copy genes in fungal phylogenies and systematics. *Persoonia* 23: 35-40.

SELBMANN, L., L. ZUCCONI, S. RUISI, M. GRUBE, M. CARDINALE, and S. ONOFRI. 2010. Culturable bacteria associated with Antarctic lichens: affiliation and psychrotolerance. *Polar Biology* 33: 71-83.

ST. CLAIR, L., J. JOHANSEN, S. ST. CLAIR, and K. KNIGHT. 2007. The Influence of Grazing and Other Environmental Factors on Lichen Community Structure along an Alpine Tundra Ridge in the Uinta Mountains, Utah, U.S.A. *Arctic, Antarctic, and Alpine Research* 39: 603-613.

STAMATAKIS, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

STAMATAKIS, A., P. HOOVER, and J. ROUGEMONT. 2008. A Rapid Bootstrap Algorithm for the RAxML Web Servers. *Systematic Biology* 57: 758-771.

SULLIVAN, J., and P. JOYCE. 2005. Model selection in phylogenetics. *Annual Review of Ecology, Evolution, and Systematics* 36: 445-466.

SWOFFORD, D. L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Ver. 4.0.

TEHLER, A., and M. KÄLLERSJÖ. 2001. *Parmeliopsis ambigua* and *P. hyperopta* (Parmeliaceae): species or chemotypes? *The Lichenologist* 33: 403-408.

THELL, A. 1999. Group I Intron Versus its Sequences in Phylogeny of Cetrarioid Lichens. *The Lichenologist* 31: 441-449.

THELL, A., J. A. ELIX, and U. SØCHTING. 2009. *Xanthoparmelia lineola* s. l. in Australia and North America. *Bibliothecia Lichenologica* 99: 393-404.

THELL, A., T. FEUERER, J. A. ELIX, and I. KÄRNEFELT. 2006. A contribution to the phylogeny and taxonomy of *Xanthoparmelia* (Ascomycota, Parmeliaceae). *Journal of the Hattori Botanical Laboratory* 100: 797-807.

THOMAS, A., and R. ROSENTRETER. 1992. Antelope utilization of lichen in the Birch Creek Valley of Idaho. *In* E. Raper [ed.], Proceedings - Symposium of the 15th Biennial Pronghorn Antelope Workshop, Rocksprings Wyoming, June 9-11, 1992., 6-12. Wyoming Game and Fish Department, Rock Springs, Wyoming, USA.

TRUONG, C., Y. NACIRI, and P. CLERC. 2009. Multivariate analysis of anatomical characters confirms the differentiation of two morphologically close species, *Melanohalea olivacea* (L.) O. Blanco et al. and *M. septentrionalis* (Lynge) O. Blanco et al. *The Lichenologist* 41: 649-661.

VELMALA, S., L. MYLLYS, P. HALONEN, T. GOWARD, and T. AHTI. 2009. Molecular data show that *Bryoria fremontii* and *B. tortuosa* (Parmeliaceae) are conspecific. *The Lichenologist* 41: 231-242.

WEBER, W. A. 1967. Environmental modification in crustose lichens II. Fruticose growth forms in *Aspicilia*. *Aquile, Ser. Botanica* 6: 43-51.

_____. 1977. Environmental Modifications. *In* M. R. D. Seaward [ed.], Lichen Ecology, 9-29. Academic Press, New York, New York, USA.

WEDIN, M., M. WESTBERG, A. T. CREWE, A. TEHLER, and O. W. PURVIS. 2009. Species delimitation and evolution of metal bioaccumulation in the lichenized *Acarospora smaragdula* (Ascomycota, Fungi) complex. *Cladistics* 25: 161-172.

WHEELER, D. L., T. BARRETT, D. A. BENSON, S. H. BRYANT, K. CANESE, V. CHETVERNIN, D. M. CHURCH, et al. 2006. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*: gkl1031.

WHITE, T. J., T. D. BRUNS, S. LEE, and J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press, San Diego, California, USA.

WIENS, J. J. 1998. Combining Data Sets with Different Phylogenetic Histories. *Systematic Biology* 47: 568-581.

WIRTZ, N., C. PRINTZEN, and H. T. LUMBSCH. 2008. The delimitation of Antarctic and bipolar species of neuropogonoid *Usnea* (Ascomycota, Lecanorales): a cohesion approach of species recognition for the *Usnea perpusilla* complex. *Mycological Research* 112: 472-484.

ZWICKL, D. J., and D. M. HILLIS. 2002. Increased Taxon Sampling Greatly Reduces Phylogenetic Error. *Systematic Biology* 51: 588-598.

Table 2.1. Primers used for PCR amplification and sequencing of the nuclear ribosomal IGS, ITS, and group I intron markers and low-copy protein-coding markers β-tubulin and *MCM7*.

Marker	Primer name	Forward primer sequence	Annealing temperature (°C)	Reference
IGS	IGS12	5'-AGTCTGTGGATTAGTGGCCG-3'	66- 56 (touchdown)	Carbone & Kohn 1999
	NS1R	5'-GAGACAAGCATATGACTAC-3'		Carbone & Kohn 1999
	X_IGS_R	5'-TAC TGG CAG AAT CAR CCA GG-3'		Leavitt (2010)
ITS/group I intron	ITS1F	5'-CTT GGT CAT TTA GAG GAA GTA A-3'	55-60	(Gardes and Bruns, 1993)
	ITS4	5'- TCC TCC GCT TAT TGA TAT GC-3'		(White et al., 1990)
LSU	LROR	5'-ACC CGC TGA ACT TAA GC-3'	55-60	Vilgalys unpublished
	LR5	5'-ATC CTG AGG GAA ACT TC-3'		Vilgalys unpublished
β-tubulin	Bt3-LM	5'-GAACGTCTACTTCAACGAG-3'	55-60	(Myllys, Lohtander, and Tehler, 2001)
	Bt10-LM	5'-TCGGAAGCAGCCATCATGTTCTT-3'		(Myllys, Lohtander, and Tehler, 2001)
	BT_rhizo_F	5'-GCA ACA AGT ATG TTC CTC GTG C-3'	66- 56 (touchdown)	Leavitt (2010)
	BT_rhizo_R	5'-GTAAGAGGTGCGAAGCCAACC-3'		Leavitt (2010)
MCM7	*MCM7*-709for	5'-ACI MGI GTI TCV GAY GTH AARCC-3'	56	Schmitt et al., 2009a
	MCM7-1348rev	5'-GAY TTD GCI ACI CCI GGR TCW CCC AT-3'		Schmitt et al., 2009a
	X_ *MCM7*_F	5'- CGT ACA CYT GTG ATC GAT GTG -3'	66- 56 (touchdown)	Leavitt (2010)
	X_ *MCM7*_R	5'- GTC TCC ACG TAT TCG CAT TCC-3'		Leavitt (2010)

Table 2.2. Genetic variability of sampled loci - N, number of sequences; aligned basepairs (bp), total alignment length; number of variable sites and parsimony informative (PI) sites for each sampled locus; and model of evolution selected for each locus.

Locus	**N**	**aligned bp**	**# of variable sites**	**# PI sites**	**Model selected**
ITS	427	598	224	166	GTR+I+G
LSU	422	851	116	72	GTR+I+G
IGS	391	389	148	102	GTR+G
group I intron	311	417	121	80	SYM+G
β-tubulin	389	787	180	108	GTR+I+G
MCM7	353	541	156	104	GTR+I+G
Total	432	3583	945	632	na

Table 2.3. Genetic variability of defined clades: N, number of OTUs assigned membership in each define clade; aligned basepairs (bp), total clade-specific alignment length; number of variable sites and parsimony informative (PI) sites for each sampled locus.

Clade	**N**	**aligned bp**	**# of variable sites**	**# PI sites**
***X*-I**	34	3074	77	55
***X*-II**	23	3457	167	126
***X*-III**	34	3459	195	87
***X*-IV**	120	3487	376	231
***X*-V**	52	3476	216	119
***X*-VI**	146	3493	299	161
Total tree	432	3583	945	632

Figure 2.1. Geographic distribution of sampled *Xanthoparmelia* specimens in western North America. Sampled localities not shown include: Cherokee and Rutherford counties, North Carolina and Puebla, Mexico.

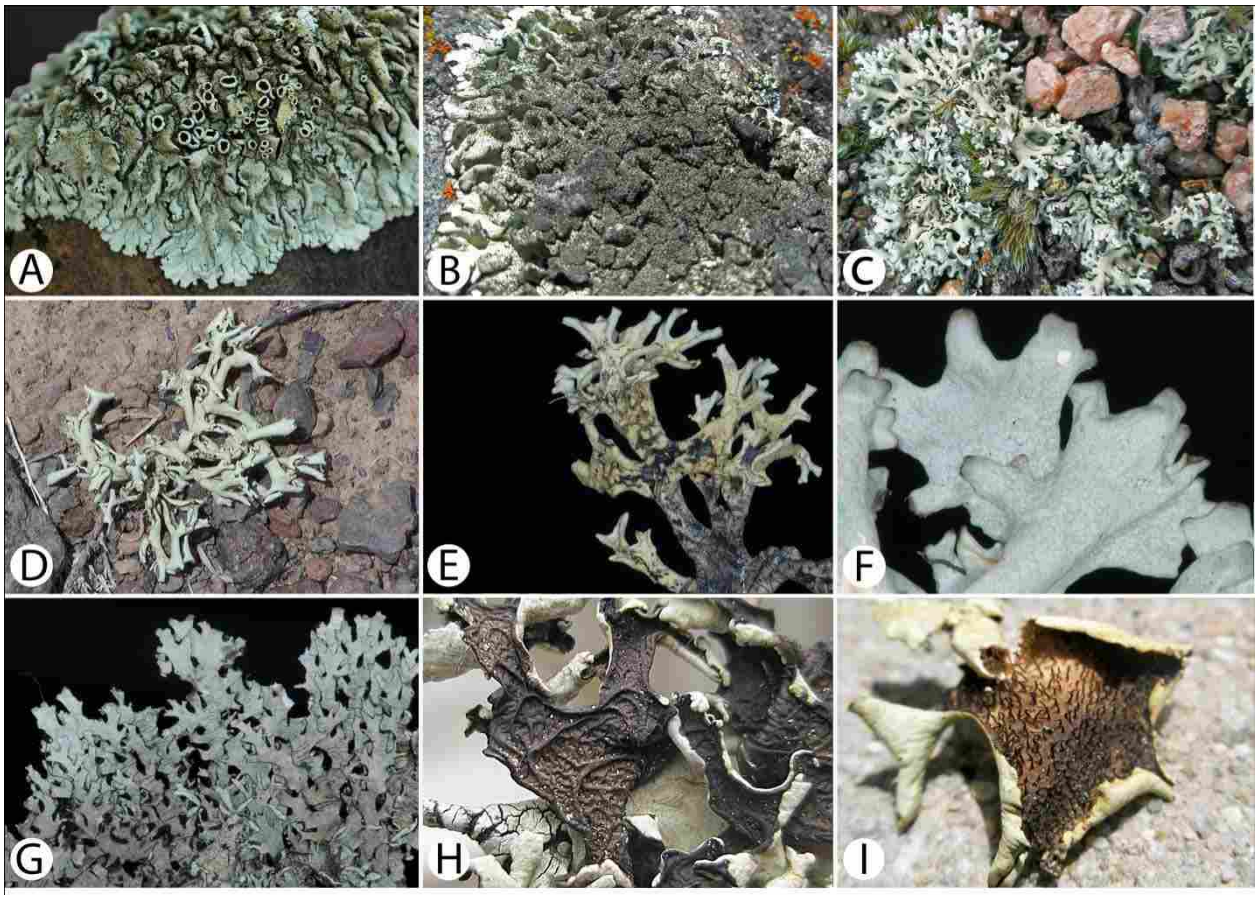


Figure 2.2. Variation in morphology and habit within sampled *Xanthoparmelia* in western North America. (A) saxicolous attached taxon *X. cumberlandia* sensu lato (s. l.) with sexual reproductive structures (apothecia) producing ascospores (B) saxicolous attached taxon *X. mexicana* with specialized vegetative reproductive structures (isidia) containing propagules of both symbionts, (C) terricolous taxon *X. wyomingica* s. l., an intermediate growth-form between attached and vagrant forms, (D) vagrant taxon *X. chlorochroa* s. l., (E) unique morphology of rare vagrant or semi-attached taxon *X. idahoensis* s. l. known from fine calcareous soils, (F) white-maculate upper cortex on *X. camtschadalis* s. l., (G) lobe morphology and emaculate surface on *X. stenophylla*, (H) erhizinate lower surface of vagrant taxon *X. norchlorochroa* s. l., (I) rhizine characters on vagrant taxon *X. chlorochroa* s. l.

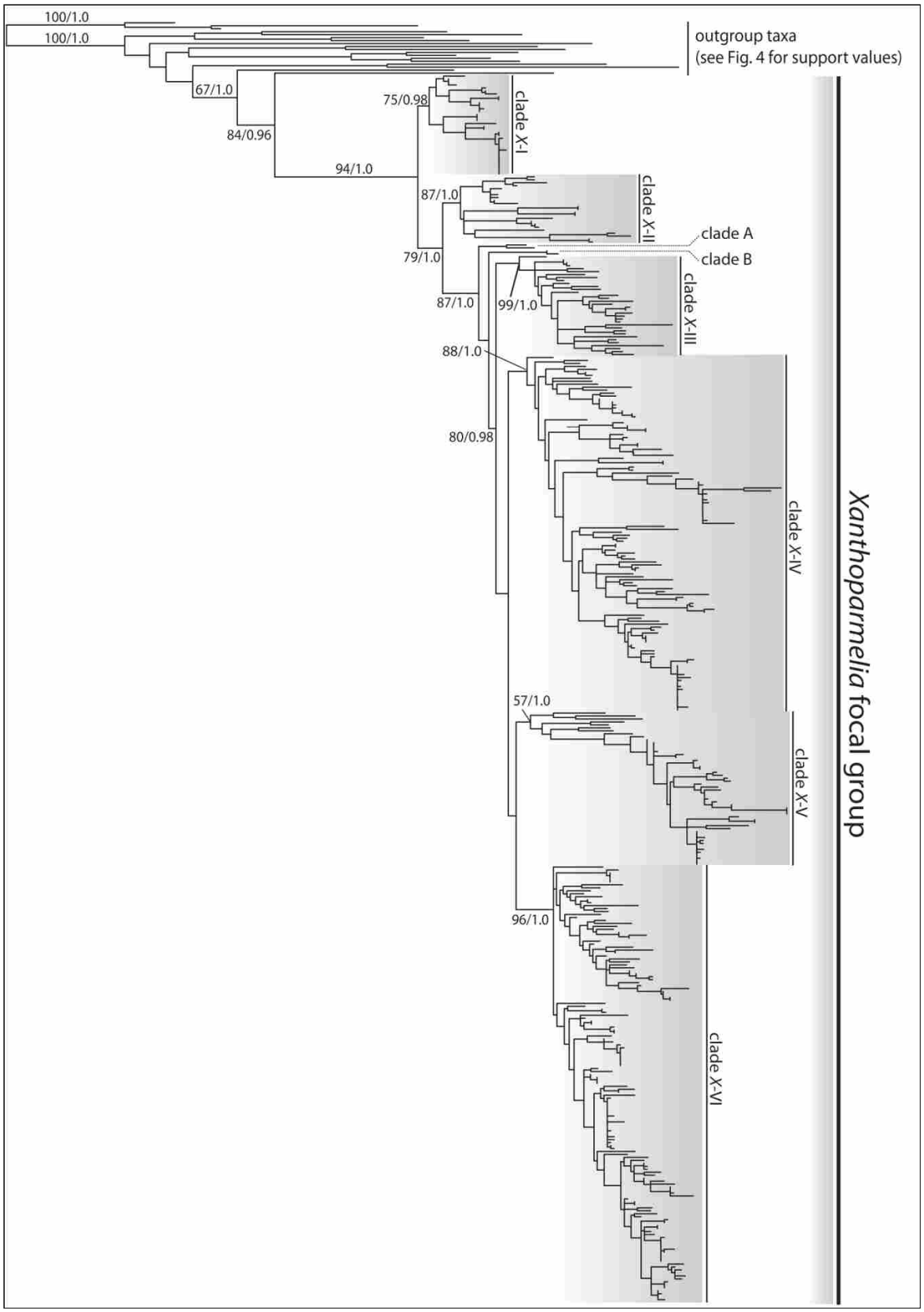

100/1.0
100/1.0
outgroup taxa
(see Fig. 4 for support values)
67/1.0
75/0.98
clade X-I
84/0.96
94/1.0
87/1.0
clade X-II
clade A
clade B
79/1.0
87/1.0
99/1.0
clade X-III
88/1.0
80/0.98
clade X-IV
57/1.0
clade X-V
96/1.0
clade X-VI
Xanthoparmelia focal group

Figure 2.3 (on previous page). Simplified ML topology indicating relationships of *Xanthoparmelia* taxa inferred from a combined analysis of nuclear ribosomal markers ITS, IGS, LSU, and intron and protein-coding fragments from β-tubulin and *MCM7* genes representing 432 OTUs. Values at each major node indicate maximum likelihood non-parametric –bootstrap support (BS) / Bayesian posterior probability (PP); only BS values ≥ 50 and PP ≥ 0.5 are shown; and scale indicates substitutions per site. Clades *X*-I through *X*-V are discussed in the text, and detailed relationships within each defined clade are shown in Figures 5 and 6.

Figure 2.4. ML topology indicting the intrageneric relationship of western North America *Xanthoparmelia* focal group to outgroup taxa. Values at each node indicate maximum likelihood non-parametric bootstrap support (BS) / Bayesian posterior probability (PP); only BS values ≥ 50 and PP ≥ 0.5 are shown; and scale bar indicates substitutions per site.

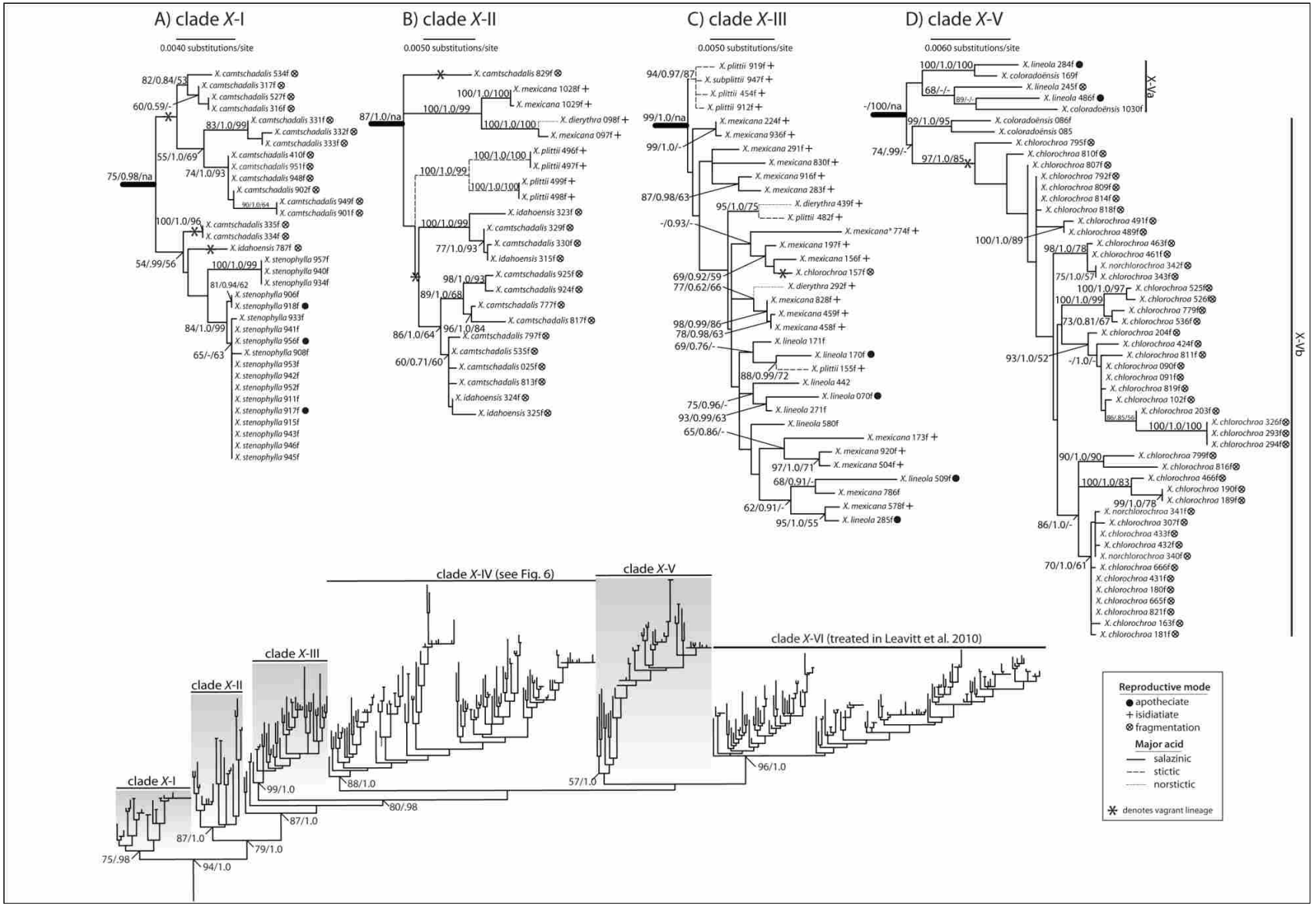

A) clade X-I
0.0040 substitutions/site
B) clade X-II
0.0050 substitutions/site
C) clade X-III
0.0050 substitutions/site
D) clade X-V
0.0060 substitutions/site
X-Va
X-Vb
clade X-IV (see Fig. 6)
clade X-V
clade X-VI (treated in Leavitt et al. 2010)
clade X-III
clade X-II
clade X-I
Reproductive mode
apotheciate
isidiatiate
fragmentation
Major acid
salazinic
stictic
norstictic
denotes vagrant lineage

Figure 2.5 (on previous page). ML topology indicating relationships in clade *X*-I (Fig. 5A), *X*-II (Fig. 5B), *X*-III (Fig. 5C), and *X*-V (Fig. 5D). Values at each node indicate maximum likelihood (ML) non-parametric bootstrap support (BS) / Bayesian posterior probability (PP) / maximum parsimony (MP) non-parametric bootstrap (BS); only ML and MP BS values ≥ 50 and Bayesian PP ≥ 0.5 are shown; and scale bar indicates substitutions per site.

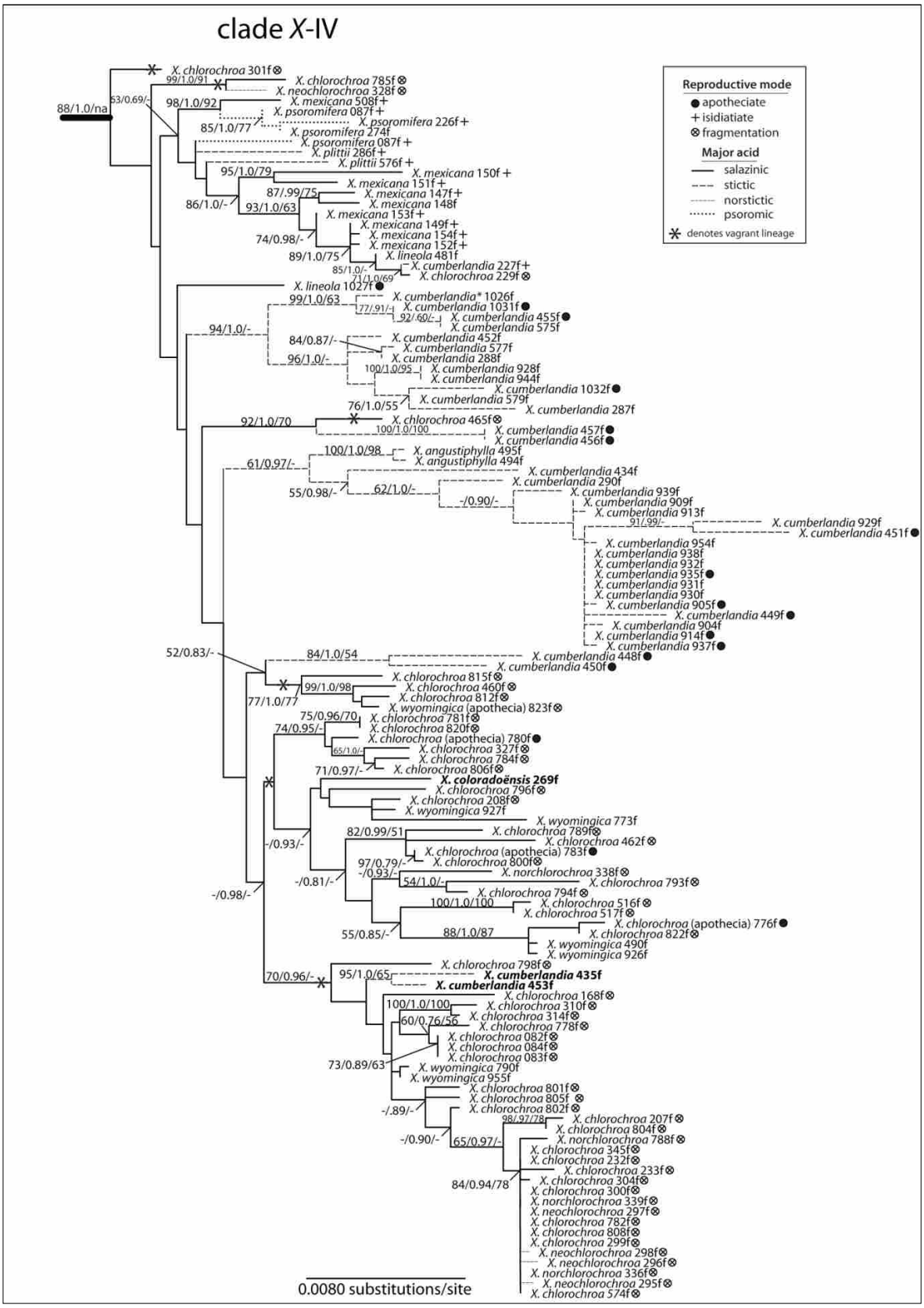

clade X-IV
Reproductive mode
apotheciate
+ isidiatiate
⊗ fragmentation
Major acid
salazinic
stictic
norstictic
psoromic
denotes vagrant lineage
X. chlorochroa 301f⊗
X. chlorochroa 785f⊗
X. neochlorochroa 328f⊗
X. mexicana 508f+
X. psoromifera 087f+
X. psoromifera 226f+
X. psoromifera 274f
X. psoromifera 087f+
X. plittii 286f+
X. plittii 576f+
X. mexicana 150f+
X. mexicana 151f+
X. mexicana 147f+
X. mexicana 148f
X. mexicana 153f+
X. mexicana 149f+
X. mexicana 154f+
X. mexicana 152f+
X. lineola 481f
X. cumberlandia 227f+
X. chlorochroa 229f⊗
X. lineola 1027f●
X. cumberlandia* 1026f
X. cumberlandia 1031f●
X. cumberlandia 455f●
X. cumberlandia 575f
X. cumberlandia 452f
X. cumberlandia 577f
X. cumberlandia 288f
X. cumberlandia 928f
X. cumberlandia 944f
X. cumberlandia 1032f●
X. cumberlandia 579f
X. cumberlandia 287f
X. chlorochroa 465f⊗
X. cumberlandia 457f●
X. cumberlandia 456f●
X. angustiphylla 495f
X. angustiphylla 494f
X. cumberlandia 434f
X. cumberlandia 290f
X. cumberlandia 939f
X. cumberlandia 909f
X. cumberlandia 913f
X. cumberlandia 929f
X. cumberlandia 451f●
X. cumberlandia 954f
X. cumberlandia 938f
X. cumberlandia 932f
X. cumberlandia 935f●
X. cumberlandia 931f
X. cumberlandia 930f
X. cumberlandia 905f●
X. cumberlandia 449f●
X. cumberlandia 904f
X. cumberlandia 914f●
X. cumberlandia 937f●
X. cumberlandia 448f●
X. cumberlandia 450f●
X. chlorochroa 815f⊗
X. chlorochroa 460f⊗
X. chlorochroa 812f⊗
X. wyomingica (apothecia) 823f⊗
X. chlorochroa 781f⊗
X. chlorochroa 820f⊗
X. chlorochroa (apothecia) 780f●
X. chlorochroa 327f⊗
X. chlorochroa 784f⊗
X. chlorochroa 806f⊗
X. coloradoënsis 269f
X. chlorochroa 796f⊗
X. chlorochroa 208f⊗
X. wyomingica 927f
X. wyomingica 773f
X. chlorochroa 789f⊗
X. chlorochroa 462f⊗
X. chlorochroa (apothecia) 783f●
X. chlorochroa 800f⊗
X. norchlorochroa 338f⊗
X. chlorochroa 793f⊗
X. chlorochroa 794f⊗
X. chlorochroa 516f⊗
X. chlorochroa 517f⊗
X. chlorochroa (apothecia) 776f●
X. chlorochroa 822f⊗
X. wyomingica 490f
X. wyomingica 926f
X. chlorochroa 798f⊗
X. cumberlandia 435f
X. cumberlandia 453f
X. chlorochroa 168f⊗
X. chlorochroa 310f⊗
X. chlorochroa 314f⊗
X. chlorochroa 778f⊗
X. chlorochroa 082f⊗
X. chlorochroa 084f⊗
X. chlorochroa 083f⊗
X. wyomingica 790f
X. wyomingica 955f
X. chlorochroa 801f⊗
X. chlorochroa 805f ⊗
X. chlorochroa 802f⊗
X. chlorochroa 207f⊗
X. chlorochroa 804f⊗
X. norchlorochroa 788f⊗
X. chlorochroa 345f⊗
X. chlorochroa 232f⊗
X. chlorochroa 233f⊗
X. chlorochroa 304f⊗
X. chlorochroa 300f⊗
X. norchlorochroa 339f⊗
X. neochlorochroa 297f⊗
X. chlorochroa 782f⊗
X. chlorochroa 808f⊗
X. chlorochroa 299f⊗
X. neochlorochroa 298f⊗
X. neochlorochroa 296f⊗
X. norchlorochroa 336f⊗
X. neochlorochroa 295f⊗
X. chlorochroa 574f⊗
0.0080 substitutions/site

Figure 2.6 (on previous page). ML topology indicating relationships in clade *X*-IV. Values at each node indicate maximum likelihood (ML) non-parametric bootstrap support (BS) / Bayesian posterior probability (PP) / maximum parsimony (MP) non-parametric bootstrap (BS); only ML and MP BS values ≥ 50 and Bayesian PP ≥ 0.5 are shown; and scale bar indicates substitutions per site.

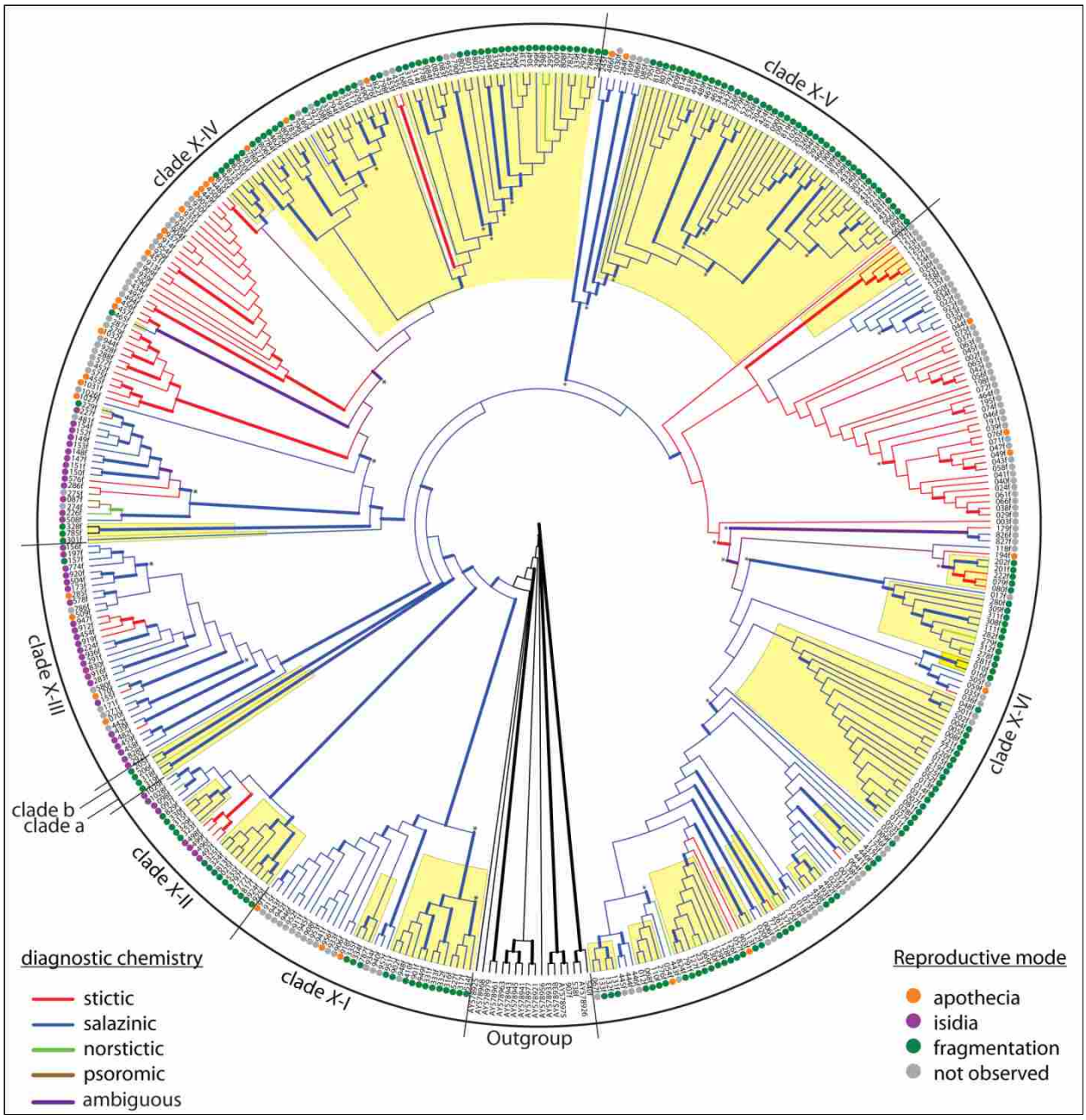


Figure 2.7. Evolution of morphological and chemical characters in the vagrant *Xanthoparmelia* complex mapped on ML topology inferred from a combined analysis of nuclear ribosomal markers ITS, IGS, LSU, and intron and protein-coding fragments from β-tubulin and *MCM7* genes representing 432 OTU. Thickened branches indicate BS and PP values ≥ 70/0.95; thickened branches marked with '*' indicate PP values ≥ 0.95 and BS < 70; clades highlighted in yellow indicate independent origins of vagrant lineages.

Supplementary data 2.1. Collection information for all *Xanthoparmelia* specimens included in the present study: ID, individual code; species; Brigham Young University Herbarium of Non-vascular Cryptogams voucher accession number; major acid, diagnostic secondary chemistry; Location; Lat., latitude; Lon., longitude; Ele., altitude in meters a.s.l.; collector(s).

ID	Species (sensu lato)	Herbarium Accession No.	Major Acid	Reproductive mode	Location	Lat.	Lon.	Ele.	Collector (s)
001f	*X. coloradoënsis*	BRY-55151	salazinic	not observed	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
002f	*X. cumberlandia*	BRY-55152	stictic	not observed	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
003f	*X. cumberlandia*	BRY-55153	stictic	not observed	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
004f	*X. chlorochroa*	BRY-55154	salazinic	fragmentation	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
005f	*X. chlorochroa*	BRY-55155	salazinic	fragmentation	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
006f	*X. coloradoënsis*	BRY-55156	salazinic	not observed	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
007f	*X. norchlorochroa*	BRY-55157	salazinic	fragmentation	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
008f	*X. chlorochroa*	BRY-55158	salazinic	fragmentation	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
009f	*X. chlorochroa*	BRY-55159	salazinic	fragmentation	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
010f	*X. chlorochroa*	BRY-55160	salazinic	fragmentation	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
011f	*X. chlorochroa*	BRY-55161	salazinic	fragmentation	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
012f	*X. coloradoënsis*	BRY-55162	salazinic	not observed	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
013f	*X. norchlorochroa*	BRY-55163	salazinic	fragmentation	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
014f	*X. chlorochroa*	BRY-55164	salazinic	fragmentation	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
015f	*X. chlorochroa*	BRY-55165	salazinic	fragmentation	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.

016f	*X. chlorochroa*	BRY-55166	salazinic	fragmentation	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
017f	*X. coloradoënsis*	BRY-55167	salazinic	not observed	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
018f	*X. coloradoënsis*	BRY-55168	salazinic	not observed	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
019f	*X. coloradoënsis*	BRY-55169	salazinic	not observed	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
020f	*X. coloradoënsis*	BRY-55170	salazinic	not observed	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
022f	*X. coloradoënsis*	BRY-55171	salazinic	not observed	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
023f	*X. coloradoënsis*	BRY-55172	salazinic	not observed	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
024f	*X. cumberlandia*	BRY-55173	stictic	not observed	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
025f	*X. camtschadalis*	BRY-55174	salazinic	fragmentation	USA, MT, Broadwater Co.	45.9584	-111.6108	1440 m	B. McCune 29230
027f	*X. chlorochroa*	BRY-55175	salazinic	fragmentation	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
028f	*X. chlorochroa*	BRY-55176	salazinic	fragmentation	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
029f	*X. cumberlandia**	BRY-55177	stictic	not observed	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
030f	*X. coloradoënsis*	BRY-55178	salazinic	not observed	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
031f	*X. chlorochroa*	BRY-55179	salazinic	fragmentation	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
032f	*X. coloradoënsis*	BRY-55180	salazinic	not observed	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
033f	*X. coloradoënsis*	BRY-55181	salazinic*	not observed	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
034f	*X. coloradoënsis*	BRY-55182	salazinic*	not observed	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
035f	*X. coloradoënsis**	BRY-55183	salazinic	not observed	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
036f	*X. cumberlandia*	BRY-55184	stictic	not observed	USA, UT,	38.1202	111.5071	3300 m	Leavitt et al.

037f	*X. californica**	BRY-55185	norstictic	not observed	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
038f	*X. cumberlandia*	BRY-55186	stictic	not observed	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
039f	*X. cumberlandia**	BRY-55187	stictic	not observed	USA, UT, Wayne Co.	38.1220	111.5071	3300 m	Leavitt et al.
040f	*X. cumberlandia*	BRY-55188	stictic	not observed	USA, UT, Wayne Co.	38.1308	-111.4695	3300 m	Leavitt et al.
041f	*X. cumberlandia*	BRY-55189	stictic	not observed	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
042f	*X. cumberlandia**	BRY-55190	stictic	not observed	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
043f	*X. cumberlandia*	BRY-55191	stictic	not observed	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
044f	*X. cumberlandia*	BRY-55192	stictic	apothecia	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
045f	*X. cumberlandia*	BRY-55193	stictic	not observed	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
046f	*X. neowyomingica**	BRY-55194	stictic	not observed	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
047f	*X. cumberlandia*	BRY-55195	stictic	not observed	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
048f	*X. chlorochroa*	BRY-55196	salazinic	fragmentation	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
049f	*X. cumberlandia*	BRY-55197	stictic	apothecia	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
052f	*X. chlorochroa*	BRY-55198	salazinic	fragmentation	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
053f	*X. chlorochroa*	BRY-55199	salazinic	fragmentation	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
054f	*X. coloradoënsis*	BRY-55200	salazinic	apothecia	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
055f	*X. coloradoënsis**	BRY-55201	salazinic	not observed	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
056f	*X. cumberlandia*	BRY-55202	stictic	not observed	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.

057f	*X. cumberlandia*	BRY-55203	stictic	not observed	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
058f	*X. cumberlandia*	BRY-55204	stictic	not observed	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
059f	*X. coloradoënsis*	BRY-55205	salazinic	apothecia	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
061f	*X. cumberlandia*	BRY-55206	stictic	not observed	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
062f	*X. cumberlandia*	BRY-55207	stictic	not observed	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
063f	*X. cumberlandia*	BRY-55208	stictic	not observed	USA, UT, Wayne Co.	38.1309	-111.46945	3300 m	Leavitt et al.
064f	*X. coloradoënsis**	BRY-55209	salazinic	not observed	USA, UT, Wayne Co.	38.1625	-111.53581	3300 m	Leavitt et al.
065f	*X. cumberlandia*	BRY-55210	stictic	not observed	USA, UT, Summit Co.	40.7743	-109.82444	3410 m	EA 80-1103
066f	*X. cumberlandia*	BRY-55211	stictic	not observed	USA, UT, Summit Co.	40.7743	-109.82444	3410 m	EA 80-1104
067f	*X. coloradoënsis*	BRY-55212	salazinic	not observed	USA, UT, Summit Co.	40.8047	-110.0213	3360 m	EA 80-1108
068f	*X. chlorochroa*	BRY-55213	salazinic	fragmentation	USA, WY, Uinta Co.	41.3769	-110.6621	2057 m	SDL, LLS
069f	*X. chlorochroa*	BRY-55214	salazinic	fragmentation	UT, Duchesne Co.	40.3699	-110.41279	2005 m	SDL, MFR
070f	*X. lineola*	BRY-55215	salazinic	Apothecia	UT, Duchesne Co.	40.3698	-110.41282	2005 m	SDL, MFR
071f	*X. cumberlandia*	BRY-55216	stictic	not observed	USA, UT, Wayne Co.	38.5812	-111.7700	3040 m	Leavitt et al.
072f	*X. cumberlandia*	BRY-55217	stictic	not observed	USA, UT, Wayne Co.	38.5812	-111.7700	3040 m	Leavitt et al.
073f	*X. coloradoënsis*	BRY-55218	salazinic	not observed	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
074f	*X. cumberlandia*	BRY-55219	sticitic	not observed	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
075f	*X. cumberlandia*	BRY-55220	stictic	not observed	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
076f	*X. cumberlandia*	BRY-55221	stictic	apothecia	USA, UT,	38.4097	-111.4757	3300 m	Leavitt et al.

					Wayne Co.				
079f	*X. vagans*	BRY-55222	stictic	fragmentation	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
080f	*X. vagans*	BRY-55223	stictic	fragmentation	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
081f	*X. chlorochroa*	BRY-55224	salazinic	fragmentation	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
082f	*X. chlorochroa*	BRY-55225	salazinic	fragmentation	USA, UT, Wayne Co.	38.2757	-111.6081	2347 m	Leavitt et al.
083f	*X. chlorochroa*	BRY-55226	salazinic	fragmentation	USA, UT, Wayne Co.	38.2757	-111.6081	2347 m	Leavitt et al.
084f	*X. chlorochroa*	BRY-55227	salazinic	fragmentation	USA, UT, Wayne Co.	38.2757	-111.6081	2347 m	Leavitt et al.
085f	*X. coloradoënsis*	BRY-55228	salazinic	not observed	USA, UT, Wayne Co.	38.2757	-111.6081	2347 m	Leavitt et al.
086f	*X. coloradoënsis*	BRY-55229	salazinic	not observed	USA, UT, Wayne Co.	38.2757	-111.6081	2347 m	Leavitt et al.
087f	*X. lavicola*	BRY-55230	psoromic	isidia	USA, UT, Wayne Co.	38.2757	-111.6081	2347 m	Leavitt et al.
090f	*X. chlorochroa*	BRY-55231	salazinic	fragmentation	USA, ID, Lemhi Co.	44.6812	-113.3623	1820 m	Leavitt et al.
091f	*X. chlorochroa*	BRY-55232	salazinic	fragmentation	USA, ID, Lemhi Co.	44.6812	-113.3623	1820 m	Leavitt et al.
097f	*X. mexicana*	BRY-55233	salazinic	isidia	Mex, Puebla	19.2990	-97.1193	1740 m	Leavitt et al.
098f	*X. dierythra*	BRY-55234	norstictic	isidia/apothecia	Mex, Puebla	19.2990	-97.1193	1740 m	Leavitt et al.
102f	*X. chlorochroa*	BRY-55235	salazinic	fragmentation	USA, ID, Lemhi Co:	44.6811	-113.3623	1820 m	Leavitt et al.
110f	*X. chlorochroa*	BRY-55236	salazinic	fragmentation	USA, WY, Uinta Co.	41.3769	-110.6621	2057 m	Leavitt et al.
111f	*X. chlorochroa*	BRY-55237	salazinic	fragmentation	USA, WY, Uinta Co.	41.3769	-110.6621	2057 m	Leavitt et al.
112f	*X. chlorochroa*	BRY-55238	salazinic	fragmentation	USA, ID, Owyhee Co.	43.3202	-116.9795	1271 m	Leavitt et al.
113f	*X. chlorochroa*	BRY-55239	salazinic	fragmentation	USA, ID, Owyhee Co.	43.3202	-116.9795	1271 m	Leavitt et al.
118f	*X. coloradoënsis*	BRY-55240	salazinic	not observed	USA, ID, Lemhi Co.	44.6812	-113.3623	1820 m	SDL, LLS, KBK

120f	*X. coloradoënsis*	BRY-55241	salazinic	not observed	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
121f	*X. neowyomingica*	BRY-55242	stictic	not observed	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
122f	*X. neowyomingica*	BRY-55243	stictic	not observed	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
123f	*X. neowyomingica*	BRY-55244	stictic	not observed	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
124f	*X. neowyomingica*	BRY-55245	stictic	not observed	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
125f	*X. neowyomingica*	BRY-55246	stictic	not observed	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
126f	*X. chlorochroa*	BRY-55247	salazinic	fragmentation	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
127f	*X. chlorochroa*	BRY-55248	salazinic	fragmentation	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
128f	*X. chlorochroa*	BRY-55249	salazinic	fragmentation	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
129f	*X. chlorochroa*	BRY-55250	salazinic	fragmentation	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
130f	*X. chlorochroa*	BRY-55251	salazinic	fragmentation	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
131f	*X. chlorochroa*	BRY-55252	salazinic	fragmentation	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
132f	*X. chlorochroa*	BRY-55253	salazinic	fragmentation	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
133f	*X. chlorochroa*	BRY-55254	salazinic	fragmentation	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
135f	*X. coloradoënsis*	BRY-55255	salazinic	not observed	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
136f	*X. wyominigica*	BRY-55256	salazinic	not observed	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
138f	*X. cumberlandia*	BRY-55257	stictic	not observed	USA, UT, Utah Co.	40.0847	-111.3401	1750 m	SDL, MJF
147f	*X. mexicana*	BRY-55258	salazinic	isidia	USA, AZ, Mojave Co.	36.9739	-113.6444	890 m	Leavitt et al.
148f	*X. mexicana*	BRY-55259	salazinic	isidia	USA, AZ,	36.9739	-113.6444	890 m	Leavitt et al.

					Mojave Co.				
149f	*X. mexicana*	BRY-55260	salazinic	isidia	USA, AZ, Mojave Co.	36.9739	-113.6444	890 m	Leavitt et al.
150f	*X. mexicana*	BRY-55261	salazinic	isidia	USA, AZ,	36.9739	-113.6444	890 m	Leavitt et al.
151f	*X. mexicana*	BRY-55262	salazinic	isidia	USA, AZ, Mojave Co.	36.9739	-113.6444	890 m	Leavitt et al.
152f	*X. mexicana*	BRY-55263	salazinic	isidia	USA, AZ, Mojave Co.	36.9739	-113.6444	890 m	Leavitt et al.
153f	*X. mexicana*	BRY-55264	salazinic	isidia	USA, AZ, Mojave Co.	36.9739	-113.6444	890 m	Leavitt et al.
154f	*X. mexicana*	BRY-55265	salazinic	isidia	USA, AZ, Mojave Co.	36.9739	-113.6444	890 m	Leavitt et al.
155f	*X. plittii*	BRY-55266	stictic	isidia	USA, UT, Wayne Co.	38.2879	-111.2274	1641 m	Leavitt et al.
156f	*X. mexicana*	BRY-55267	salazinic	isidia	USA, UT, Wayne Co.	38.2879	-111.2274	1641 m	Leavitt et al.
157f	*X. chlorochroa*	BRY-55268	salazinic	fragmentation	USA, NM, McKinley Co.	35.5500	-107.6666	2060 m	BRY-SL10275
163f	*X. chlorochroa*	BRY-55269	salazinic	fragmentation	USA, AZ, Coconino Co.	35.8083	-112.0325	1950 m	BRY-C21648
168f	*X. chlorochroa*	BRY-55270	salazinic	fragmentation	USA, WY, Sweetwater Co.	41.9861	110.0417	1950 m	BRY-C18517
169f	*X. coloradoënsis*	BRY-55271	salazinic	not observed	USA, NM, Grant Co.	33.2187	-108.7988	1560 m	BRY-C32565
170f	*X. lineola*	BRY-55272	salazinic	apothecia	USA, NM, Grant Co.	33.1915	-108.6682	1770 m	BRY-C32565
171f	*X. lineola*	BRY-55273	salazinic	apothecia	USA, NM, Grant Co.	33.1797	-108.0465	2048 m	EA49-519
173f	*X. mexicana*	BRY-55274	salazinic	isidia	USA, UT, Washington Co.	37.2047	-113.6417	1030 m	EA49-525
175f	*X. cumberlandia*	BRY-55275	stictic	apothecia	USA, ID, Elmore Co.	43.8167	-115.0861	1682 m	EA69-949
179f	*X. cumberlandia*	BRY-55276	stictic	not observed	USA, UT, Summit Co.	40.7882	-110.6982	3060 m	EA80-1118
180f	*X. chlorochroa*	BRY-55277	salazinic	fragmentation	USA, UT, Toole Co.	40.2967	-112.2785	1653 m	EA50-535

181f	*X. chlorochroa*	BRY-55278	salazinic	fragmentation	USA, UT, Toole Co.	40.2967	-112.2785	1653 m	EA50-544
189f	*X. chlorochroa*	BRY-55279	salazinic	fragmentation	USA, CO, Montrose Co.	38.4377	-107.9560	1880 m	EA49-526
190f	*X. chlorochroa*	BRY-55280	salazinic	fragmentation	USA, CO, Montrose Co.	38.4377	-107.9560	1880 m	EA49-526
191f	*X. cumberlandia*	BRY-55281	stictic	not observed	USA, CO, Dolores Co.	37.6939	-108.3233	2622 m	EA53-602
192f	*X. cumberlandia*	BRY-55282	stictic	not observed	USA, CO, Dolores Co.	37.6939	-108.3233	2622 m	EA53-598
194f	*X. cumberlandia*	BRY-55283	stictic	apothecia	USA, CO, Saguache Co.	37.8564	-105.4317	3030 m	EA55-634
195f	*X. cumberlandia*	BRY-55284	stictic	not observed	USA, CO, Archuleta Co:	37.3884	-107.0918	2657 m	EA57-681
197f	*X. mexicana*	BRY-55285	salazinic	isidia	USA, UT, San Juan Co.	37.7807	-109.8587	2133 m	EA67-899
198f	*X. cumberlandia*	BRY-55286	stictic	not observed	USA, UT, San Juan Co.	37.7807	-109.8587	2133 m	EA67-893
201f	*X. chlorochroa*	BRY-55287	salazinic	fragmentation	USA, MT, Beaverhead Co.	44.6225	-113.0520	2715 m	St. Clair et al.
202f	*X. chlorochroa*	BRY-55288	salazinic	fragmentation	USA, MT, Beaverhead Co.	44.6225	-113.0520	2715 m	St. Clair et al.
203f	*X. chlorochroa*	BRY-55289	salazinic	fragmentation	USA, ID, Lemhi Co.	44.6516	-113.2238	1971 m	St. Clair et al.
204f	*X. chlorochroa*	BRY-55290	salazinic	fragmentation	USA, ID, Lemhi Co.	44.6516	-113.2238	1971 m	St. Clair et al.
205f	*X. camtschadalis*	BRY-55291	salazinic	fragmentation	Canada, Saskatchewan.	50.6432	-107.9702	569 m	de Vries, B., s.n.
206f	*X. camtschadalis*	BRY-55292	salazinic	fragmentation	Canada, Saskatchewan.	50.6432	-107.9702	569 m	de Vries, B., s.n.
207f	*X. chlorochroa*	BRY-55293	salazinic	fragmentation	USA, WY, Carbon Co.	41.7708	-107.4778	2040 m	s.n.
208f	*X. chlorochroa*	BRY-55294	salazinic	fragmentation	USA, WY, Carbon Co.	41.7708	-107.4778	2040 m	s.n.
219f	*X. chlorochroa*	BRY-55295	salazinic	fragmentation	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	SDL
220f	*X. chlorochroa*	BRY-55296	salazinic	fragmentation	USA, UT,	38.4097	-111.4757	3300 m	SDL

					Wayne Co.				
221f	*X. chlorochroa*	BRY-55297	salazinic	fragmentation	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	SDL
222f	*X. vagans*	BRY-55298	stictic	fragmentation	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	SDL
224f	*X. mexicana*	BRY-55299	salazinic	isidia	USA, CA, Riverside Co.	33.7491	-116.7146	1660 m	Leavitt et al.
226f	*X. dierythra*	BRY-55300	norstictic	isidia	USA, UT, Wayne Co.	38.2736	-111.6106	2340 m	SDL
227f	*X. cumberlandia*	BRY-55301	stictic	isidia	USA, UT, Wayne Co.	38.2736	-111.6106	2340 m	SDL
229f	*X. chlorochroa*	BRY-55302	salazinic	fragmentation	USA, UT, Wayne Co.	38.4941	-111.5357	2471 m	SDL
231f	*X. neochlorochroa*	BRY-55303	norstictic	fragmentation	USA, UT, Wayne Co.	38.4941	-111.5357	2471 m	SDL
232f	*X. chlorochroa*	BRY-55304	salazinic	fragmentation	USA, UT, Wayne Co.	38.4347	-111.6992	2330 m	SDL
233f	*X. chlorochroa*	BRY-55305	salazinic	fragmentation	USA, UT, Wayne Co.	38.4347	-111.6992	2330 m	SDL
245f	*X. lineola*	BRY-55306	salazinic	apothecia	USA, AZ, Cochise Co.	32.0055	-109.3610	5400 m	EA31-259
247f	*X. cumberlandia*	BRY-55307	stictic	apothecia	USA, ID, Idaho Co.	46.3353	-115.3145	640 m	EA32-280
258f	*X. coloradoënsis*	BRY-55308	salazinic	not observed	USA, ID, Custer Co.	44.7833	-114.6875	2479 m	EA46-467
261f	*X. vagans*	BRY-55309	stictic	fragmentation	USA, ID, Lemhi Co.	44.1578	-113.8794	2069 m	EA47-485
269f	*X. coloradoënsis*	BRY-55310	salazinic	not observed	USA, UT, Washington Co.	37.2845	-113.0966	1540 m	SDL
271f	*X. lineola*	BRY-55311	salazinic	not observed	USA, UT, Washington Co.	37.3474	-113.1010	2110 m	Leavitt et al.
272f	*X. coloradoënsis*	BRY-55312	salazinic	not observed	USA, UT, Washington Co.	37.3474	-113.1010	2110 m	Leavitt et al.
274f	*X. psoromifera*	BRY-55313	psoromic	not observed	USA, UT, Wayne Co.	38.2757	-111.6081	2347 m	Leavitt et al.
275f	*X. psoromifera*	BRY-55314	psoromic	not observed	USA, UT, Wayne Co.	38.2757	-111.6081	2347 m	Leavitt et al.

276f	*X. chlorochroa*	BRY-55315	salazinic	fragmentation	USA, WY, Lincoln Co.	41.6257	-110.6270	2050 m	SDL, JHL
278f	*X. neochlorochroa*	BRY-55316	norstictic	fragmentation	USA, WY, Lincoln Co.	41.6387	-110.5699	2018 m	SDL, JHL
279f	*X. neochlorochroa*	BRY-55317	norstictic	fragmentation	USA, WY, Lincoln Co.	41.6254	-110.6270	2050 m	SDL, JHL
280f	*X. lipochlorochroa* *type locality	BRY-55318	fatty acids	fragmentation	USA, WY, Lincoln Co.	41.6388	-110.5699	2018 m	SDL, JHL
281f	*X. lipochlorochroa* *type locality	BRY-55319	fatty acids	fragmentation	USA, WY, Lincoln Co.	41.6388	-110.5699	2018 m	SDL, JHL
282f	*X. lipochlorochroa* *type locality	BRY-55320	fatty acids	fragmentation	USA, WY, Lincoln Co.	41.6254	-110.6270	2050 m	SDL, JHL
283f	*X. mexicana*	BRY-55321	salazinic	isidia	USA, CA, Sonoma Co.	38.5309	-122.8947	99 m	Leavitt et al.
284f	*X. lineola*	BRY-55322	salazinic	apothecia	USA, CA, Sonoma Co.	38.5309	-122.8947	99 m	Leavitt et al.
285f	*X. lineola*	BRY-55323	salazinic	apothecia	USA, CA, Sonoma Co.	38.5309	-122.8947	99 m	Leavitt et al.
286f	*X. plittii*	BRY-55324	stictic	isidia	USA, CA, Sonoma Co.	38.5309	-122.8947	99 m	SDL
287f	*X. cumberlandia*	BRY-55325	stictic	not observed	USA, CA, Sonoma Co.	38.5309	-122.89465	99 m	SDL
288f	*X. cumberlandia*	BRY-55326	stictic	not observed	USA, CA, Sonoma Co.	38.5309	-122.89465	99 m	SDL
290f	*X. cumberlandia*	BRY-55327	stictic	not observed	USA, WA, Spokane Co.	47.6385	-117.37667	99 m	HCL, JHL, DJH
291f	*X. mexicana*	BRY-55328	salazinic	isidia	USA, NV, Elko Co.	41.9421	114.688278	1569 m	SDL
292f	*X. dierythra*	BRY-55329	norstictic	isidia	USA, NV, Elko Co.	41.9421	114.688278	1569 m	SDL
293f	*X. chlorochroa*	BRY-55330	salazinic	fragmentation	USA, NV, Elko Co.	41.9494	-114.68194	1577 m	SDL
294f	*X. chlorochroa*	BRY-55331	salazinic	fragmentation	USA, NV, Elko Co.	41.9494	-114.68194	1577 m	SDL

295f	*X. neochlorochroa*	BRY-55332	norstictic	fragmentation	USA, NV, Elko Co.	41.9494	-114.68194	1577 m	SDL
296f	*X. neochlorochroa*	BRY-55333	norstictic	fragmentation	USA, NV, Elko Co.	41.9494	-114.68194	1577 m	SDL
297f	*X. neochlorochroa*	BRY-55334	norstictic	fragmentation	USA, NV, White Pine Co.	39.0699	-114.4472	1760 m	SDL
298f	*X. neochlorochroa*	BRY-55335	norstictic	fragmentation	USA, NV, White Pine Co.	39.0699	-114.4472	1760 m	SDL
299f	*X. chlorochroa*	BRY-55336	salazinic	fragmentation	USA, NV, White Pine Co.	39.0699	-114.4472	1760 m	SDL
300f	*X. chlorochroa*	BRY-55337	salazinic	fragmentation	USA, NV, White Pine Co..	39.0699	-114.4472	1760 m	SDL
301f	*X. chlorochroa*	BRY-55338	salazinic	fragmentation	USA, ID, Lemhi Co.	44.1944	-112.9424	1951 m	A. DeBolt 754
304f	*X.chlorochroa*	BRY-55339	salazinic	fragmentation	USA, ID, Custer Co.	44.3323	-114.0501	2490 m	Rosentreter 4385
307f	*X. chlorochroa*	BRY-55340	salazinic	fragmentation	USA, UT, San Juan Co.	37.9346	-109.8296	1524 m	A. DeBolt 754
308f	*X. chlorochroa*	BRY-55341	salazinic	fragmentation	USA, MT, Beaverhead Co.	44.4876	-112.8269	2120 m	McCune 21280
309f	*X. chlorochroa*	BRY-55342	salazinic	fragmentation	USA, MT, Beaverhead Co.	44.4876	-112.8269	2120 m	McCune 21280
310f	*X. chlorochroa*	BRY-55343	salazinic	fragmentation	USA, WY, Park Co.	44.9779	-110.7047	1920 m	Rosentreter 13610
311f	*X. chlorochroa*	BRY-55344	salazinic	fragmentation	USA, WY, Fremont Co.	43.5774	-109.73670	2469 m	Rosentreter 15445
312f	*X. chlorochroa*	BRY-55345	salazinic	fragmentation	USA, WY, Fremont Co.	43.5774	-109.7370	2469 m	Rosentreter 15445
314f	*X. chlorochroa*	BRY-55346	salazinic	fragmentation	USA, WY, Park Co.	44.9779	-110.7047	1920 m	Rosentreter 13610
315f	*X. idahoensis*	BRY-55347	salazinic	fragmentation	USA, ID, Lemhi Co.	44.9316	-113.7674	1858 m	Rosentreter 13897
316f	*X. camtschadalis*	BRY-55348	salazinic	fragmentation	USA, ID, Lemhi Co.	45.0536	-113.7065	1420 m	Rosentreter 4520
317f	*X. camtschadalis*	BRY-55349	salazinic	fragmentation	USA, ID, Lemhi Co.	45.0536	-113.7065	1420 m	Rosentreter 4520
318f	*X. idahoensis*	BRY-55350	salazinic	fragmentation	USA, ID, Lemhi	45.1204	-113.8624	1219 m	Rosentreter

	*type locality				Co.				3828
319f	*X. idahoensis*	BRY-55351	salazinic	fragmentation	USA, ID, Lemhi Co.	45.1204	-113.8624	1219 m	Rosentreter 3828
	*type locality								
323f	*X. idahoensis*	BRY-55352	salazinic	fragmentation	USA, CO, Grand Co.	40.1093	-106.4262	2320 m	Rosentreter 9339
324f	*X. idahoensis*	BRY-55353	salazinic	fragmentation	Canada, Saskatchewan.	49.2666	-107.6369	8310 m	Rosentreter, s.n.
325f	*X. idahoensis*	BRY-55354	salazinic	fragmentation	Canada, Saskatchewan.	49.2666	-107.6369	8310 m	Rosentreter, s.n.
326f	*X. chlorochroa*	BRY-55355	salazinic	fragmentation	USA, ID, Twin Falls Co.	42.0340	-114.7219	1888 m	Rosentreter 8205
327f	*X. chlorochroa*	BRY-55356	salazinic	fragmentation	USA, CO, Weld Co.	40.4249	-104.7092	1420 m	Rosentreter 7135
328f	*X. neochlorochroa*	BRY-55357	norstictic	fragmentation	USA, CO, Weld Co.	40.4249	-104.7092	1420 m	Rosentreter 7135
329f	*X. camtschadalis*	BRY-55358	salazinic	fragmentation	USA, Lemhi Co.	45.1738	-113.8064	1340 m	Rosentreter 16240
330f	*X. camtschadalis*	BRY-55359	salazinic	fragmentation	USA, Lemhi Co.	45.1738	-113.8064	1340 m	Rosentreter 16240
331f	*X. camtschadalis*	BRY-55360	salazinic	fragmentation	USA, MT, Jefferson Co.	45.8385	-111.8674	1620 m	Rosentreter 14671
332f	*X. camtschadalis*	BRY-55361	salazinic	fragmentation	USA, MT, Jefferson Co.	45.8385	-111.8674	1620 m	Rosentreter 14671
333f	*X. camtschadalis*	BRY-55362	salazinic	fragmentation	USA, CO, Grand Co.	40.4058	-105.6246	2600 m	Rosentreter 14787
334f	*X. camtschadalis*	BRY-55363	salazinic	fragmentation	USA, ID, Owyhee Co.	42.4737	-116.6630	1600 m	Rosentreter 15083
335f	*X. camtschadalis*	BRY-55364	salazinic	fragmentation	USA, ID, Owyhee Co.	42.4737	-116.6630	1600 m	Rosentreter 15083
336f	*X. norchlorochroa*	BRY-55365	salazinic	fragmentation	USA, WY, Sweetwater Co.	41.4193	-108.0524	2100 m	Rosentreter, s.n.
337f	*X. neochlorochroa*	BRY-55366	norstictic	fragmentation	USA, WY, Laramie Co.	41.2916	-105.5245	2137 m	Rosentreter, s.n.
338f	*X. norchlorochroa*	BRY-55367	salazinic	fragmentation	USA, ID, Clark Co.	44.1567	-112.9093	1860 m	Rosentreter, s.n.
339f	*X. norchlorochroa*	BRY-55368	salazinic	fragmentation	USA, ID, Clark Co.	44.1567	-112.9093	1860 m	Rosentreter, s.n.

340f	*X. norchlorochroa*	BRY-55369	salazinic	fragmentation	USA, UT, San Juan Co.	38.3291	-109.4298	1780 m	Belnap, J., s.n.
341f	*X. norchlorochroa*	BRY-55370	salazinic	fragmentation	USA, UT, San Juan Co.	38.3291	-109.4298	1780 m	Belnap, J., s.n.
342f	*X. norchlorochroa*	BRY-55371	salazinic	fragmentation	USA, UT, San Juan Co.	38.3839	-109.4529	1580 m	Rosentreter 8230
343f	*X. chlorochroa*	BRY-55372	salazinic	fragmentation	USA, ID, Lemhi Co.	45.0237	-113.9190	1280 m	Rosentreter 8230
345f	*X. chlorochroa*	BRY-55373	salazinic	fragmentation	USA, ID, Custer Co.	44.3590	-114.0649	1646 m	Rosentreter 4974
410f	*X. camtschadalis*	BRY-55374	salazinic	fragmentation	USA, MT, Broadwater Co.	46.1364	-111.4045	1200 m	B. McCune 29198
424f	*X. chlorochroa*	BRY-55375	salazinic	fragmentation	USA, ID, Lemhi Co.	44.6812	-113.3623	1820 m	BRY-34402
431f	*X. chlorochroa*	BRY-55376	salazinic	fragmentation	USA, UT, Toole Co.	40.2967	-112.2785	1650 m	SDL, LLS
432f	*X. chlorochroa*	BRY-55377	salazinic	fragmentation	USA, UT, Toole Co.	40.2967	-112.2785	1650 m	Leavitt et al.
433f	*X. chlorochroa*	BRY-55378	salazinic	fragmentation	USA, UT, Toole Co.	40.2967	-112.2785	1650 m	Leavitt et al.
434f	*X. cumberlandia*	BRY-55379	stictic	not observed	USA, ID, Idaho Co.	45.4549	-115.9448	603 m	Leavitt et al.
435f	*X. cumberlandia*	BRY-55380	stictic	not observed	USA, ID, Idaho Co.	45.4549	-115.9448	603 m	Leavitt et al.
437f	*X. chlorochroa*	BRY-55381	salazinic	fragmentation	USA, UT, Duchesne Co.	40.2039	-110.7130	2088 m	SDL, LLS, GS
438f	*X. chlorochroa*	BRY-55382	salazinic	fragmentation	USA, UT, Duchesne Co.	40.2039	-110.7130	2088 m	SDL, LLS, GS
439f	*X. dierythra*	BRY-55383	norstictic	isidia	USA, UT, near Weasel Point	40.2039	-110.7130	2060 m	Leavitt et al.
440f	*X. chlorochroa*	BRY-55384	salazinic	fragmentation	USA, UT, Duchesne Co.	40.5444	-110.2852	2517 m	Leavitt et al.
441f	*X. chlorochroa*	BRY-55385	salazinic	fragmentation	USA, UT, Duchesne Co.	40.5444	-110.2852	2517 m	Leavitt et al.
442f	*X. lineola*	BRY-55386	salazinic	not observed	USA, UT, Duchesne Co.	40.5260	-110.3529	2426 m	Leavitt et al.
443f	*X. californica*	BRY-55387	norstictic	not observed	USA, UT,	40.2052	-110.7133	2088 m	Leavitt et al.

444f	*X. coloradoënsis**	BRY-55388	stictic	not observed	USA, UT, Duchesne Co.	40.5351	-110.2233	2413 m	Leavitt et al.
445f	*X. coloradoënsis**	BRY-55389	salazinic	not observed	USA, UT, Duchesne Co.	40.5351	-110.2233	2413 m	Leavitt et al.
446f	*X. coloradoënsis**	BRY-55390	salazinic	not observed	USA, UT, Duchesne Co.	40.5351	-110.2233	2413 m	Leavitt et al.
448f	*X. cumberlandia*	BRY-55391	stictic	apothecia	USA, ID, Idaho Co.	46.4301	-115.1341	814 m	Leavitt et al.
449f	*X. cumberlandia*	BRY-55392	stictic	apothecia	USA, ID, Idaho Co.	46.4301	-115.1341	814 m	Leavitt et al.
450f	*X. subcumberlandia*	BRY-55393	stictic	apothecia	USA, ID, Idaho Co.	46.0425	-115.2767	750 m	Leavitt et al.
451f	*X. cumberlandia*	BRY-55394	stictic	apothecia	USA, ID, Idaho Co.	46.0425	-115.2767	750 m	Leavitt et al.
452f	*X. cumberlandia*	BRY-55395	stictic	not observed	USA, ID, Idaho Co.	45.9254	-116.1305	974 m	Leavitt et al.
453f	*X. cumberlandia*	BRY-55396	stictic	not observed	USA, ID, Idaho Co.	45.9254	-116.1305	974 m	Leavitt et al.
454f	*X. plittii*	BRY-55397	stictic	isidia	USA, ID, Idaho Co.	45.4549	-115.9448	603 m	Leavitt et al.
455f	*X. cumberlandia*	BRY-55398	stictic	apothecia	USA, ID, Idaho Co.	45.4549	-115.9448	603 m	Leavitt et al.
456f	*X. cumberlandia*	BRY-55399	stictic	apothecia	USA, CA, Marin Co.	38.0929	-122.8860	308 m	Leavitt et al.
457f	*X. cumberlandia*	BRY-55400	stictic	apothecia	USA, CA, Marin Co.	38.0929	-122.8860	308 m	Leavitt et al.
458f	*X. mexicana*	BRY-55401	salazinic	isidia	USA, ID, Lemhi Co.	45.0611	-113.7130	1362 m	Leavitt et al.
459f	*X. mexicana*	BRY-55402	salazinic	isidia	USA, ID, Lemhi Co.	45.0611	-113.7130	1362 m	Leavitt et al.
460f	*X. chlorochroa*	BRY-55403	salazinic	fragmentation	USA, ID, Lemhi Co.	45.0611	-113.7130	1362 m	Leavitt et al.
461f	*X. chlorochroa*	BRY-55404	salazinic	fragmentation	USA, ID, Lemhi Co.	45.0611	-113.7130	1362 m	Leavitt et al.
462f	*X. chlorochroa*	BRY-55405	salazinic	fragmentation	USA, ID, Lemhi Co.	45.0611	-113.7130	1362 m	Leavitt et al.

463f	*X. chlorochroa*	BRY-55406	salazinic	fragmentation	USA, ID, Lemhi Co.	45.0611	-113.7130	1362 m	Leavitt et al.
464f	*X. neowyomingica**	BRY-55407	stictic	not observed	USA, UT, Summit Co.	40.8581	-110.5012	3645 m	Leavitt et al.
465f	*X. chlorochroa*	BRY-55408	salazinic	fragmentation	USA, NM, San Juan Co.	36.1167	-107.8333	1940 m	BRY-10272
466f	*X. chlorochroa*	BRY-55409	salazinic	fragmentation	USA, NM, Navajo Indian Reservation.	36.3833	-108.2167	1910 m	BRY-10274
481f	*X. lineola*	BRY-55410	salazinic	not observed	USA, UT, Utah Co.	40.4897	-111.7747	1740 m	Leavitt et al.
482f	*X. plittii*	BRY-55411	stictic	isidia	USA, UT, Utah Co.	40.4897	111.7747	1740 m	Leavitt et al.
486f	*X. lineola*	BRY-55412	salazinic	apothecia	USA, AZ, Gila Co.	34.1437	-111.5646	1650 m	EA7-58
489f	*X. chlorochroa*	BRY-55413	salazinic	fragmentation	USA, MT, McCone Co.	48.0100	-106.3888	732 m	B. McCune 29318
490f	*X. wyomingica*	BRY-55414	salazinic	not observed	USA, MT, Phillips Co.	48.4568	-107.6567	720 m	B. MCCune 29317
491f	*X. chlorochroa*	BRY-55415	salazinic	fragmentation	USA, MT, Fallon Co.	46.5050	-104.1770	1036 m	McCune 28170
492f	*X. chlorochroa*	BRY-55416	salazinic	fragmentation	USA, UT, Utah Co.	39.8426	-111.1298	2393 m	SDL & JHL
493f	*X. chlorochroa*	BRY-55417	salazinic	fragmentation	USA, UT, Utah Co.	39.8426	-111.1298	2393 m	SDL & JHL
494f	*X. angustiphylla*	BRY-55418	stictic	not observed	USA, NC, Cherokee Co.	35.0316	-83.2387	1029 m	SDL
495f	*X. angustiphylla*	BRY-55419	stictic	not observed	USA, NC, Cherokee Co.	35.0316	-83.2387	1029 m	SDL
496f	*X. plittii*	BRY-55420	stictic	isidiate	USA, NC, Rutherford Co.	35.4327	-82.2505	680 m	Leavitt et al.
497f	*X. plittii*	BRY-55421	stictic	isidiate	USA, NC, Rutherford Co.	35.4327	-82.2505	680 m	Leavitt et al.
498f	*X. plittii*	BRY-55422	stictic	isidiate	USA, NC, Avery Co.	36.0953	-81.8292	1530 m	Leavitt et al.
499f	*X. plittii*	BRY-55423	stictic	isidiate	USA, NC, Avery Co.	36.0953	-81.8292	1530 m	Leavitt et al.

501f	*X. wyomingica*	BRY-55424	salazinic	not observed	USA, WA, Lincoln Co.	47.3894	-117.8357	689 m	HCH, DJH
502f	*X. wyomingica*	BRY-55425	salazinic	not observed	USA, WA, Lincoln Co.	47.3894	-117.8357	689 m	HCH, DJH
504f	*X. mexicana*	BRY-55426	salazinic	isidia	USA, AZ, Coconino Co.	37.7117	-111.5944	1955 m	J. Hollinger 20080608.18
505f	*X. coloradoënsis*	BRY-55427	salazinic	not observed	USA, AZ, Coconino Co.	35.1534	-111.7409	2220 m	J. Hollinger 20080624.27
508f	*X. mexicana*	BRY-55428	salazinic	isidia	USA, UT, Wayne Co.	38.2454	-111.3768	2127 m	J. Hollinger 20080606.64
509f	*X. lineola*	BRY-55429	salazinic	apothecia	USA, UT, Wayne Co.	38.2454	-111.3768	2127 m	J. Hollinger 20080606.63
516f	*X. chlorochroa*	BRY-55430	salazinic	fragmentation	USA, ND, Slope Co.	46.4564	-103.9277	830 m	J. Hertz 2075
517f	*X. chlorochroa*	BRY-55431	salazinic	fragmentation	USA, ND, Slope Co.	46.4564	-103.9277	830 m	J. Hertz 2075
525f	*X. chlorochroa*	BRY-55432	salazinic	fragmentation	USA, ND, Dunn Co.	47.3721	-102.9963	610 m	Esslinger 16617
526f	*X. chlorochroa*	BRY-55433	salazinic	fragmentation	USA, ND, Dunn Co.	47.3721	-102.9963	610 m	Esslinger 16617
527f	*X. camtschadalis*	BRY-55434	salazinic	fragmentation	USA, MT, Stillwater Co.	45.6011	-109.0660	1110 m	Esslinger 12685
534f	*X. camtschadalis*	BRY-55435	salazinic	fragmentation	USA, ND, Dunn Co.	47.5048	-102.6341	730 m	G. Lind 1213
535f	*X. camtschadalis*	BRY-55436	salazinic	fragmentation	USA, ND, Dunn Co.	47.5048	-102.6341	730 m	G. Lind 1213
536f	*X. chlorochroa*	BRY-55437	salazinic	fragmentation	USA, ND, Dunn Co.	47.5048	-102.6341	730 m	G. Lind 1213
574f	*X. chlorochroa*	BRY-55438	salazinic	fragmentation	USA, UT, Millard Co.	38.5945	-113.7430	760 m	Leavitt et al.
575f	*X. cumberlandia*	BRY-55439	stictic	not observed	USA, CA, San Diego Co.	32.9185	-117.2553	90 m	SDL, DHL, AB
576f	*X. plittii*	BRY-55440	stictic	isidia	USA, CA, San Diego Co.	32.9185	-117.2553	90 m	Leavitt et al.
577f	*X cumberlandia*	BRY-55441	stictic	not observed	USA, CA, Marin Co.	37.9111	-122.6243	592 m	SDL
578f	*X. mexicana*	BRY-55442	salazinic	not observed	USA, CA, Marin	37.9111	-122.6243	605 m	SDL

					Co.				
579f	*X. cumberlandia*	BRY-55443	stictic	not observed	USA, CA, Marin Co.	37.9978	-123.0118	142 m	SDL
580f	*X. lineola*	BRY-55444	salazinic	not observed	USA, AZ, Maricopa Co.	33.8474	-111.4720	1150 m	R. Fuller
665f	*X. chlorochroa*	BRY-55445	salazinic	fragmentation	USA, CO, Archuleta Co.	37.2051	-107.3274	1995 m	SDL & HCL
666f	*X. chlorochroa*	BRY-55446	salazinic	fragmentation	USA, CO, Archuleta Co.	37.2051	-107.3274	1995 m	SDL & HCL
771f	*X. norchlorochroa*	BRY-55447	norstictic	fragmentation	USA, CO, Rio Blano Co.	39.8278	-107.2985	3020 m	SDL, LLS, GS
772f	*X. chlorochroa*	BRY-55448	salazinic	fragmentation	USA, UT, Piute/Beaver Co.	38.2328	-112.3652	3035 m	M. Greenwood
773f	*X. wyomingica*	BRY-55449	salazinic	not observed	USA, MT, Lewis and Clark Co.	46.8206	-111.8160	1280 m	LLS, RCS, GS, SDL
774f	*X. mexicana**	BRY-55450	salazinic	isidia	USA, MT, Lewis and Clark Co.	46.8206	-111.8160	1280 m	LLS, RCS, GS, SDL
775f	*X. chlorochroa*	BRY-55451	salazinic	fragmentation	USA, CO, Summit Co.	39.8790	-106.2781	2447 m	SDL
776f	*X. chlorochroa (apotheciate)*	BRY-55452	salazinic	apothecia/fragmentation	USA, CO, Teller Co.	38.9275	-106.2824	2545 m	SDL
777f	*X. camtschadalis*	BRY-55453	salazinic	fragmentation	USA, SD. Perkins Co.	45.9230	-102.3628	760 m	SDL
778f	*X. chlorochroa*	BRY-55454	salazinic	fragmentation	USA, SD, Harding Co.	45.3998	-103.1636	991 m	SDL
779f	*X. chlorochroa*	BRY-55455	salazinic	fragmentation	USA, SD, Butte Co.	45.0651	-103.3813	890 m	SDL
780f	*X. chlorochroa (apotheciate)*	BRY-55456	salazinic	apothecia/fragmentation	USA, ND, Dunn Co.	47.3578	-103.0523	751 m	SDL
781f	*X. chlorochroa*	BRY-55457	salazinic	fragmentation	USA, ND, Billings Co.	46.7874	-103.3164	847 m	SDL
782f	*X. chlorochroa*	BRY-55458	salazinic	fragmentation	USA, NV, White Pine Co.	39.3035	-114.3727	1706 m	SDL and LLS
783f	*X. chlorochroa (apotheciate)*	BRY-55459	salazinic	apothecia/fragmentation	USA, NE, Souix Co.	42.1191	-103.6791	1431 m	SDL
784f	*X. chlorochroa*	BRY-55460	salazinic	fragmentation	USA, NE, Souix Co.	42.4657	-103.7942	1423 m	SDL

785f	*X. chlorochroa*	BRY-55461	salazinic	fragmentation	USA, ND, Morton Co.	46.8908	101.4294	650 m	SDL
786f	*X. mexicana*	BRY-55462	salazinic	isidia	USA, ND, Mercer Co.	47.4202	-101.6317	641 m	SDL
787f	*X. idahoensis*	BRY-55463	salazinic	not observed	USA, WY, Albany Co.	41.3240	-105.7434	2240 m	SDL
788f	*X. norchlorochroa*	BRY-55464	salazinic	fragmentation	USA, WY, Sweetwater Co.	41.0765	-108.1540	1576 m	J. Munsha
789f	*X. chlorochroa*	BRY-55465	salazinic	fragmentation	USA, WY, Hot Springs Co.	43.5916	-107.8383	1576 m	J. Munsha
790f	*X. wyomingica*	BRY-55466	salazinic	not observed	USA, WY, Johnson Co.	44.3385	-106.7656	1581 m	SDL
791f	*X. chlorochroa*	BRY-55467	salazinic	fragmentation	USA, WY, Lincoln Co.	41.8246	-110.7632	2019 m	SDL
792f	*X. chlorochroa*	BRY-55468	salazinic	fragmentation	USA, MT, Custer Co.	46.3748	-105.8818	673 m	J. Munsha
793f	*X. chlorochroa*	BRY-55469	salazinic	fragmentation	USA, MT, Custer Co.	46.3955	-105.7800	853 m	J. Munsha
794f	*X. chlorochroa*	BRY-55470	salazinic	fragmentation	USA, MT, Bighorn Co.	45.1064	-106.7873	1058 m	SDL
795f	*X. chlorochroa*	BRY-55471	salazinic	fragmentation	USA, MT, Custer Co.	46.3187	-105.9884	814 m	SDL
796f	*X. chlorochroa*	BRY-55472	salazinic	fragmentation	USA, CO, Arapahoe Co.	39.7319	-103.9356	1585 m	SDL
797f	*X. camtschadalis*	BRY-55473	salazinic	fragmentation	USA, CO, Larimie Co.	40.8532	-105.2568	1920 m	SDL
798f	*X. chlorochroa*	BRY-55474	salazinic	fragmentation	USA, CO, Elbert Co.	39.4477	-103.9247	1674 m	SDL
799f	*X. chlorochroa*	BRY-55475	salazinic	fragmentation	USA, CO, Elbert Co.	39.3425	-104.5777	2013 m	SDL
800f	*X. chlorochroa*	BRY-55476	salazinic	fragmentation	USA, CO, Weld Co.	40.6403	-104.4489	1519 m	SDL
801f	*X. chlorochroa*	BRY-55477	salazinic	fragmentation	USA, WY, Sweetwater Co.	42.2370	-109.1712	2112 m	SDL
802f	*X. chlorochroa*	BRY-55478	salazinic	fragmentation	USA, WY, Crook Co.	44.2751	-104.9885	1293 m	SDL
804f	*X. chlorochroa*	BRY-55479	salazinic	fragmentation	USA, WY,	43.2021	-107.9202	1569 m	SDL

					Fremont Co.				
805f	*X. chlorochroa*	BRY-55480	salazinic	fragmentation	USA, WY, Natroma Co.	43.0346	-106.8668	1713 m	SDL
806f	*X. chlorochroa*	BRY-55481	salazinic	fragmentation	USA, WY, Albany Co.	41.7412	-104.8854	1661 m	SDL
807f	*X. chlorochroa*	BRY-55482	salazinic	fragmentation	USA, WY, Converse Co.	42.7963	-105.6146	1584 m	SDL
808f	*X. chlorochroa*	BRY-55483	salazinic	fragmentation	USA, WY, Lincoln Co.	41.9526	-110.2440	2046 m	SDL
809f	*X. chlorochroa*	BRY-55484	salazinic	fragmentation	USA, WY, Johnson Co.	44.2165	-106.3028	1418 m	SDL
810f	*X. chlorochroa*	BRY-55485	salazinic	fragmentation	USA, WY, Cambell Co.	44.2854	-105.1447	1304 m	SDL
811f	*X. chlorochroa*	BRY-55486	salazinic	fragmentation	USA, WY, Niobara Co.	42.7607	-104.9120	1535 m	SDL
812f	*X. chlorochroa*	BRY-55487	salazinic	fragmentation	USA, WY, Fremont Co.	42.9370	-108.4622	1576 m	SDL
813f	*X. camtschadalis*	BRY-55488	salazinic	fragmentation	USA, WY, Albany Co.	40.9999	-105.4130	2310 m	SDL
814f	*X. chlorochroa*	BRY-55489	salazinic	fragmentation	USA, WY, Cambell Co.	44.2052	-105.8470	1417 m	SDL
815f	*X. chlorochroa*	BRY-55490	salazinic	fragmentation	USA, WY, Albany Co.	41.3239	-105.7434	2235 m	SDL
816f	*X. chlorochroa*	BRY-55491	salazinic	fragmentation	USA, WY, Converse Co.	43.6905	-105.4714	1497 m	SDL
817f	*X. camtschadalis*	BRY-55492	salazinic	not observed	USA, WY, Platte Co.	41.8191	-105.2622	2150 m	SDL
818f	*X. chlorochroa*	BRY-55493	salazinic	fragmentation	USA, WY, Natroma Co.	43.0836	-107.2107	1862 m	SDL
819f	*X. chlorochroa*	BRY-55494	salazinic	fragmentation	USA, WY, Albany Co.	41.5827	-105.6372	2177 m	SDL
820f	*X. chlorochroa*	BRY-55495	salazinic	fragmentation	USA, CO, Weld Co.	40.6097	-103.8026	1431 m	SDL
821f	*X. chlorochroa*	BRY-55496	salazinic	fragmentation	USA, CO, Park Co.	39.0254	-105.8137	2733 m	SDL
822f	*X. chlorochroa*	BRY-55497	salazinic	fragmentation	USA, CO, Chaffee Co.	38.8411	106.0059	2673 m	SDL

823f	*X. wyominigica (with apothecia)*	BRY-55498	salazinic	apothecia	USA, CO, Chaffee Co.	38.8411	-106.0059	2673 m	SDL
824f	*X. chlorochroa*	BRY-55499	salazinic	fragmentation	USA, CO, Moffat Co.	40.6206	-107.4658	1942 m	SDL
825f	*X. chlorochroa*	BRY-55500	salazinic	fragmentation	USA, CO, Jackson Co.	40.4252	-106.5233	2553 m	SDL
826f	*X. wyomingica (type)*	BRY-55501	salazinic	not observed	USA, WY, Johnson Co.	44.3394	-106.9768	2462 m	SDL
827f	*X. wyomingica (type)*	BRY-55502	salazinic	not observed	USA, WY, Johnson Co.	44.3394	-106.9768	2462 m	SDL
828f	*X. mexicana*	BRY-55503	salazinic	isidia	USA, WY, Johnson Co.	44.3394	-106.9768	2462 m	SDL
829f	*X. camtschadalis*	BRY-55504	salazinic	fragmentation	USA, ND, Billings Co.	47.6020	-103.4499	740 m	SDL
830f	*X. mexicana*	BRY-55505	salazinic	isidia	USA, NV, White Pine Co.	39.2478	-114.1195	2326 m	LLS and SDL
901f	*X. camtschadalis*	BRY-55506	salazinic	not observed	Canada, BC, Kamloops.	50.7607	-118.8457	2080 m	C. Bjork 2008, s. n.
902f	*X. camtschadalis*	BRY-55507	salazinic	not observed	Canada, BC, Kamloops.	50.7607	-118.8457	2080 m	T. Goward 2008, s.n.
903f	*X. cumberlandia*	BRY-55508	stictic	apothecia	Canada, BC, Osoyoos	49.0320	-119.4660	1300'	C. Bjork 2007-15213
904f	*X. cumberlandia*	BRY-55509	sticitic	not observed	Canada, BC, Table Mountain	51.8643	-119.9833	1027 m	T. Goward 2008, s.n.
905f	*X. cumberlandia*	BRY-55510	sticitic	apothecia	Canada, BC, Frogpond Trail	51.8654	-120.0405	692 m	T. Goward 2008, s.n.
906f	*X. stenophylla*	BRY-55511	salazinic	not observed	Canada, BC, Edgewood	51.8686	-120.0215	714 m	T. Goward 2008, s.n.
908f	*X. stenophylla*	BRY-55512	salazinic	not observed	Canada, BC, Boulder City	51.8699	-120.0257	715 m	T. Goward 2008, s.n.
909f	*X. cumberlandia*	BRY-55513	sticitic	not observed	Canada, BC, Table Mtn	51.8643	-119.9833	1027 m	T. Goward 2008, s.n.
911f	*X. stenophylla*	BRY-55514	salazinic	not observed	Canada, BC, Fage Bluffs	51.8024	-120.0295	640 m	T. Goward 2008, s.n.
912f	*X.plittii*	BRY-55515	stictic	Isidia	Canada, BC, Kamloops.	50.7607	-118.8457	2080 m	T. Goward 2008, s.n.
913f	*X. cumberlandia*	BRY-55516	sticitic	not observed	Canada, BC,	51.8024	-120.0295	640 m	T. Goward

					Fage Bluffs				2008, s.n.
914f	*X. cumberlandia*	BRY-55517	sticitic	apothecia	Canada, BC, Edgewood	51.8686	-120.0215	714 m	T. Goward 2008, s.n.
915f	*X. stenophylla*	BRY-55518	salazinic	not observed	Canada, BC, Fage Bluffs	51.8024	-120.0295	640 m	T. Goward 2008, s.n.
916f	*X. mexicana*	BRY-55519	salazinic	isidia	WA, Spokane Co.	47.4189	-117.5688	700 m	C. Bjork 17714
917f	*X. stenophylla*	BRY-55520	salazinic	apothecia	Canada, BC, Edgewood West	51.8706	-120.0305	714 m	J. Hollinger 17714
918f	*X. stenophylla*	BRY-55521	salazinic	apothecia	Canada, BC, WGP: Edgewood	51.8686	-120.0215	714 m	T. Goward 2008, s.n.
919f	*X. plittii*	BRY-55522	stictic	isidia	USA, MT, Mineral Co.	47.2254	-114.9657	820 m	T. Goward 2008, s.n.
920f	*X. mexicana*	BRY-55523	salazinic	isidia	USA, MT, Carter Co.	45.8192	-104.4400	1100 m	T. Wheeler 1875
922f	*X. coloradoënsis*	BRY-55524	salazinic	not observed	USA, MT, Sanders Co.	48.0413	-115.7517	1630 m	T. Wheeler 1371
923f	*X. coloradoënsis*	BRY-55525	salazinic	not observed	USA, MT, Lake Co.	47.2952	-113.8312	2370 m	T. Wheeler 1409
924f	*X. camtschadalis*	BRY-55526	salazinic	fragmentation	Canada, BC,	55.1945	-123.2966	970 m	C. Bjork 16372
925f	*X. camtschadalis*	BRY-55527	salazinic	fragmentation	Canada, Saskatchewan	55.5717	-123.2966	1280 m	McCintosh 8828e
926f	*X. wyomingica*	BRY-55528	salazinic	not observed	USA, MT, Russell Co	47.7561	-110.8991	830 m	T. Wheeler 2006, s.n.
927f	*X. wyomingica*	BRY-55529	salazinic	not observed	USA, MT, Russell Co	47.7561	-110.8991	830 m	T. Wheeler 2006, s.n.
928f	*X. cumberlandia*	BRY-55530	sticitic	not observed	Canada, BC, Blue Bluffs	51.8000	-120.0203	496 m	T. Goward 2008, s.n.
929f	*X. cumberlandia*	BRY-55531	sticitic	not observed	Canada, BC, WGP: Edgewood	51.8706	-120.0305	714 m	T. Goward 2008, s.n.
930f	*X. cumberlandia*	BRY-55532	sticitic	not observed	USA, WA, Spokane Co.	47.4189	-117.5688	700 m	C. Bjork 17719
931f	*X. cumberlandia*	BRY-55533	sticitic	not observed	Canada, BC, Kamloops	50.7607	-118.8457	2080 m	T. Goward 2008, s.n.
932f	*X. cumberlandia*	BRY-55534	sticitic	not observed	USA, WA, Spokane Co.	47.3631	-117.5804	700 m	C. Bjork 16671

933f	*X. stenophylla*	BRY-55535	salazinic	not observed	Canada, BC, Edgewood	51.8686	-120.0215	714 m	T. Goward 2008, s.n.
934f	*X. stenophylla*	BRY-55536	salazinic	not observed	Canada, BC, Boulder City	51.8699	-120.0257	715 m	T. Goward 2008, s.n.
935f	*X. cumberlandia*	BRY-55537	sticitic	apothecia	Canada, BC, Fage Bluffs	51.8024	-120.0295	640 m	T. Goward 2008, s.n.
936f	*X. mexicana*	BRY-55538	salazinic	isidia	USA, WA, Grand Co.	47.9449	-119.0282	510 m	C. Bjork 17707
937f	*X. cumberlandia*	BRY-55539	sticitic	apothecia	Canada, BC, Frogpond Trail	51.8654	-120.0405	692 m	J. Hollinger, s.n.
938f	*X. cumberlandia*	BRY-55540	sticitic	not observed	Canada, BC, Edgewood	51.8686	-120.0215	714 m	T. Goward 2008, s.n.
939f	*X. cumberlandia*	BRY-55541	sticitic	not observed	Canada, BC, Boulder City	51.8699	-120.0257	715 m	T. Goward 2008, s.n.
940f	*X. stenophylla*	BRY-55542	salazinic	not observed	Canada, BC, Boulder City	51.8699	-120.0257	715 m	T. Goward 2008, s.n.
941f	*X. stenophylla*	BRY-55543	salazinic	not observed	Canada, BC, Boulder City	51.8699	-120.0257	715 m	T. Goward 2008, s.n.
942f	*X. stenophylla*	BRY-55544	salazinic	not observed	Canada, BC, Edgewood	51.8686	-120.0215	714 m	T. Goward 2008, s.n.
943f	*X. stenophylla*	BRY-55545	salazinic	not observed	Canada, BC, Boulder City	51.8699	-120.0257	715 m	T. Goward 2008, s.n.
944f	*X. cumberlandia*	BRY-55546	sticitic	not observed	Canada, BC, Frogpond Trail	51.8654	-120.0405	692 m	J. Hollinger, s.n.
945f	*X. stenophylla*	BRY-55547	salazinic	not observed	Canada, BC, Frogpond Trail	51.8654	-120.0405	692 m	T. Goward 2008, s.n.
946f	*X. stenophylla*	BRY-55548	salazinic	not observed	Canada, BC, Edgewood West	51.8706	-120.0305	714 m	T. Goward 2008, s.n.
947f	*X. subplittii*	BRY-55549	stictic	isidia	Canada, BC, Fage Bluffs	51.8024	-120.0295	640 m	T. Goward 2008, s.n.
948f	*X. camtschadalis*	BRY-55550	salazinic	not observed	Canada, BC, Kamloops.	50.6880	-120.4685	410 m	T. Goward 2008, s.n.
949f	*X. camtschadalis*	BRY-55551	salazinic	not observed	Canada, BC, Kamloops.	50.6880	-120.4685	410 m	T. Goward 2008, s.n.
950f	*X. wyomingica*	BRY-55552	salazinic	not observed	USA, WA, Lincoln Co.	47.5902	-118.5359	670 m	C. Bjork 2008 15542
951f	*X. stenophylla*	BRY-55553	salazinic	not observed	Canada, BC,	50.6880	-120.4685	670 m	T. Goward

					Kamloops.				2008, s.n.
952f	*X. stenophylla*	BRY-55554	salazinic	not observed	Canada, BC, Edgewood	51.8686	-120.0215	715 m	T. Goward 2008, s.n.
953f	*X. stenophylla*	BRY-55555	salazinic	not observed	Canada, BC, Edgewood West	51.8706	-120.0305	714 m	T. Goward 2008, s.n.
954f	*X. cumberlandia*	BRY-55556	sticitic	not observed	Canada, BC, Table Mtn	51.8643	-119.9833	1027 m	T. Goward 2008, s.n.
955f	*X. wyomingica*	BRY-55557	salazinic	not observed	USA, MT, Russell Co.	47.7561	-110.8991	830 m	T. Wheeler 2006 s.n.
956f	*X. stenophylla*	BRY-55558	salazinic	apothecia	Canada, BC, Edgewood West	51.8706	-120.0305	714 m	T. Goward 2008, s.n.
957f	*X. stenophylla*	BRY-55559	salazinic	not observed	Canada, BC, Edgewood West	51.8706	-120.0305	714 m	T. Goward 2008, s.n.
1026f	*X. cumberlandia**	BRY-55560	stictic	not observed	USA, CA, San Luis Obispo Co.	35.3566	-120.6558	710 m	SDL & LG
1027f	*X. lineola*	BRY-55561	salazinic	apothecia	USA, CA, San Luis Obispo Co.	35.3566	-120.6558	710 m	SDL & LG
1028f	*X. mexicana*	BRY-55562	salazinic	isidia/apothecia	USA, CA, San Luis Obispo Co.	35.4778	-120.9923	20 m	SDL & LG
1029f	*X. mexicana*	BRY-55563	salazinic	isidia	USA, CA, San Luis Obispo Co.	35.4778	-120.9923	20 m	SDL & LG
1030f	*X. coloradoënsis*	BRY-55564	salazinic	not observed	USA, CA, San Luis Obispo Co.:	35.4778	-120.9923	20 m	SDL & LG
1031f	*X. cumberlandia*	BRY-55565	stictic	apothecia	USA, CA, San Luis Obispo Co.	35.4778	-120.9923	20 m	SDL & LG
1032f	*X. cumberlandia*	BRY-55566	stictic	apothecia	USA, CA, San Luis Obispo Co.	35.4778	-120.9923	20 m	SDL & LG
Outgroup taxa									
	Karoowia saxeti		-	-	Taiwan, Pingtung Co.	-	-	-	
538f	*Karoowia saxeti*	BRY-55567	-	-	Uruguay, Florida	34.20576	-55.97073		Leavitt et al.
540f	*Karoowia saxeti*	BRY-55568	-	-	Uruguay, Florida	34.20576	-55.97073		Leavitt et al.
-	*X. brachinaensis*	CANB	-	-	Australia, Flinders Ranges	-	-	-	GenBank
-	*X. convoluta*	GZU 46511	-	-	Namibia, Swakopmund	-	-	-	GenBank
-	*X. crespoae*	MAF 7524	-	-	Australia, New	-	-	-	GenBank

					south Wales				
-	*X. lithophila*	MAF 6900	-	-	Australia, New South Wales	-	-	-	GenBank
-	*X. loxodes*	MAF 6206	-	-	Spain, Zamora	-	-	-	GenBank
907f	*X. mougeotii*	BRY-55569	-	-	USA, WA, Spokane Co.	47.41892	-117.56883	700 m	C. Bjork 17756
-	*X. murina*	MAF 9915	-	-	Australia, Norton National Park	-	-	-	GenBank
-	*X. notata*	CANB	-	-	Australia, Australian Capital Territories	-	-	-	GenBank
-	*X. scotophylla*	CANB	-	-	Australia, Mount Remarkable National Park	-	-	-	GenBank
-	*X. semiviridis*	MAF 6876	-	-	Australia, New South Wales	-	-	-	GenBank
-	*X. subprolixa*	MAF 7667	-	-	Australia, Australian Capital Territory	-	-	-	GenBank
-	*X. tegeta*	MAF 7523	-	-	Australia, Australian Capital Territories	-	-	-	GenBank
-	*X. tinctina*	MAF 6070	-	-	Spain, Gerona	-	-	-	GenBank
-	*X. transvaalensis*	MAF 9841	-	-	Spain, Zaragoza	-	-	-	GenBank
-	*X. verrucigera*	MAF 9920	-	-	Spain, Gerona	-	-	-	GenBank

Supplementary data 2.2. GenBank accession numbers for all *Xanthoparmelia* specimens included in the present study: ID, individual code; Brigham Young University Herbarium of Non-vascular Cryptogams (BRY) voucher accession number; GenBank accession numbers for LSU, ITS, IGS, group I intron, *MCM7*, and β-tubulin markers.

ID	Species (sensu lato)	Herbarium Acc. No.	LSU	ITS	IGS	intron	*MCM7*	β-tubulin
001f	*X. coloradoënsis*	BRY-55151	HM579019	HM578607	HM577905	HM578296	HM579426	HM577516
002f	*X. cumberlandia*	BRY-55152	HM579020	HM578608	HM577906	HM578297	HM579427	HM577517
003f	*X. cumberlandia*	BRY-55153	HM579021	HM578609	HM577907	HM578298	HM579428	HM577518
004f	*X. chlorochroa*	BRY-55154	HM579022	HM578610	HM577908	HM578299	HM579429	HM577519
005f	*X. chlorochroa*	BRY-55155	HM579023	HM578611	HM577909	HM578300	HM579430	HM577520
006f	*X. coloradoënsis*	BRY-55156	HM579024	HM578612	HM577910	HM578301	HM579431	HM577521
007f	*X. norchlorochroa*	BRY-55157	HM579025	HM578613	HM577911	HM578302	HM579432	HM577522
008f	*X. chlorochroa*	BRY-55158	HM579026	HM578614	HM577912	HM578303	HM579433	HM577523
009f	*X. chlorochroa*	BRY-55159	HM579027	HM578615	HM577913	HM578304	HM579434	HM577524
010f	*X. chlorochroa*	BRY-55160	HM579028	HM578616	HM577914	HM578305	HM579435	HM577525
011f	*X. chlorochroa*	BRY-55161	HM579029	HM578617	HM577915	HM578306	HM579436	HM577526
012f	*X. coloradoënsis*	BRY-55162	HM579030	HM578618	HM577916	HM578307	HM579437	HM577527
013f	*X. norchlorochroa*	BRY-55163	HM579031	HM578619	HM577917	HM578308	HM579438	HM577528
014f	*X. chlorochroa*	BRY-55164	HM579032	HM578620	HM577918	HM578309	HM579439	HM577529
015f	*X. chlorochroa*	BRY-55165	HM579033	HM578621	HM577919	-	HM579440	HM577530
016f	*X. chlorochroa*	BRY-55166	HM579034	HM578622	HM577920	HM578310	HM579441	HM577531
017f	*X. coloradoënsis*	BRY-55167	HM579035	HM578623	HM577921	HM578311	HM579442	HM577532
018f	*X. coloradoënsis*	BRY-55168	HM579036	HM578624	HM577922	HM578312	HM579443	HM577533
019f	*X. coloradoënsis*	BRY-55169	HM579037	HM578625	-	HM578313	HM579444	HM577534
020f	*X. coloradoënsis*	BRY-55170	HM579038	HM578626	HM577923	HM578314	HM579445	HM577535
022f	*X. coloradoënsis*	BRY-55171	HM579039	HM578627	HM577924	HM578315	HM579446	HM577536
023f	*X. coloradoënsis*	BRY-55172	HM579040	HM578628	HM577925	HM578316	HM579447	HM577537
024f	*X. cumberlandia*	BRY-55173	HM579041	HM578629	HM577926	-	HM579448	HM577538
025f	*X. camtschadalis*	BRY-55174	HM579042	HM578630	HM577927	-	HM579449	HM577539
027f	*X. chlorochroa*	BRY-55175	HM579043	HM578631	HM577928	HM578317	HM579450	HM577540
028f	*X. chlorochroa*	BRY-55176	HM579044	HM578632	HM577929	HM578318	HM579451	HM577541
029f	*X. cumberlandia*	BRY-55177	HM579045	HM578633	HM577930	-	HM579452	HM577542
030f	*X. coloradoënsis*	BRY-55178	HM579046	HM578634	HM577931	HM578319	HM579453	HM577543
031f	*X. chlorochroa*	BRY-55179	HM579047	HM578635	HM577932	HM578320	HM579454	HM577544
032f	*X. coloradoënsis*	BRY-55180	HM579048	HM578636	HM577933	HM578321	HM579455	HM577545
033f	*X. coloradoënsis*	BRY-55181	HM579049	HM578637	HM577934	HM578322	HM579456	HM577546
034f	*X. coloradoënsis*	BRY-55182	HM579050	HM578638	HM577935	HM578323	HM579457	HM577547
035f	*X. coloradoënsis*	BRY-55183	HM579051	HM578639	HM577936	HM578324	HM579458	HM577548
036f	*X. cumberlandia*	BRY-55184	HM579052	HM578640	HM577937	HM578325	HM579459	HM577549
037f	*X. californica*	BRY-55185	HM579053	HM578641	HM577938	HM578326	HM579460	HM577550
038f	*X. cumberlandia*	BRY-55186	HM579054	HM578642	HM577939	-	HM579461	HM577551
039f	*X. cumberlandia*	BRY-55187	HM579055	HM578643	HM577940	HM578327	HM579462	HM577552
040f	*X. cumberlandia*	BRY-55188	HM579056	HM578644	HM577941	-	HM579463	HM577553
041f	*X. cumberlandia*	BRY-55189	HM579057	HM578645	HM577942	HM578328	HM579464	HM577554
042f	*X. cumberlandia*	BRY-55190	HM579058	HM578646	HM577943	-	-	HM577555
043f	*X. cumberlandia*	BRY-55191	HM579059	HM578647	HM577944	HM578329	HM579465	HM577556
044f	*X. cumberlandia*	BRY-55192	HM579060	HM578648	-	HM578330	HM579466	HM577557
045f	*X. cumberlandia*	BRY-55193	HM579061	HM578649	-	-	HM579467	-
046f	*X. neowyomingica*	BRY-55194	HM579062	HM578650	HM577945	HM578331	HM579468	HM577558
047f	*X. cumberlandia*	BRY-55195	HM579063	HM578651	HM577946	HM578332	HM579469	HM577559
048f	*X. chlorochroa*	BRY-55196	HM579064	HM578652	HM577947	HM578333	HM579470	HM577560
049f	*X. cumberlandia*	BRY-55197	HM579065	HM578653	HM577948	HM578334	HM579471	HM577561
052f	*X. chlorochroa*	BRY-55198	HM579066	HM578654	HM577949	HM578335	HM579472	HM577562
053f	*X. chlorochroa*	BRY-55199	HM579067	HM578655	HM577950	HM578336	HM579473	HM577563
054f	*X. coloradoënsis*	BRY-55200	HM579068	HM578656	HM577951	HM578337	HM579474	HM577564
055f	*X. coloradoënsis*	BRY-55201	HM579069	HM578657	HM577952	HM578338	HM579475	HM577565
056f	*X. cumberlandia*	BRY-55202	HM579070	HM578658	HM577953	HM578339	HM579476	-
057f	*X. cumberlandia*	BRY-55203	HM579071	HM578659	HM577954	HM578340	HM579477	HM577566

058f	*X. cumberlandia*	BRY-55204	HM579072	HM578660	HM577955	HM578341	HM579478	HM577567
059f	*X. coloradoënsis*	BRY-55205	HM579073	HM578661	HM577956	HM578342	HM579479	HM577568
061f	*X. cumberlandia*	BRY-55206	-	HM578662	-	-	-	-
063f	*X. cumberlandia*	BRY-55208	HM579074	HM578663	HM577957	HM578343	HM579480	HM577569
064f	*X. coloradoënsis*	BRY-55209	HM579075	HM578664	HM577958	HM578344	HM579481	-
065f	*X. cumberlandia*	BRY-55210	HM579076	HM578665	-	HM578345	-	HM577570
066f	*X. cumberlandia*	BRY-55211	-	HM578666	-	-	-	HM577571
067f	*X. coloradoënsis*	BRY-55212	HM579077	HM578667	HM577959	HM578346	HM579482	HM577572
068f	*X. chlorochroa*	BRY-55213	HM579078	HM578668	HM577960	HM578347	HM579483	HM577573
069f	*X. chlorochroa*	BRY-55214	HM579079	HM578669	HM577961	HM578348	HM579484	HM577569
070f	*X. lineola*	BRY-55215	HM579080	HM578670	HM577962	-	HM579485	HM577575
071f	*X. cumberlandia*	BRY-55216	HM579081	HM578671	-	HM578349	HM579486	-
072f	*X. cumberlandia*	BRY-55217	HM579082	HM578672	HM577963	HM578350	HM579487	-
073f	*X. coloradoënsis*	BRY-55218	HM579083	HM578673	HM577964	HM578351	HM579488	HM577576
074f	*X. cumberlandia*	BRY-55219	HM579084	HM578674	-	HM578352	HM579489	-
075f	*X. cumberlandia*	BRY-55220	HM579085	HM578675	HM577965	HM578353	HM579490	HM577577
076f	*X. cumberlandia*	BRY-55221	HM579086	HM578676	HM577966	HM578354	HM579491	HM577578
079f	*X. vagans*	BRY-55222	HM579087	HM578677	HM577967	-	HM579492	HM577579
080f	*X. vagans*	BRY-55223	HM579088	HM578678	HM577968	-	HM579493	HM577580
081f	*X. chlorochroa*	BRY-55224	HM579089	HM578679	HM577969	HM578355	HM579494	HM577581
082f	*X. chlorochroa*	BRY-55225	HM579090	HM578680	HM577970	HM578356	HM579495	HM577582
083f	*X. chlorochroa*	BRY-55226	HM579091	HM578681	HM577971	HM578357	HM579496	HM577583
084f	*X. chlorochroa*	BRY-55227	HM579092	HM578682	HM577972	HM578358	HM579497	HM577584
085f	*X. coloradoënsis*	BRY-55228	HM579093	HM578683	HM577973	-	HM579498	HM577585
086f	*X. coloradoënsis*	BRY-55229	HM579094	HM578684	HM577974	-	HM579499	HM577586
087f	*X. lavicola*	BRY-55230	HM579095	HM578685	HM577975	HM578359	HM579500	HM577587
090f	*X. chlorochroa*	BRY-55231	HM579096	HM578686	HM577976	HM578360	HM579501	HM577588
091f	*X. chlorochroa*	BRY-55232	HM579097	HM578687	HM577977	HM578361	HM579502	HM577589
097f	*X. mexicana*	BRY-55233	HM579098	HM578688	HM577978	HM578362	HM579503	HM577590
098f	*X. dierythra*	BRY-55234	HM579099	HM578689	HM577979	HM578363	HM579504	HM577591
102f	*X. chlorochroa*	BRY-55235	HM579100	HM578690	HM577980	HM578364	-	HM577592
110f	*X. chlorochroa*	BRY-55236	HM579101	HM578691	HM577981	HM578365	HM579505	HM577593
111f	*X. chlorochroa*	BRY-55237	HM579102	HM578692	HM577982	HM578366	HM579506	HM577594
112f	*X. chlorochroa*	BRY-55238	HM579103	HM578693	HM577983	HM578367	HM579507	HM577595
113f	*X. chlorochroa*	BRY-55239	HM579104	HM578694	HM577984	HM578368	HM579508	HM577596
118f	*X. coloradoënsis*	BRY-55240	HM579105	HM578695	HM577985	-	HM579509	HM577597
120f	*X. coloradoënsis*	BRY-55241	HM579106	HM578696	HM577986	HM578369	HM579510	HM577598
121f	*X. neowyomingica*	BRY-55242	HM579107	HM578697	HM577987	-	HM579511	HM577599
122f	*X. neowyomingica*	BRY-55243	HM579108	HM578698	HM577988	HM578370	HM579512	HM577600
123f	*X. neowyomingica*	BRY-55244	HM579109	HM578699	HM577989	HM578371	HM579513	HM577601
124f	*X. neowyomingica*	BRY-55245	HM579110	HM578700	HM577990	HM578372	HM579514	HM577602
125f	*X. neowyomingica*	BRY-55246	HM579111	HM578701	HM577991	HM578373	HM579515	HM577603
126f	*X. chlorochroa*	BRY-55247	HM579112	HM578702	HM577992	HM578374	HM579516	HM577604
127f	*X. chlorochroa*	BRY-55248	HM579113	HM578703	HM577993	HM578375	HM579517	HM577605
128f	*X. chlorochroa*	BRY-55249	HM579114	HM578704	HM577994	HM578376	HM579518	HM577606
129f	*X. chlorochroa*	BRY-55250	HM579115	HM578705	HM577995	HM578377	HM579519	HM577607
130f	*X. chlorochroa*	BRY-55251	HM579116	HM578706	HM577996	HM578378	HM579520	HM577608
131f	*X. chlorochroa*	BRY-55252	HM579117	HM578707	HM577997	HM578379	HM579521	HM577609
132f	*X. chlorochroa*	BRY-55253	HM579118	HM578708	HM577998	HM578380	HM579522	HM577610
133f	*X. chlorochroa*	BRY-55254	HM579119	HM578709	HM577999	HM578381	HM579523	HM577611
135f	*X. coloradoënsis*	BRY-55255	HM579120	HM578710	HM578000	HM578382	HM579524	HM577612
136f	*X. wyominigica*	BRY-55256	HM579121	HM578711	HM578001	HM578383	HM579525	HM577613
138f	*X. cumberlandia*	BRY-55257	HM579122	HM578712	HM578002	HM578384	HM579526	HM577614
147f	*X. mexicana*	BRY-55258	HM579123	HM578713	HM578003	HM578385	HM579527	HM577615
148f	*X. mexicana*	BRY-55259	HM579124	HM578714	HM578004	HM578386	HM579528	HM577616
149f	*X. mexicana*	BRY-55260	HM579125	HM578715	HM578005	HM578387	HM579529	HM577617
150f	*X. mexicana*	BRY-55261	HM579126	HM578716	HM578006	HM578388	HM579530	HM577618
151f	*X. mexicana*	BRY-55262	HM579127	HM578717	HM578007	HM578389	HM579531	HM577619
152f	*X. mexicana*	BRY-55263	HM579128	HM578718	HM578008	HM578390	HM579532	HM577620
153f	*X. mexicana*	BRY-55264	HM579129	-	HM578009	-	HM579533	HM577621

154f	*X. mexicana*	BRY-55265	HM579130	HM578719	HM578010	HM578391	HM579534	HM577622
155f	*X. plittii*	BRY-55266	HM579131	HM578720	HM578011	HM578392	HM579535	HM577623
156f	*X. mexicana*	BRY-55267	HM579132	HM578721	HM578012	HM578393	HM579536	HM577624
157f	*X. chlorochroa*	BRY-55268	HM579133	HM578722	HM578013	HM578394	HM579537	HM577625
163f	*X. chlorochroa*	BRY-55269	HM579134	HM578723	HM578014	HM578395	HM579538	HM577626
168f	*X. chlorochroa*	BRY-55270	HM579135	HM578724	HM578015	HM578396	HM579539	HM577627
169f	*X. coloradoënsis*	BRY-55271	HM579136	HM578725	HM578016	HM578397	HM579540	-
170f	*X. lineola*	BRY-55272	HM579137	HM578726	HM578017	HM578398	HM579541	HM577628
171f	*X. lineola*	BRY-55273	HM579138	HM578727	HM578018	HM578399	HM579542	HM577629
173f	*X. mexicana*	BRY-55274	HM579139	-	HM578019	-	HM579543	HM577630
175f	*X. cumberlandia*	BRY-55275	HM579140	HM578728	HM578020	HM578400	HM579544	HM577631
179f	*X. cumberlandia*	BRY-55276	HM579141	HM578729	HM578021	HM578401	HM579545	HM577632
180f	*X. chlorochroa*	BRY-55277	HM579142	HM578730	HM578022	HM578402	HM579546	HM577633
181f	*X. chlorochroa*	BRY-55278	HM579143	HM578731	HM578023	HM578403	HM579547	HM577634
189f	*X. chlorochroa*	BRY-55279	HM579144	HM578732	HM578024	HM578404	HM579548	-
190f	*X. chlorochroa*	BRY-55280	HM579145	HM578733	-	HM578405	HM579549	-
191f	*X. cumberlandia*	BRY-55281	HM579146	HM578734	-	HM578406	HM579550	-
192f	*X. cumberlandia*	BRY-55282	HM579147	HM578735	-	HM578407	HM579551	-
194f	*X. cumberlandia*	BRY-55283	HM579148	HM578736	-	-	HM579552	HM577635
195f	*X. cumberlandia*	BRY-55284	HM579149	HM578737	-	HM578408	HM579553	HM577636
197f	*X. mexicana*	BRY-55285	HM579150	HM578738	-	HM578409	HM579554	HM577637
198f	*X. cumberlandia*	BRY-55286	HM579151	HM578739	HM578025	HM578410	HM579555	HM577638
201f	*X. chlorochroa*	BRY-55287	HM579152	HM578740	HM578026	-	HM579556	HM577639
202f	*X. chlorochroa*	BRY-55288	HM579153	HM578741	HM578027	-	HM579557	HM577640
203f	*X. chlorochroa*	BRY-55289	HM579154	HM578742	HM578028	HM578411	HM579558	HM577641
204f	*X. chlorochroa*	BRY-55290	HM579155	HM578743	HM578029	HM578412	HM579559	HM577642
205f	*X. camtschadalis*	BRY-55291	HM579156	HM578744	HM578030	-	HM579560	HM577643
206f	*X. camtschadalis*	BRY-55292	HM579157	HM578745	HM578031	-	HM579561	HM577644
207f	*X. chlorochroa*	BRY-55293	HM579158	HM578746	HM578032	HM578413	HM579562	HM577645
208f	*X. chlorochroa*	BRY-55294	HM579159	HM578747	HM578033	HM578414	HM579563	HM577646
219f	*X. chlorochroa*	BRY-55295	HM579160	HM578748	HM578034	HM578415	HM579564	HM577647
220f	*X. chlorochroa*	BRY-55296	HM579161	HM578749	HM578035	HM578416	HM579565	HM577648
221f	*X. chlorochroa*	BRY-55297	HM579162	HM578750	HM578036	HM578417	HM579566	HM577649
222f	*X. vagans*	BRY-55298	HM579163	HM578751	HM578037	-	HM579567	HM577650
224f	*X. mexicana*	BRY-55299	HM579164	HM578752	HM578038	HM578418	HM579568	HM577651
226f	*X. dierythra*	BRY-55300	HM579165	HM578753	HM578039	HM578419	HM579569	HM577652
227f	*X. cumberlandia*	BRY-55301	HM579166	HM578754	HM578040	HM578420	HM579570	HM577653
229f	*X. chlorochroa*	BRY-55302	HM579167	HM578755	HM578041	HM578421	HM579571	HM577654
231f	*X. neochlorochroa*	BRY-55303	HM579168	HM578756	HM578042	HM578422	HM579572	HM577655
232f	*X. chlorochroa*	BRY-55304	HM579169	HM578757	HM578043	HM578423	HM579573	HM577656
233f	*X. chlorochroa*	BRY-55305	HM579170	HM578758	HM578045	HM578424	HM579574	HM577657
245f	*X. lineola*	BRY-55306	HM579171	HM578759	HM578046	HM578425	HM579575	-
247f	*X. cumberlandia*	BRY-55307	-	-	HM578047	-	-	-
258f	*X. coloradoënsis*	BRY-55308	HM579172	HM578760	HM578048	HM578426	HM579546	HM577658
261f	*X. vagans*	BRY-55309	HM579173	HM578761	HM578047	-	HM579577	HM577659
269f	*X. coloradoënsis*	BRY-55310	HM579174	HM578762	HM578048	HM578427	HM579578	HM577660
271f	*X. lineola*	BRY-55311	HM579175	HM578763	HM578049	-	HM579579	HM577661
272f	*X. coloradoënsis*	BRY-55312	HM579176	HM578764	HM578050	HM578428	HM579580	HM577660
274f	*X. psoromifera*	BRY-55313	HM579177	HM578765	HM578051	HM578429	HM579581	HM577663
275f	*X. psoromifera*	BRY-55314	HM579178	HM578766	HM578052	-	HM579582	HM577664
276f	*X. chlorochroa*	BRY-55315	HM579179	HM578767	HM578053	HM578430	HM579583	HM577665
278f	*X. neochlorochroa*	BRY-55316	HM579180	HM578768	HM578054	HM578431	HM579584	HM577666
279f	*X. neochlorochroa*	BRY-55317	HM579181	HM578769	HM578055	HM578432	HM579585	HM577667
280f	*X. lipochlorochroa*	BRY-55318	HM579182	HM578770	HM578056	HM578433	HM579586	HM577668
281f	*X. lipochlorochroa*	BRY-55319	HM579183	HM578771	HM578057	HM578434	HM579587	HM577669
282f	*X. lipochlorochroa*	BRY-55320	HM579184	HM578772	HM578058	HM578435	HM579588	HM577670
283f	*X. mexicana*	BRY-55321	HM579185	HM578773	HM578059	HM578436	HM579589	HM577671
284f	*X. lineola*	BRY-55322	HM579186	HM578774	HM578060	HM578437	HM579590	HM577672
285f	*X. lineola*	BRY-55323	HM579187	HM578775	HM578061	HM578438	HM579591	HM577673
286f	*X. plittii*	BRY-55324	HM579188	HM578776	HM578062	HM578438	HM579592	-

287f	*X. cumberlandia*	BRY-55325	HM579189	HM578777	HM578063	HM578440	HM579593	HM577674
288f	*X. cumberlandia*	BRY-55326	HM579190	HM578778	HM578064	HM578441	HM579594	HM577675
290f	*X. cumberlandia*	BRY-55327	HM579191	HM578779	HM578065	HM578442	HM579595	HM577676
291f	*X. mexicana*	BRY-55328	HM579192	HM578780	HM578066	HM578443	HM579596	HM577677
292f	*X. dierythra*	BRY-55329	HM579193	HM578781	-	HM578444	HM579597	HM577653
293f	*X. chlorochroa*	BRY-55330	HM579194	HM578782	HM578067	HM578445	HM579598	HM577679
294f	*X. chlorochroa*	BRY-55331	HM579195	HM578783	HM578068	HM578446	HM579599	HM577680
295f	*X. neochlorochroa*	BRY-55332	HM579196	-	HM578069	-	HM579600	HM577695
296f	*X. neochlorochroa*	BRY-55333	HM579197	HM578784	HM578070	HM578447	HM579601	HM577682
297f	*X. neochlorochroa*	BRY-55334	HM579198	HM578785	HM578071	HM578448	HM579602	HM577683
298f	*X. neochlorochroa*	BRY-55335	HM579199	HM578786	HM578072	HM578449	HM579603	HM577684
299f	*X. chlorochroa*	BRY-55336	HM579200	HM578787	HM578073	HM578450	HM579604	HM577685
300f	*X. chlorochroa*	BRY-55337	HM579201	HM578788	HM578074	HM578451	HM579605	HM577686
301f	*X. chlorochroa*	BRY-55338	HM579202	HM578789	HM578075	-	HM579606	HM577687
304f	*X.chlorochroa*	BRY-55339	-	HM578790	HM578076	HM578452	HM579607	HM577688
307f	*X. chlorochroa*	BRY-55340	HM579203	HM578791	-	HM578453	-	-
308f	*X. chlorochroa*	BRY-55341	-	HM578792	HM578077	HM578454	HM579608	HM577689
309f	*X. chlorochroa*	BRY-55342	HM579204	HM578793	HM578078	HM578455	-	HM577690
310f	*X. chlorochroa*	BRY-55343	HM579205	HM578794	HM578079	HM578456	HM579609	HM577691
311f	*X. chlorochroa*	BRY-55344	HM579206	HM578795	HM578080	HM578457	HM579610	HM577692
312f	*X. chlorochroa*	BRY-55345	HM579207	HM578796	HM578081	HM578458	HM579611	HM577693
314f	*X. chlorochroa*	BRY-55346	HM579208	HM578797	HM578082	HM578459	HM579612	HM577694
315f	*X. idahoensis*	BRY-55347	HM579209	HM578798	HM578083	-	HM579613	HM577695
316f	*X. camtschadalis*	BRY-55348	HM579210	HM578799	HM578084	-	HM579614	HM577696
317f	*X. camtschadalis*	BRY-55349	HM579211	HM578800	HM578085	-	HM579615	HM577697
318f	*X. idahoensis(type)*	BRY-55350	HM579212	HM578801	HM578086	-	HM579616	HM577698
319f	*X. idahoensis(type)*	BRY-55351	-	HM578802	HM578087	-	HM579617	HM577699
323f	*X. idahoensis*	BRY-55352	HM579214	HM578803	HM578088	-	HM579618	HM577700
324f	*X. idahoensis*	BRY-55353	HM579215	HM578804	HM578089	-	HM579619	HM577701
325f	*X. idahoensis*	BRY-55354	HM579216	HM578805	HM578090	-	HM579620	HM577702
326f	*X. chlorochroa*	BRY-55355	HM579217	HM578806	HM578091	HM578460	HM579621	HM577703
327f	*X. chlorochroa*	BRY-55356	HM579218	HM578807	HM578092	HM578461	HM579622	HM577704
328f	*X. neochlorochroa*	BRY-55357	HM579219	HM578808	HM578093	-	-	HM577705
329f	*X. camtschadalis*	BRY-55358	HM579220	HM578809	HM578094	-	HM579623	HM577706
330f	*X. camtschadalis*	BRY-55359	HM579221	HM578810	HM578095	-	HM579624	HM577707
331f	*X. camtschadalis*	BRY-55360	HM579222	HM578811	HM578096	-	HM579625	HM577708
332f	*X. camtschadalis*	BRY-55361	HM579223	HM578812	HM578097	-	HM579626	HM577709
333f	*X. camtschadalis*	BRY-55362	HM579224	HM578813	HM578098	-	HM579627	HM577710
334f	*X. camtschadalis*	BRY-55363	HM579225	HM578814	HM578099	-	HM579628	HM577711
335f	*X. camtschadalis*	BRY-55364	HM579226	HM578815	HM578100	-	HM579629	HM577712
336f	*X. norchlorochroa*	BRY-55365	HM579227	HM578816	HM578101	HM578462	-	HM577713
337f	*X. neochlorochroa*	BRY-55366	HM579228	HM578817	HM578102	HM578463	HM579630	HM577714
338f	*X. norchlorochroa*	BRY-55367	HM579229	HM578818	HM578103	HM578464	HM579631	HM577715
339f	*X. norchlorochroa*	BRY-55368	HM579230	HM578819	HM578104	HM578465	HM579632	HM577716
340f	*X. norchlorochroa*	BRY-55369	HM579231	HM578820	-	HM578466	HM579633	HM577717
341f	*X. norchlorochroa*	BRY-55370	HM579232	HM578821	HM578105	HM578467	HM579634	HM577718
342f	*X. norchlorochroa*	BRY-55371	HM579233	-	HM578106	-	HM579635	HM577719
343f	*X. chlorochroa*	BRY-55372	HM579234	HM578822	HM578107	HM578468	HM579636	HM577720
345f	*X. chlorochroa*	BRY-55373	HM579235	HM578823	HM578108	HM578469	HM579637	HM577721
410f	*X. camtschadalis*	BRY-55374	HM579236	HM578824	HM578109	-	HM579638	HM577722
424f	*X. chlorochroa*	BRY-55375	HM579237	HM578825	HM578110	HM578470	HM579639	HM577723
431f	*X. chlorochroa*	BRY-55376	HM579238	HM578826	HM578111	HM578471	HM579640	HM577724
432f	*X. chlorochroa*	BRY-55377	HM579239	HM578827	HM578112	HM578472	HM579641	HM577725
433f	*X. chlorochroa*	BRY-55378	HM579240	HM578828	-	HM578473	HM579642	HM577726
434f	*X. cumberlandia*	BRY-55379	HM579241	HM578829	HM578113	HM578474	HM579643	HM577727
435f	*X. cumberlandia*	BRY-55380	HM579242	HM578830	HM578114	HM578475	-	HM577728
437f	*X. chlorochroa*	BRY-55381	HM579243	HM578831	HM578115	HM578476	-	HM577729
438f	*X. chlorochroa*	BRY-55382	HM579244	HM578832	HM578116	HM578477	HM579644	HM577730
439f	*X. dierythra*	BRY-55383	HM579245	HM578833	HM578117	HM578478	-	HM577731
440f	*X. chlorochroa*	BRY-55384	HM579246	HM578834	HM578118	HM578479	HM579645	HM577732

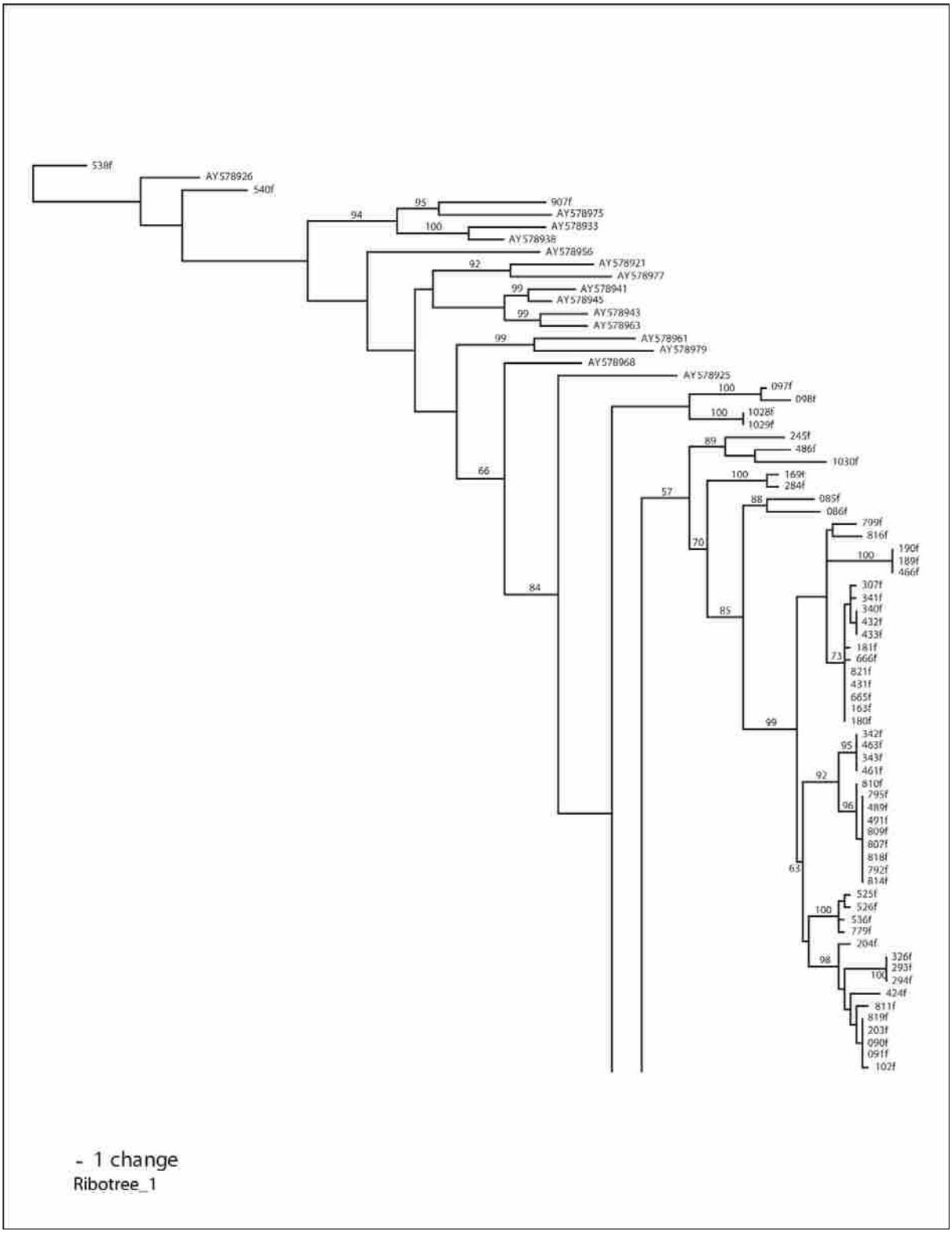
441f	*X. chlorochroa*	BRY-55385	HM579247	HM578835	HM578119	HM578480	HM579646	HM577733
442f	*X. lineola*	BRY-55386	HM579248	HM578836	HM578120	HM578481	-	HM577734
443f	*X. californica*	BRY-55387	HM579249	HM578837	-	HM578482	HM579647	HM577735
444f	*X. coloradoënsis**	BRY-55388	HM579250	HM578838	HM578121	HM578483	HM579648	HM577736
445f	*X. coloradoënsis**	BRY-55389	HM579251	HM578839	HM578122	HM578484	HM579649	HM577737
446f	*X. coloradoënsis**	BRY-55390	HM579252	HM578840	HM578123	HM578485	HM579650	HM577738
448f	*X. cumberlandia*	BRY-55391	HM579253	HM578841	HM578124	HM578486	HM579651	HM577739
449f	*X. cumberlandia*	BRY-55392	HM579254	HM578842	HM578125	HM578487	HM579652	HM577740
450f	*X. cumberlandia*	BRY-55393	HM579255	HM578843	HM578126	HM578488	HM579653	HM577741
451f	*X. cumberlandia*	BRY-55394	HM579256	HM578844	HM578127	HM578489	HM579654	HM577742
452f	*X. cumberlandia*	BRY-55395	HM579257	HM578845	HM578128	HM578490	HM579655	HM577743
453f	*X. cumberlandia*	BRY-55396	HM579258	HM578846	HM578129	HM578491	HM579656	HM577744
454f	*X. plittii*	BRY-55397	HM579259	HM578847	HM578130	HM578492	-	HM577745
455f	*X. cumberlandia*	BRY-55398	HM579260	HM578848	HM578131	HM578493	HM579657	HM577746
456f	*X. cumberlandia*	BRY-55399	HM579261	HM578849	HM578132	HM578494	HM579658	HM577747
457f	*X. cumberlandia*	BRY-55400	HM579262	HM578850	HM578133	HM578495	HM579659	HM577748
458f	*X. mexicana*	BRY-55401	HM579263	HM578851	HM578134	HM578496	HM579660	HM577749
459f	*X. mexicana*	BRY-55402	HM579264	HM578852	HM578135	HM578497	HM579661	HM577750
460f	*X. chlorochroa*	BRY-55403	HM579265	HM578853	HM578136	HM578498	HM579662	HM577751
461f	*X. chlorochroa*	BRY-55404	HM579266	HM578854	HM578137	HM578499	HM579663	HM577752
462f	*X. chlorochroa*	BRY-55405	HM579267	HM578855	HM578138	HM578500	HM579664	HM577753
463f	*X. chlorochroa*	BRY-55406	HM579268	HM578856	-	HM578501	HM579665	HM577754
464f	*X. neowyomingica*	BRY-55407	HM579269	HM578857	HM578139	HM578502	HM579666	HM577755
465f	*X. chlorochroa*	BRY-55408	HM579270	HM578858	HM578140	HM578503	HM579667	HM577756
466f	*X. chlorochroa*	BRY-55409	HM579271	HM578859	HM578141	HM578504	HM579668	HM577757
481f	*X. lineola*	BRY-55410	HM579272	HM578860	HM578142	HM578505	HM579669	HM577758
482f	*X. plittii*	BRY-55411	HM579273	HM578861	HM578143	HM578506	HM579670	HM577759
486f	*X. lineola*	BRY-55412	HM579274	HM578862	HM578144	-	HM579671	-
489f	*X. chlorochroa*	BRY-55413	-	HM578863	HM578145	-	HM579672	HM577760
490f	*X. wyomingica*	BRY-55414	HM579275	HM578864	HM578146	HM578507	HM579673	HM577761
491f	*X. chlorochroa*	BRY-55415	HM579276	HM578865	HM578147	-	HM579674	HM577762
492f	*X. chlorochroa*	BRY-55416	HM579277	HM578866	HM578148	HM578508	HM579675	HM577763
493f	*X. chlorochroa*	BRY-55417	HM579278	HM578867	HM578149	HM578509	HM579676	HM577764
494f	*X. angustiphylla*	BRY-55418	HM579279	HM578868	HM578150	HM578510	-	HM577765
495f	*X. angustiphylla*	BRY-55419	HM579280	HM578869	HM578151	HM578511	HM579677	HM577766
496f	*X. plittii*	BRY-55420	HM579281	HM578870	HM578152	-	-	HM577767
497f	*X. plittii*	BRY-55421	HM579282	HM578871	HM578153	-	HM579678	-
498f	*X. plittii*	BRY-55422	HM579283	HM578872	HM578154	-	HM579679	HM577768
499f	*X. plittii*	BRY-55423	HM579284	HM578873	HM578155	-	HM579680	HM577769
501f	*X. wyomingica*	BRY-55424	HM579285	HM578874	HM578156	HM578512	HM579681	HM577770
502f	*X. wyomingica*	BRY-55425	HM579286	HM578875	HM578157	HM578513	-	HM577771
504f	*X. mexicana*	BRY-55426	HM579287	HM578876	HM578158	HM578514	-	HM577772
505f	*X. coloradoënsis*	BRY-55427	HM579288	HM578877	HM578159	HM578515	-	HM577773
508f	*X. mexicana*	BRY-55428	HM579289	HM578878	HM578160	HM578516	HM579682	HM577774
509f	*X. lineola*	BRY-55429	HM579290	HM578879	HM578161	HM578517	-	-
516f	*X. chlorochroa*	BRY-55430	HM579291	HM578880	HM578162	HM578518	HM579683	HM577775
517f	*X. chlorochroa*	BRY-55431	HM579292	HM578881	HM578163	HM578519	HM579684	HM577776
525f	*X. chlorochroa*	BRY-55432	HM579293	HM578882	HM578164	HM578520	HM579685	HM577777
526f	*X. chlorochroa*	BRY-55433	HM579294	HM578883	HM578165	HM578521	HM579686	HM577778
527f	*X. camtschadalis*	BRY-55434	HM579295	HM578884	-	-	-	-
534f	*X. camtschadalis*	BRY-55435	HM579296	HM578885	HM578166	-	HM579687	HM577779
535f	*X. camtschadalis*	BRY-55436	HM579297	HM578886	HM578167	-	-	HM577780
536f	*X. chlorochroa*	BRY-55437	HM579298	HM578887	HM578168	HM578522	HM579688	HM577781
574f	*X. chlorochroa*	BRY-55438	HM579301	HM578890	HM578170	HM578523	M7-574	HM577783
575f	*X. cumberlandia*	BRY-55439	-	HM578891	HM578171	HM578524	-	-
576f	*X. plittii*	BRY-55440	-	HM578892	HM578172	HM578525	-	-
577f	*X. cumberlandia*	BRY-55441	HM579302	HM578893	HM578173	HM578526	-	HM577784
578f	*X. mexicana*	BRY-55442	HM579303	HM578894	HM578174	HM578527	-	-
580f	*X. lineola*	BRY-55444	HM579304	HM578896	HM578175	HM578528	HM579690	HM577785
665f	*X. chlorochroa*	BRY-55445	HM579305	HM578897	HM578176	HM578530	HM579691	HM577786

666f	*X. chlorochroa*	BRY-55446	HM579306	HM578898	HM578166	HM578531	HM579692	HM577787
771f	*X. norchlorochroa*	BRY-55447	HM579307	HM578899	HM578178	HM578532	HM579693	HM577788
772f	*X. chlorochroa*	BRY-55448	HM579308	HM578900	HM578179	HM578533	HM579694	HM577789
773f	*X. wyomingica*	BRY-55449	HM579309	HM578901	HM578180	HM578534	HM579695	HM577790
774f	*X. mexicana**	BRY-55450	HM579310	HM578902	HM578181	-	HM579696	HM577791
775f	*X. chlorochroa*	BRY-55451	HM579311	HM578903	HM578182	HM578535	HM579697	HM577792
776f	*X. chlorochroa*	BRY-55452	HM579312	HM578904	HM578183	HM578536	HM579698	HM577793
777f	*X. camtschadalis*	BRY-55453	HM579313	HM578905	HM578184	-	HM579699	HM577794
778f	*X. chlorochroa*	BRY-55454	HM579314	HM578906	HM578185	HM578537	HM579700	HM577795
779f	*X. chlorochroa*	BRY-55455	HM579315	HM578907	HM578186	HM578538	HM579701	HM577796
780f	*X. chlorochroa*	BRY-55456	HM579316	HM578908	HM578187	HM578536	HM579702	HM577797
781f	*X. chlorochroa*	BRY-55457	HM579317	HM578909	HM578188	HM578540	-	HM577798
782f	*X. chlorochroa*	BRY-55458	HM579318	HM578910	HM578189	HM578541	HM579703	HM577799
783f	*X. chlorochroa*	BRY-55459	HM579319	HM578911	HM578190	HM578542	HM579704	HM577800
784f	*X. chlorochroa*	BRY-55460	HM579320	HM578912	HM578191	HM5785543	HM579705	HM577801
785f	*X. chlorochroa*	BRY-55461	HM579321	HM578913	HM578192	-	HM579706	HM577802
786f	*X. mexicana*	BRY-55462	HM579322	HM578914	HM578193	HM578544	HM579707	HM577803
787f	*X. idahoensis*	BRY-55463	HM579323	HM578915	HM578194	-	HM579708	HM577804
788f	*X. norchlorochroa*	BRY-55464	HM579324	HM578916	HM578195	HM578545	HM579709	HM577805
789f	*X. chlorochroa*	BRY-55465	HM579325	HM578917	HM578196	HM578546	HM579710	HM577806
790f	*X. wyomingica*	BRY-55466	HM579326	HM578918	HM578197	HM578547	HM579711	HM577807
791f	*X. chlorochroa*	BRY-55467	HM579327	HM578919	HM578198	HM578548	HM579712	HM577808
792f	*X. chlorochroa*	BRY-55468	HM579328	HM578920	HM578199	-	HM579713	HM577809
793f	*X. chlorochroa*	BRY-55469	HM579329	HM578921	HM578200	-	HM579714	HM577810
794f	*X. chlorochroa*	BRY-55470	HM579330	HM578922	HM578201	HM578549	HM579715	HM577811
795f	*X. chlorochroa*	BRY-55471	HM579331	HM578923	HM578202	-	HM579716	HM577812
796f	*X. chlorochroa*	BRY-55472	HM579332	HM578924	HM578203	HM578550	HM579717	HM577813
797f	*X. camtschadalis*	BRY-55473	HM579333	HM578925	HM578204	-	HM579718	HM577814
798f	*X. chlorochroa*	BRY-55474	-	HM578926	HM578205	HM578551	HM579719	HM577815
799f	*X. chlorochroa*	BRY-55475	HM579334	HM578927	HM578206	HM578552	HM579720	HM577816
800f	*X. chlorochroa*	BRY-55476	HM579335	HM578928	HM578207	HM578553	HM579721	HM577817
801f	*X. chlorochroa*	BRY-55477	HM579336	HM578929	HM578208	HM578554	HM579722	HM577818
802f	*X. chlorochroa*	BRY-55478	HM579337	HM578930	HM578209	HM578555	HM579723	HM577819
804f	*X. chlorochroa*	BRY-55479	HM579338	HM578931	HM578210	HM578556	HM579724	HM577820
805f	*X. chlorochroa*	BRY-55480	HM579339	HM578932	HM578211	HM578557	HM579725	HM577821
806f	*X. chlorochroa*	BRY-55481	HM579340	HM578933	HM578212	HM578558	HM579726	HM577822
807f	*X. chlorochroa*	BRY-55482	HM579341	HM578934	HM578213	-	HM579727	HM577823
808f	*X. chlorochroa*	BRY-55483	HM579342	HM578935	HM578214	HM578559	HM579728	HM577824
809f	*X. chlorochroa*	BRY-55484	HM579343	HM578936	HM578215	-	HM579729	HM577825
810f	*X. chlorochroa*	BRY-55485	HM579344	HM578937	HM578216	-	HM579730	HM577826
811f	*X. chlorochroa*	BRY-55486	HM579345	HM578938	HM578217	HM578560	HM579731	HM577827
812f	*X. chlorochroa*	BRY-55487	HM579346	HM578939	HM578218	HM578561	HM579732	HM577828
813f	*X. camtschadalis*	BRY-55488	HM579347	HM578940	HM578219	-	HM579733	HM577829
814f	*X. chlorochroa*	BRY-55489	HM579348	HM578941	HM578220	-	HM579734	HM577830
815f	*X. chlorochroa*	BRY-55490	HM579349	HM578942	HM578221	HM578562	HM579735	-
816f	*X. chlorochroa*	BRY-55491	HM579350	HM578943	HM578222	HM578563	HM579736	HM577831
817f	*X. camtschadalis*	BRY-55492	HM579351	HM578944	HM578223	-	HM579737	HM577832
818f	*X. chlorochroa*	BRY-55493	HM579352	HM578945	HM578224	-	HM579738	HM577833
819f	*X. chlorochroa*	BRY-55494	HM579353	HM578946	HM578225	HM578564	HM579739	HM577834
820f	*X. chlorochroa*	BRY-55495	HM579354	HM578947	HM578226	HM578565	HM579740	HM577835
821f	*X. chlorochroa*	BRY-55496	HM579355	HM578948	HM578227	HM578566	HM579741	HM577836
822f	*X. chlorochroa*	BRY-55497	HM579356	HM578949	HM578228	HM578567	HM579742	HM577837
823f	*X. wyomingica*	BRY-55498	HM579357	HM578950	HM578229	HM578568	HM579743	HM577838
824f	*X. chlorochroa*	BRY-55499	HM579358	HM578951	HM578230	HM578569	HM579744	HM577839
825f	*X. chlorochroa*	BRY-55500	HM579359	HM578952	HM578231	HM578570	HM579745	HM577840
826f	*X. wyomingica (type)*	BRY-55501	HM579360	HM578953	HM578232	HM578571	HM579746	HM577841
827f	*X. wyomingica (type)*	BRY-55502	HM579361	HM578954	-	HM578572	HM579747	HM577842
828f	*X. mexicana*	BRY-55503	HM579362	HM578955	HM578233	HM578573	HM579748	HM577843
829f	*X. camtschadalis*	BRY-55504	HM579363	HM578956	HM578234	-	HM579729	HM577844
830f	*X. mexicana*	BRY-55505	HM579364	HM578957	HM578235	HM578574	HM579750	HM577845

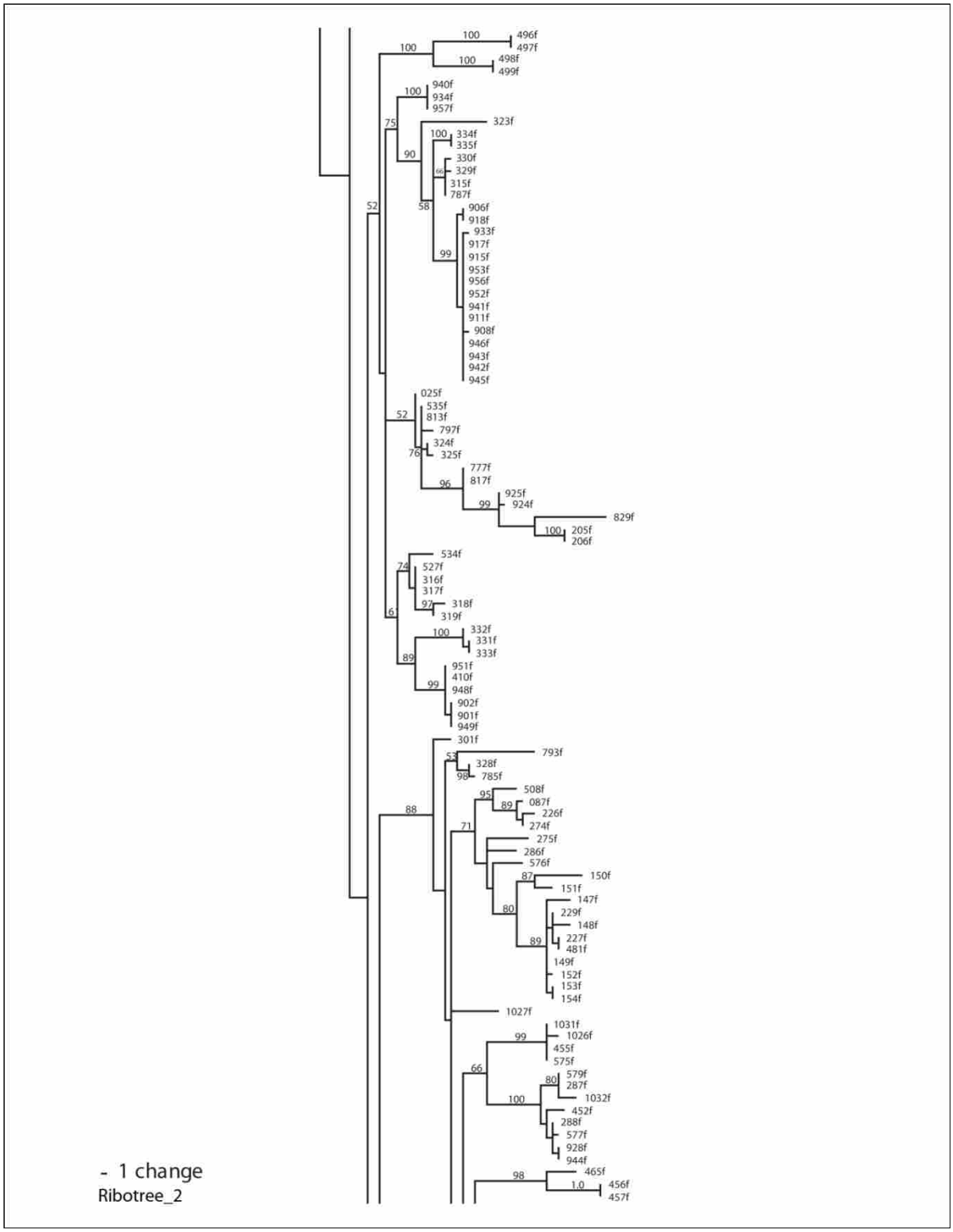
901f	*X. camtschadalis*	BRY-55506	HM579365	HM578958	HM578236	-	HM579751	HM577846
902f	*X. camtschadalis*	BRY-55507	HM579366	HM578959	-	-	HM579752	HM577847
903f	*X. cumberlandia*	BRY-55508	HM579367	HM578960	HM578237	HM578575	HM579753	HM577848
904f	*X. cumberlandia*	BRY-55509	HM579368	HM578961	HM578238	HM578576	-	-
905f	*X. cumberlandia*	BRY-55510	HM579369	HM578962	HM578239	HM578577	-	HM577849
906f	*X. stenophylla*	BRY-55511	HM579370	HM578963	HM578240	-	HM579754	HM577850
908f	*X. stenophylla*	BRY-55512	HM579372	HM578965	HM578242	-	HM579756	HM577852
909f	*X. cumberlandia*	BRY-55513	HM579373	HM578966	-	HM578578	-	HM577853
911f	*X. stenophylla*	BRY-55514	HM579374	HM578967	HM578243	-	-	HM577854
912f	*X.plittii*	BRY-55515	HM579375	HM578968	HM578244	HM578579	HM579757	HM577855
913f	*X. cumberlandia*	BRY-55516	HM579376	HM578969	HM578245	HM578580	-	HM577856
914f	*X. cumberlandia*	BRY-55517	HM579377	HM578970	HM578246	HM578581	-	-
915f	*X. stenophylla*	BRY-55518	HM579378	HM578971	HM578247	-	-	HM577857
916f	*X. mexicana*	BRY-55519	HM579379	HM578972	HM578248	HM578582	HM579758	HM577858
917f	*X. stenophylla*	BRY-55520	HM579380	HM578973	HM578249	-	-	HM577859
918f	*X. stenophylla*	BRY-55521	HM579381	HM578974	HM578250	-	-	HM577860
919f	*X. plittii*	BRY-55522	HM579382	HM578975	HM578251	HM578583	HM579759	HM577861
920f	*X. mexicana*	BRY-55523	-	HM578976	HM578252	HM578584	HM579760	-
922f	*X. coloradoënsis*	BRY-55524	HM579383	HM578977	HM578253	HM578585	HM579761	HM577862
923f	*X. coloradoënsis*	BRY-55525	HM579384	HM578978	HM578254	HM578586	HM579762	HM577863
924f	*X. camtschadalis*	BRY-55526	HM579385	HM578979	HM578255	-	HM579763	HM577864
925f	*X. camtschadalis*	BRY-55527	HM579386	HM578980	HM578256	-	HM579764	HM577865
926f	*X. wyomingica*	BRY-55528	HM579387	HM578981	HM578257	HM578587	HM579765	HM577866
927f	*X. wyomingica*	BRY-55529	HM579388	HM578982	HM578258	HM578588	-	HM577867
928f	*X. cumberlandia*	BRY-55530	HM579389	HM578983	HM578259	HM578589	-	HM577868
929f	*X. cumberlandia*	BRY-55531	HM579390	HM578984	HM578260	HM578590	HM579766	HM577869
930f	*X. cumberlandia*	BRY-55532	HM579391	HM578985	HM578261	HM578591	-	HM577870
931f	*X. cumberlandia*	BRY-55533	HM579392	HM578986	HM578262	HM578592	-	HM577871
932f	*X. cumberlandia*	BRY-55534	HM579393	HM578987	HM578263	HM578593	-	HM577872
933f	*X. stenophylla*	BRY-55535	HM579394	HM578988	HM578264	-	HM579767	HM577873
934f	*X. stenophylla*	BRY-55536	HM579395	HM578989	HM578265	-	HM579768	HM577874
935f	*X. cumberlandia*	BRY-55537	HM579396	HM578990	HM578266	HM578594	-	HM577875
936f	*X. mexicana*	BRY-55538	HM579397	HM578991	HM578267	HM578595	-	HM577876
937f	*X. cumberlandia*	BRY-55539	HM579398	HM578992	HM578268	HM578596	-	HM577877
938f	*X. cumberlandia*	BRY-55540	HM579399	HM578993	HM578269	HM578597	-	HM577878
939f	*X. cumberlandia*	BRY-55541	HM579400	HM578994	HM578270	HM578598	-	HM577879
940f	*X. stenophylla*	BRY-55542	HM579401	HM578995	HM578271	-	HM579769	HM577880
941f	*X. stenophylla*	BRY-55543	HM579402	HM578996	HM578272	-	-	HM577881
942f	*X. stenophylla*	BRY-55544	HM579403	HM578997	HM578273	-	-	HM577882
943f	*X. stenophylla*	BRY-55545	HM579404	HM578998	HM578274	-	-	HM577883
944f	*X. cumberlandia*	BRY-55546	HM579405	HM578999	HM578275	HM578599	-	HM577884
945f	*X. stenophylla*	BRY-55547	HM579406	HM579000	HM578276	-	-	HM577885
946f	*X. stenophylla*	BRY-55548	HM579407	HM579001	HM578277	-	-	HM577886
947f	*X. subplittii*	BRY-55549	HM579408	HM579002	HM578278	HM578600	-	HM577887
948f	*X. camtschadalis*	BRY-55550	HM579409	HM579003	HM578279	-	-	HM577888
949f	*X. camtschadalis*	BRY-55551	HM579410	HM579004	HM578280	-	-	HM577889
950f	*X. wyomingica*	BRY-55552	HM579411	HM579005	HM578281	-	-	HM577890
951f	*X. stenophylla*	BRY-55553	HM579412	HM579006	HM578282	-	-	HM577891
952f	*X. stenophylla*	BRY-55554	HM579413	HM579007	HM578283	-	-	HM577892
953f	*X. stenophylla*	BRY-55555	HM579414	HM579008	HM578284	-	-	HM577893
954f	*X. cumberlandia*	BRY-55556	HM579415	HM579009	HM578285	HM578601	-	HM577894
955f	*X. wyomingica*	BRY-55557	HM579416	HM579010	HM578286	HM578602	HM579770	HM577895
956f	*X. stenophylla*	BRY-55558	HM579417	HM579011	HM578287	-	-	HM577896
957f	*X. stenophylla*	BRY-55559	HM579418	HM579012	HM578288	-	-	HM577897
1026f	*X. cumberlandia**	BRY-55560	HM579419	HM579013	HM578289	HM578603	HM579771	HM577898
1027f	*X. lineola*	BRY-55561	HM579420	HM579014	HM578290	-	HM579772	HM577899
1028f	*X. mexicana*	BRY-55562	HM579421	HM579015	HM578291	HM578604	HM579773	HM577900
1029f	*X. mexicana*	BRY-55563	HM579422	HM579016	HM578292	HM578605	HM579774	HM577901
1030f	*X. coloradoënsis*	BRY-55564	HM579423	HM579017	HM578293	-	HM579775	HM577902
1031f	*X. cumberlandia*	BRY-55565	HM579424	-	HM578294	-	HM579776	HM577903

1032f	*X. cumberlandia*	BRY-55566	HM579425	HM579018	HM578295	HM578506	HM579777	HM577904
Outgroup taxa								
-	*Karoowia saxeti*	ABL	AY578926	AY581063	-	-	-	-
538f	*Karoowia saxeti*	BRY-55567	-	HM579299	HM578888	HM578169		HM579689
540f	*Karoowia saxeti*	BRY-55568	HM579300	HM578889				
-	*X. brachinaensis*	CANB	AY578925	AY581062	-	-	-	-
-	*X. convoluta*	GZU 6511	AY578956	AY581094	-	-	-	-
-	*X. lithophila*	MAF 6900	AY578941	AY581077	-	-	-	-
-	*X. loxodes*	MAF7072	AY578940	AY581076	-	-	-	-
907f	*X. mougeotii*	BRY-55569	HM579371	HM578964	HM578241		HM579755	HM577851
-	*X. murina*	MAF 9915	AY578943	AY581079	-	-	-	-
-	*X. notata*	CANB	AY578968	AY581101	-	-	-	-
-	*X. scotophylla*	CANB	AY578945	AY581081	-	-	-	-
-	*X. semiviridis*	MAF 6876	AY578921	AY581058	-	-	-	-
-	*X. subprolixa*	MAF 7667	AY578938	AY581074	-	-	-	-
-	*X. tegeta*	MAF 7523	AY578975	AY581107	-	-	-	-
-	*X. tinctina*	MAF6070	AY578976	AY581108	-	-	-	-
-	*X. verrucigera*	MAF 9920	AY578979	AY581111	-	-	-	-

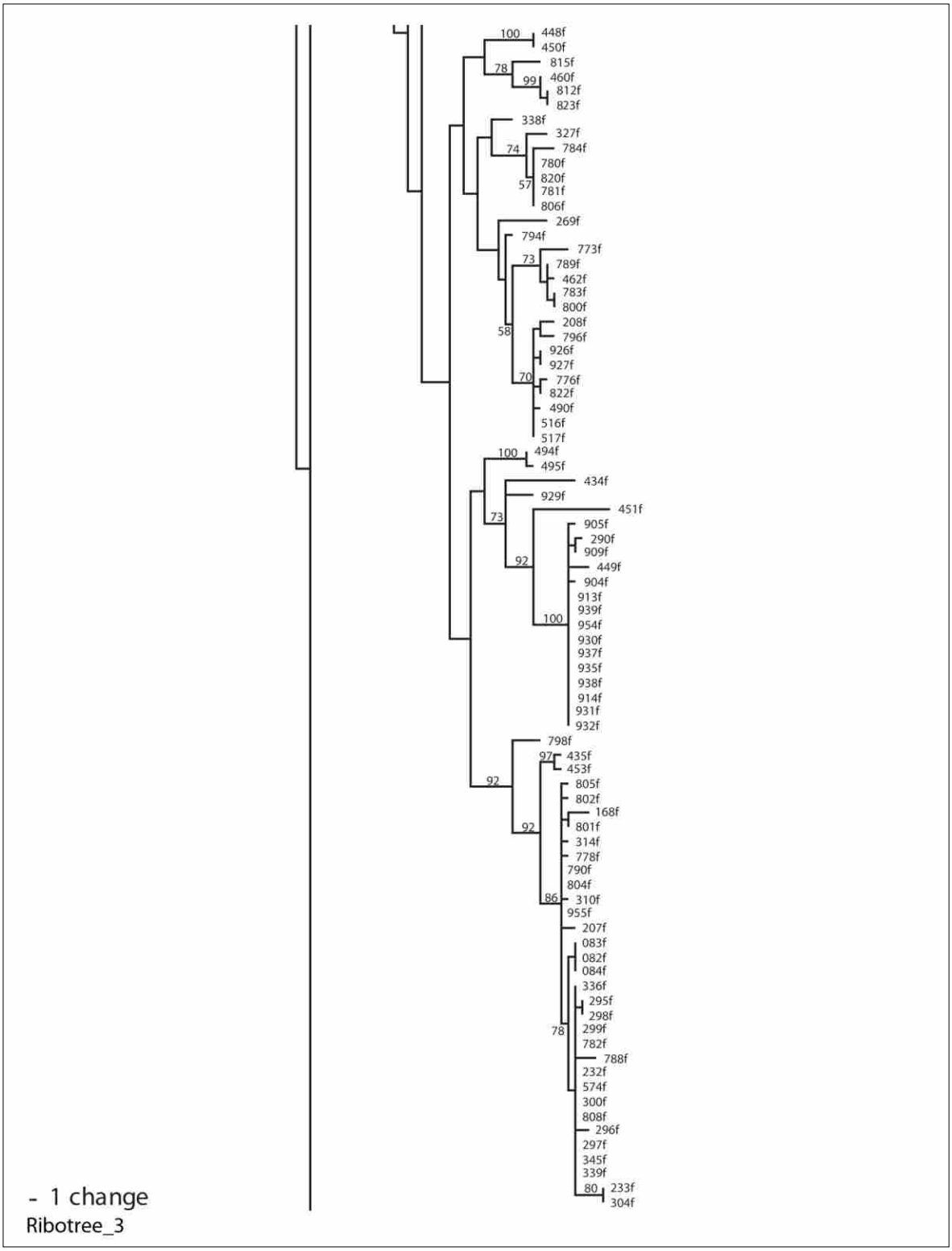
Supplementary data 2.3 (subsequent 13 pages). (A) Maximum likelihood topology of the concatenated nuclear ribosomal (IGS, ITS, LSU, and group I intron) topology, with bootstrap support indicated at nodes; (B) maximum likelihood topology estimated from the β-tubulin fragment, with bootstrap support indicated at nodes; and (C) maximum likelihood topology estimated from the *MCM7* fragment, with bootstrap support indicated at nodes.



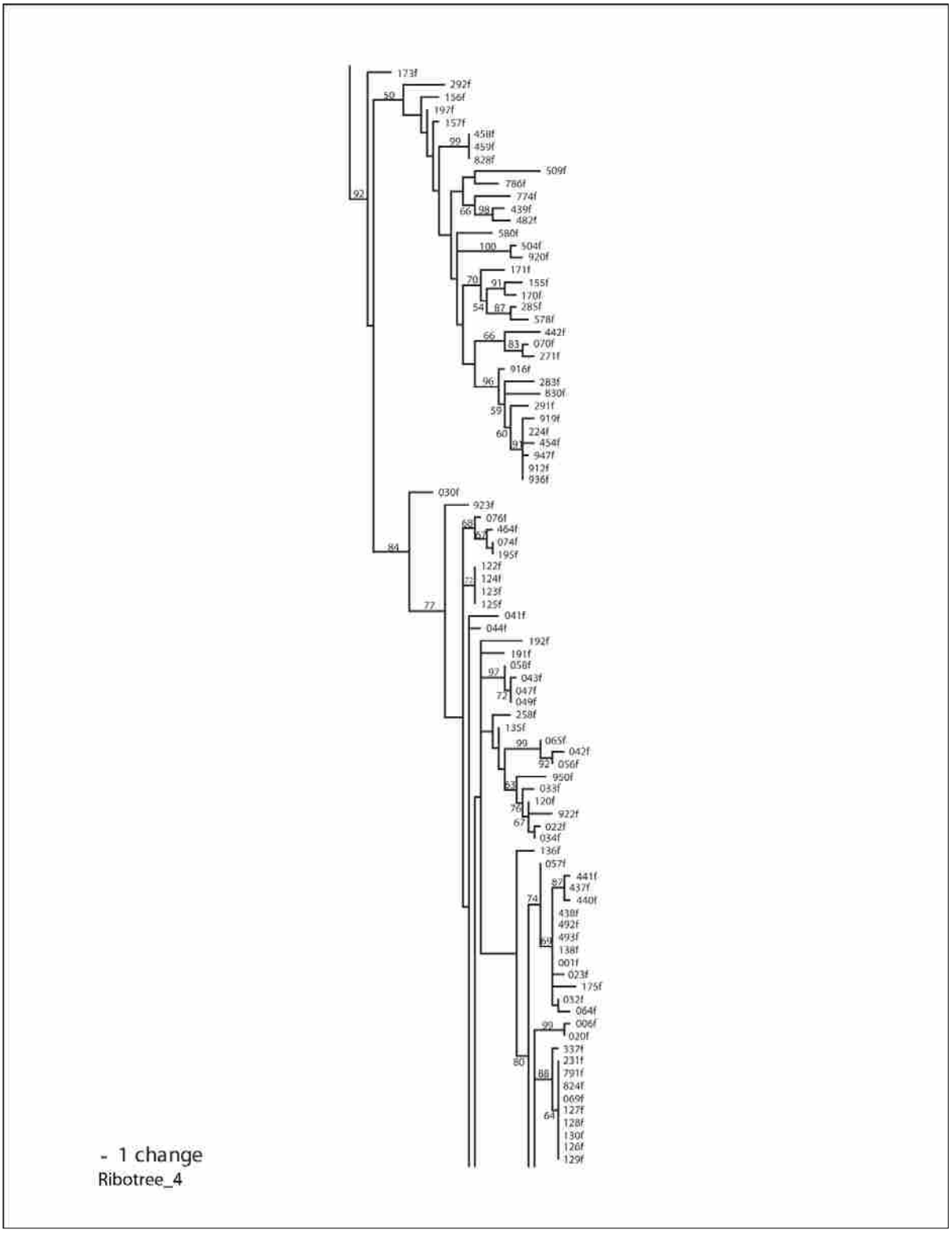
Supplementary Figure 2.3a-1. Maximum likelihood topology of the concatenated nuclear ribosomal (IGS, ITS, LSU, and group I intron) topology, with bootstrap support indicated at nodes.



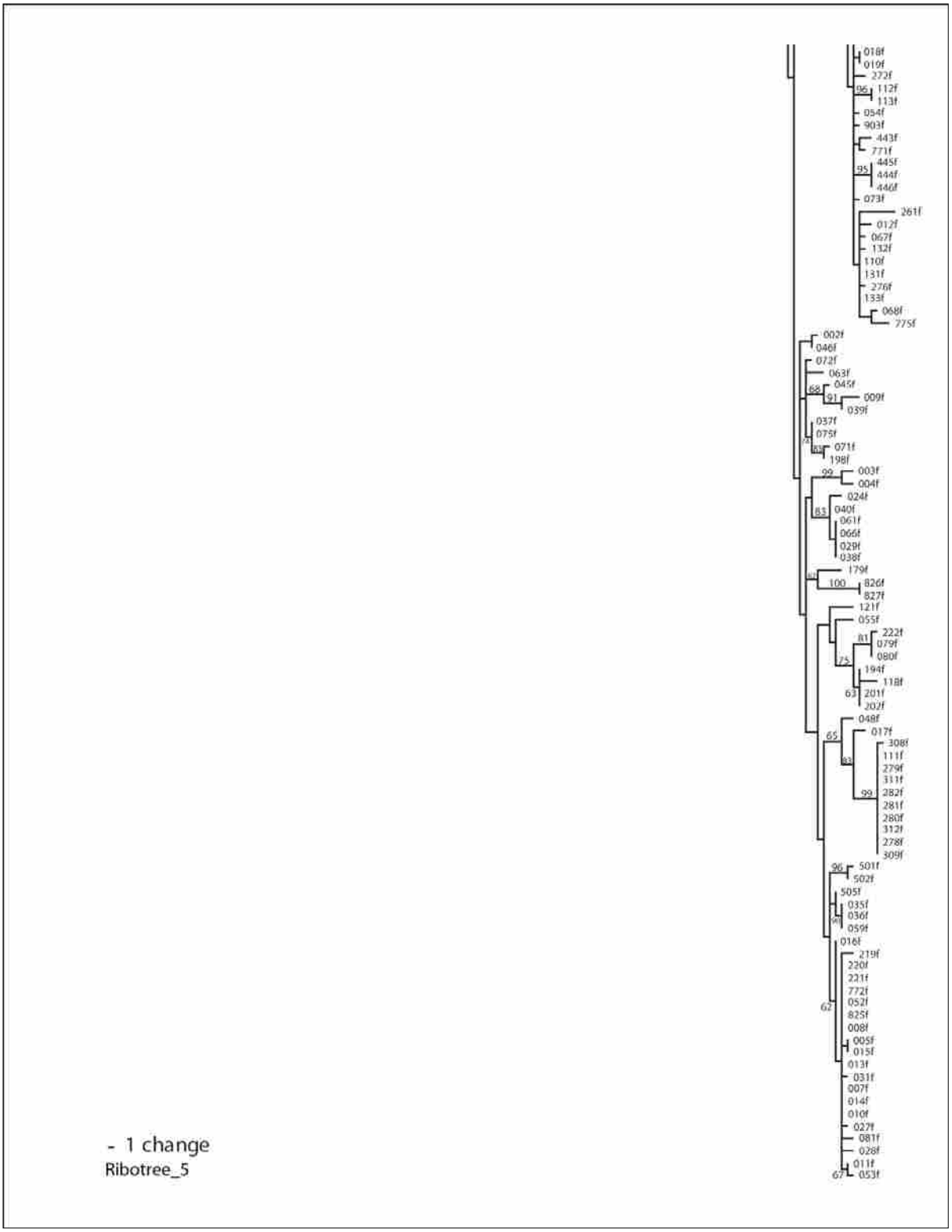
Supplementary Figure 2.3a-2. Maximum likelihood topology of the concatenated nuclear ribosomal (IGS, ITS, LSU, and group I intron) topology, with bootstrap support indicated at nodes.



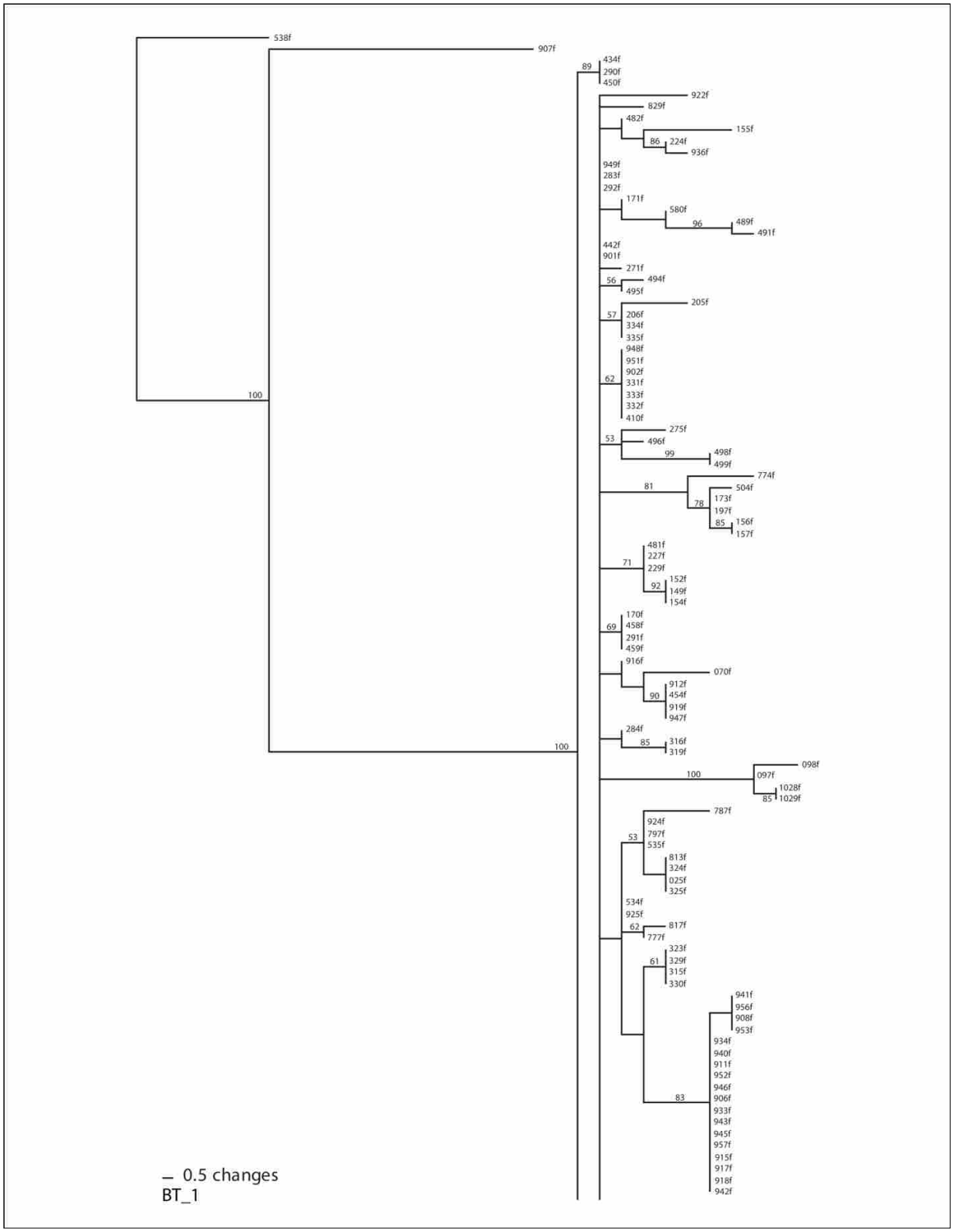
Supplementary Figure 2.3a-3. Maximum likelihood topology of the concatenated nuclear ribosomal (IGS, ITS, LSU, and group I intron) topology, with bootstrap support indicated at nodes.



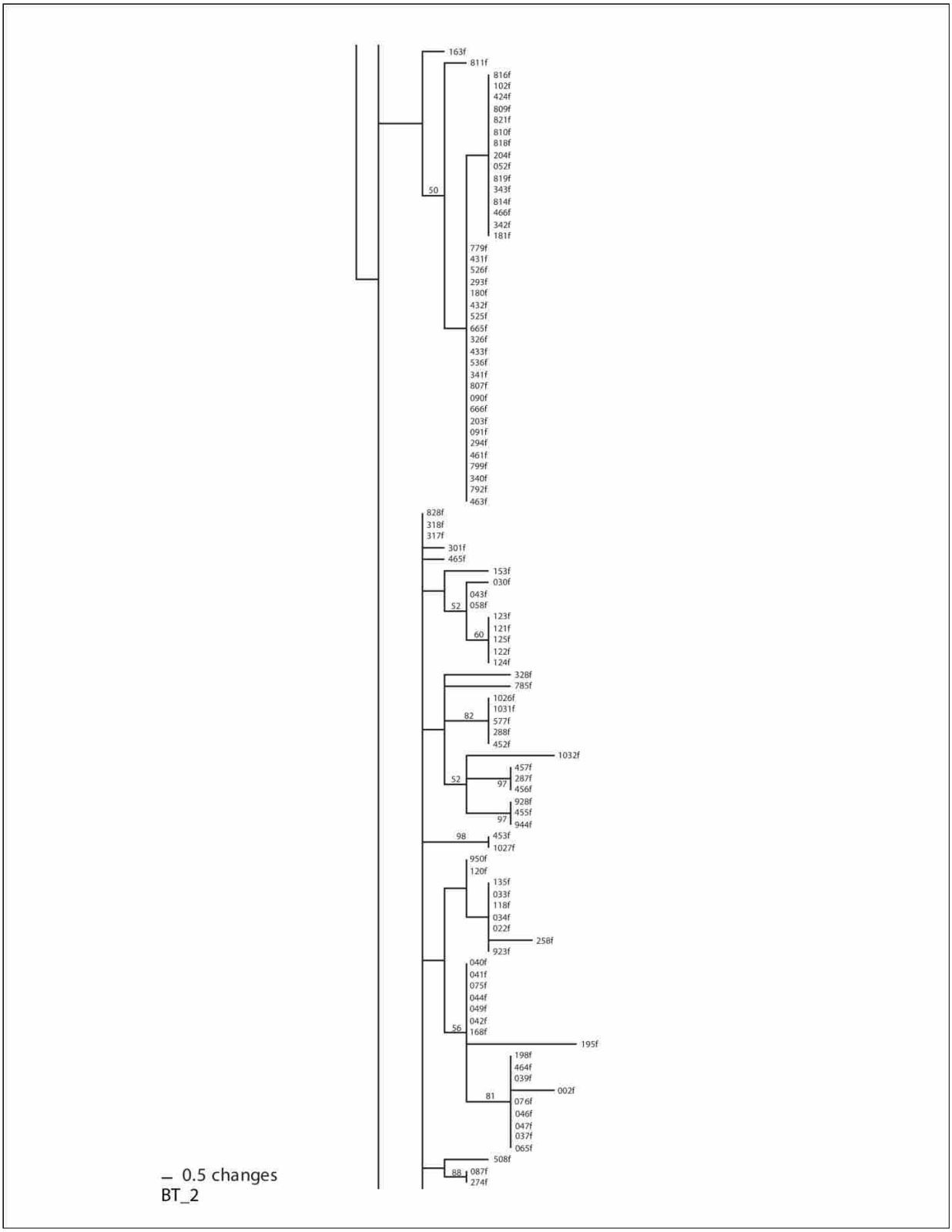
Supplementary Figure 2.3a-4. Maximum likelihood topology of the concatenated nuclear ribosomal (IGS, ITS, LSU, and group I intron) topology, with bootstrap support indicated at nodes.



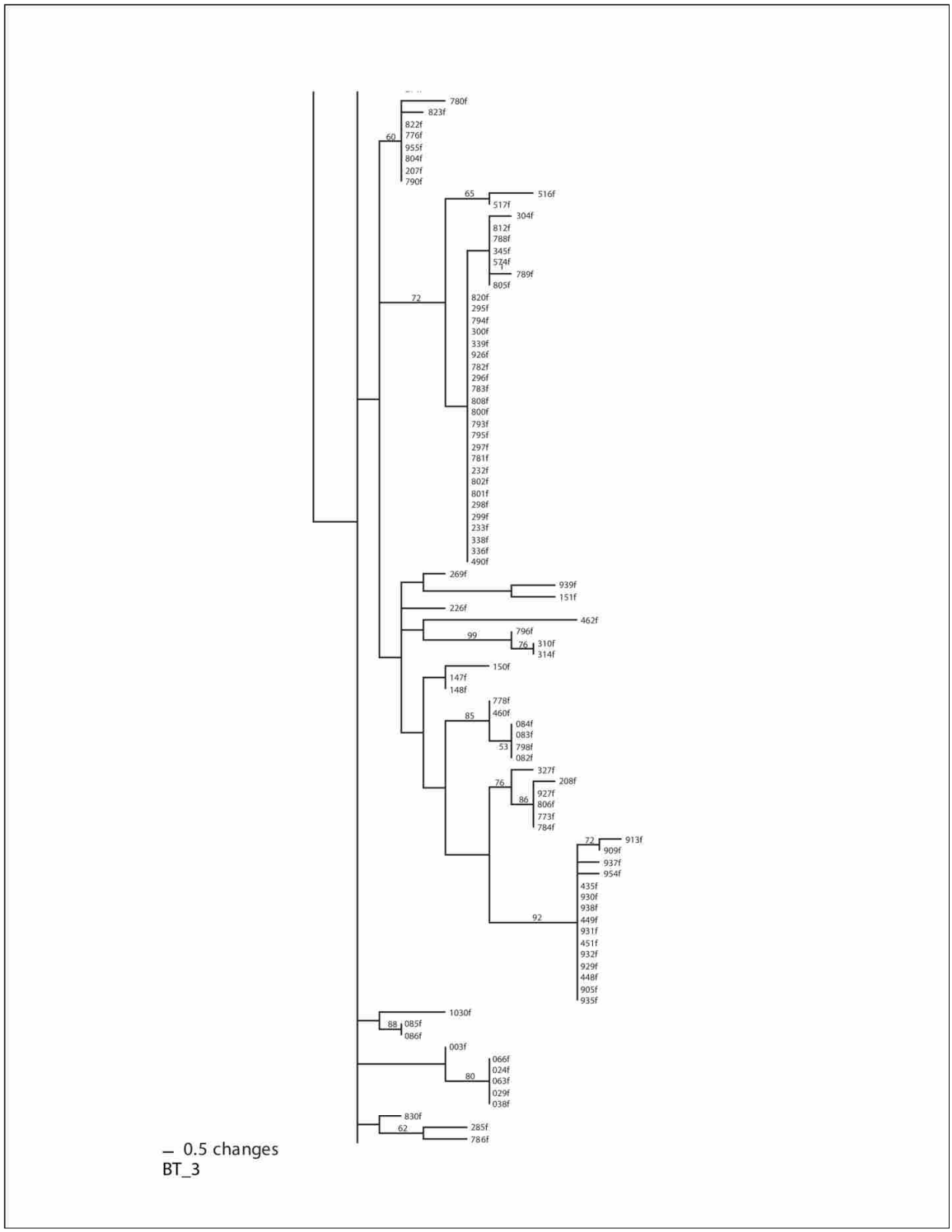
Supplementary Figure 2.3a-5. Maximum likelihood topology of the concatenated nuclear ribosomal (IGS, ITS, LSU, and group I intron) topology, with bootstrap support indicated at nodes



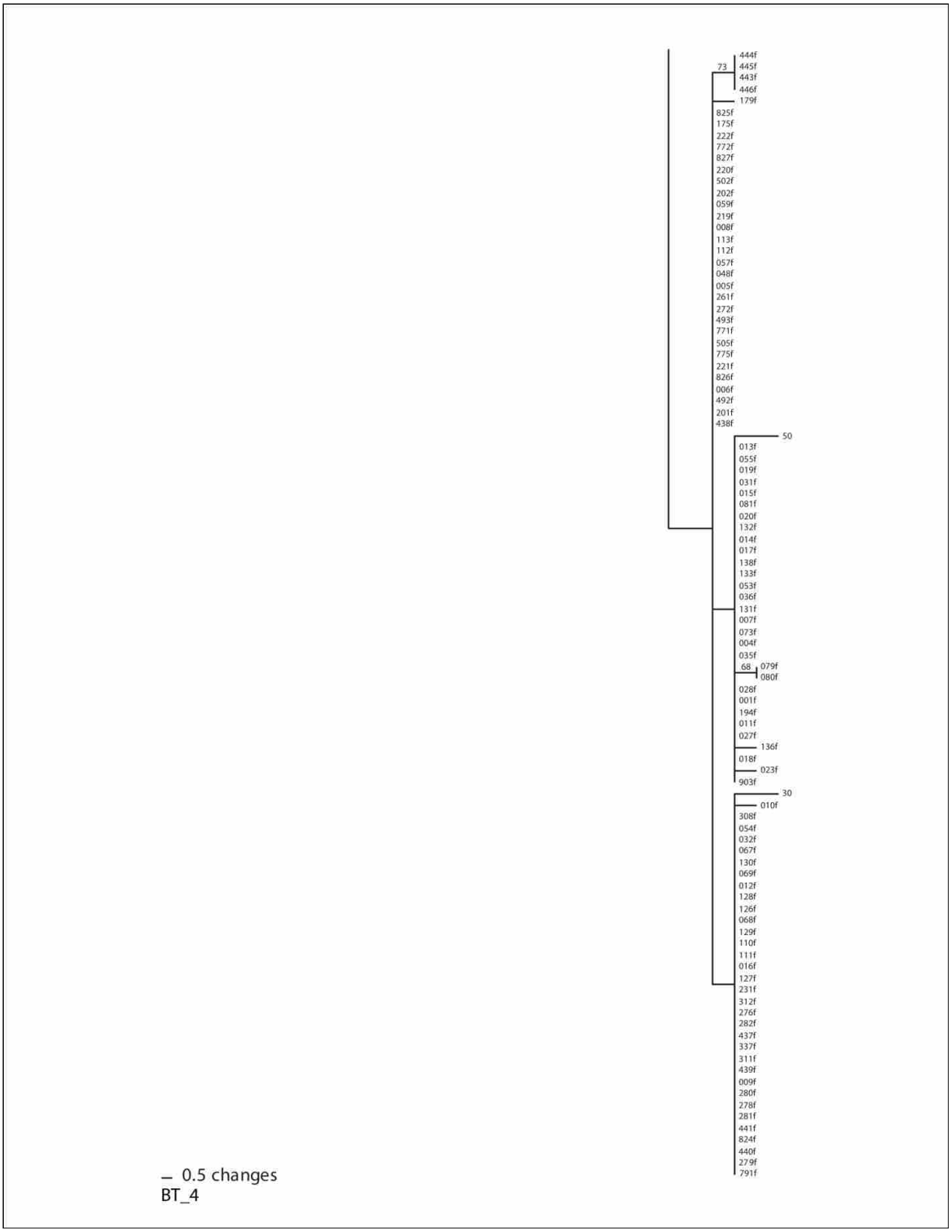
Supplementary Figure 2.3b-1. Maximum likelihood topology estimated from the β-tubulin fragment, with bootstrap support indicated at nodes.



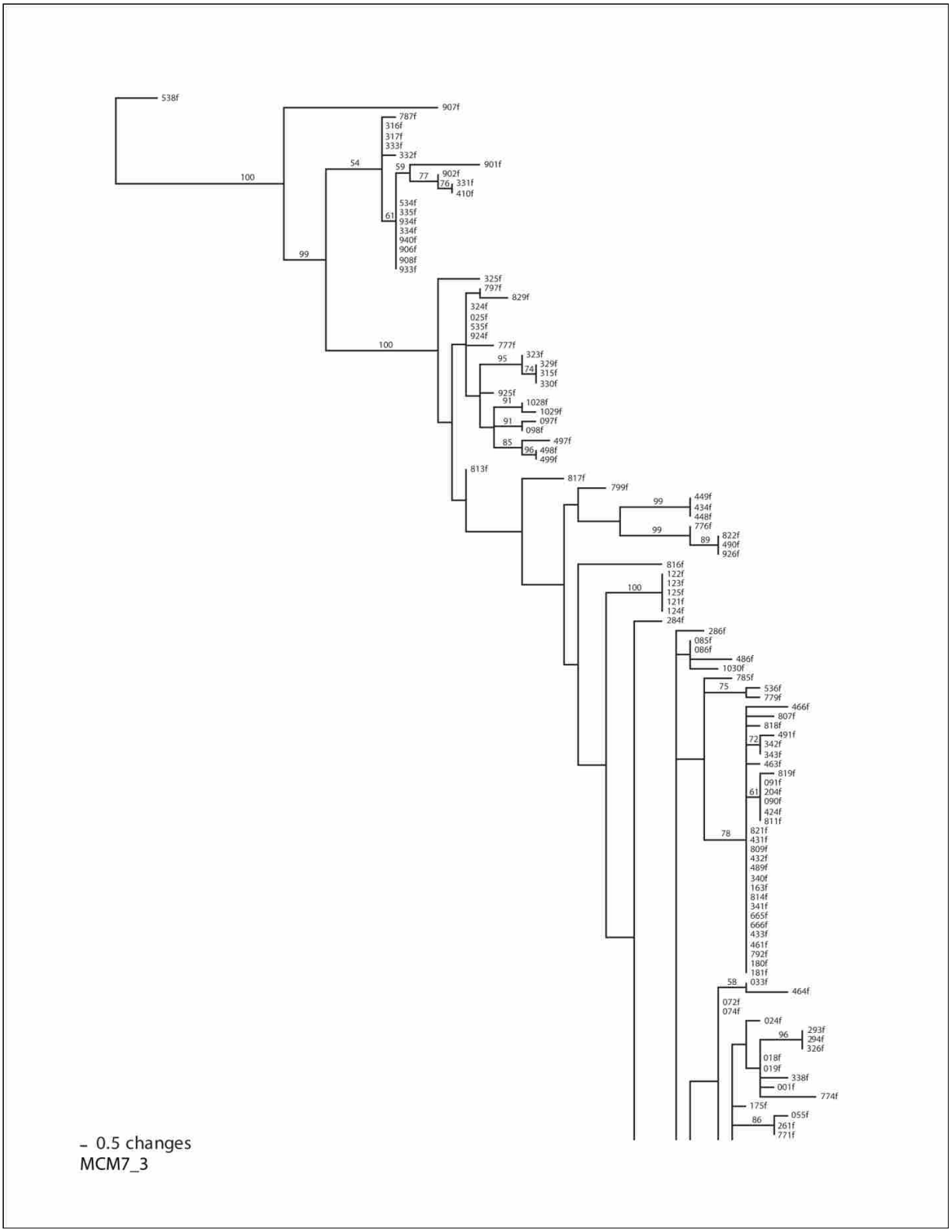
Supplementary Figure 2.3b-2. Maximum likelihood topology estimated from the β-tubulin fragment, with bootstrap support indicated at nodes.



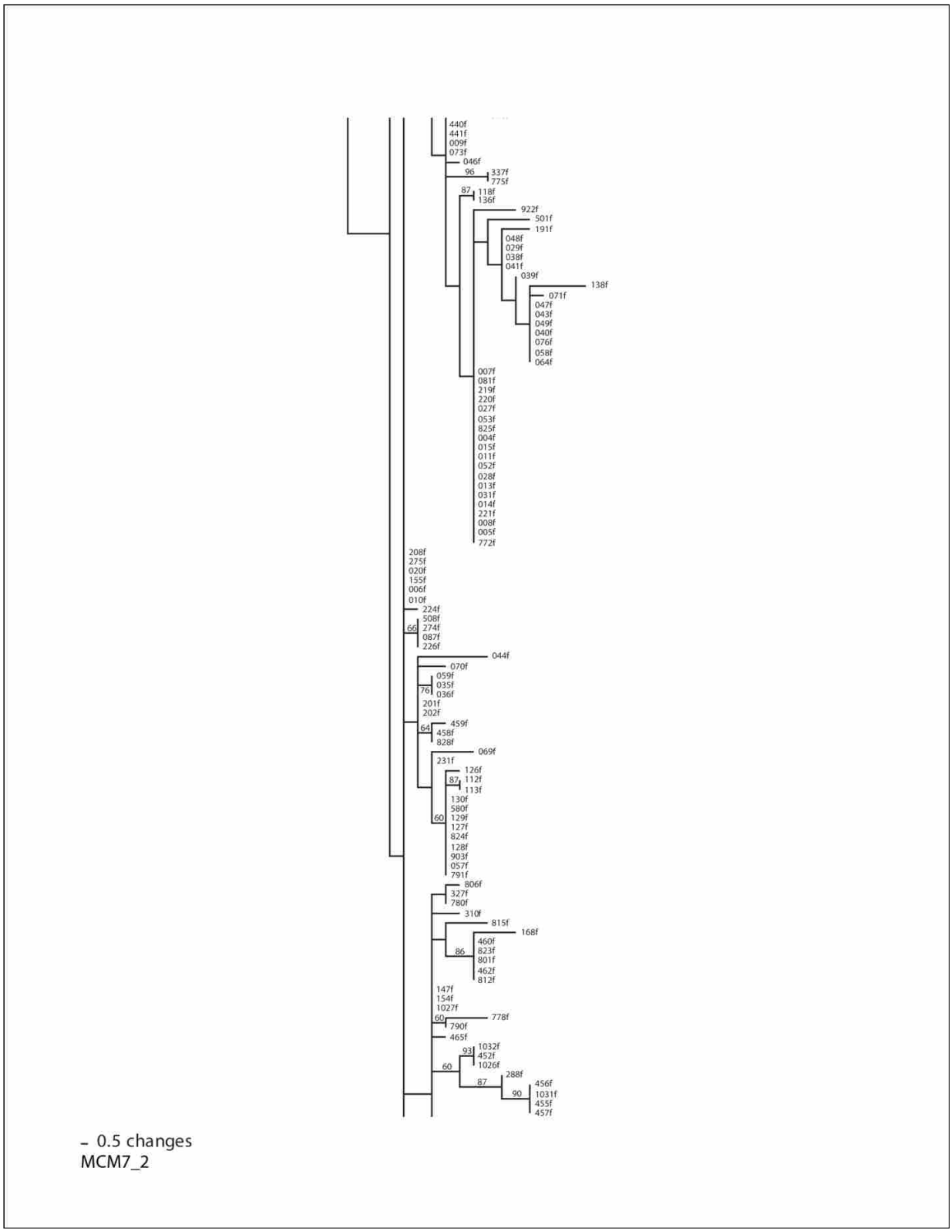
Supplementary Figure 2.3b-3. Maximum likelihood topology estimated from the β-tubulin fragment, with bootstrap support indicated at nodes.



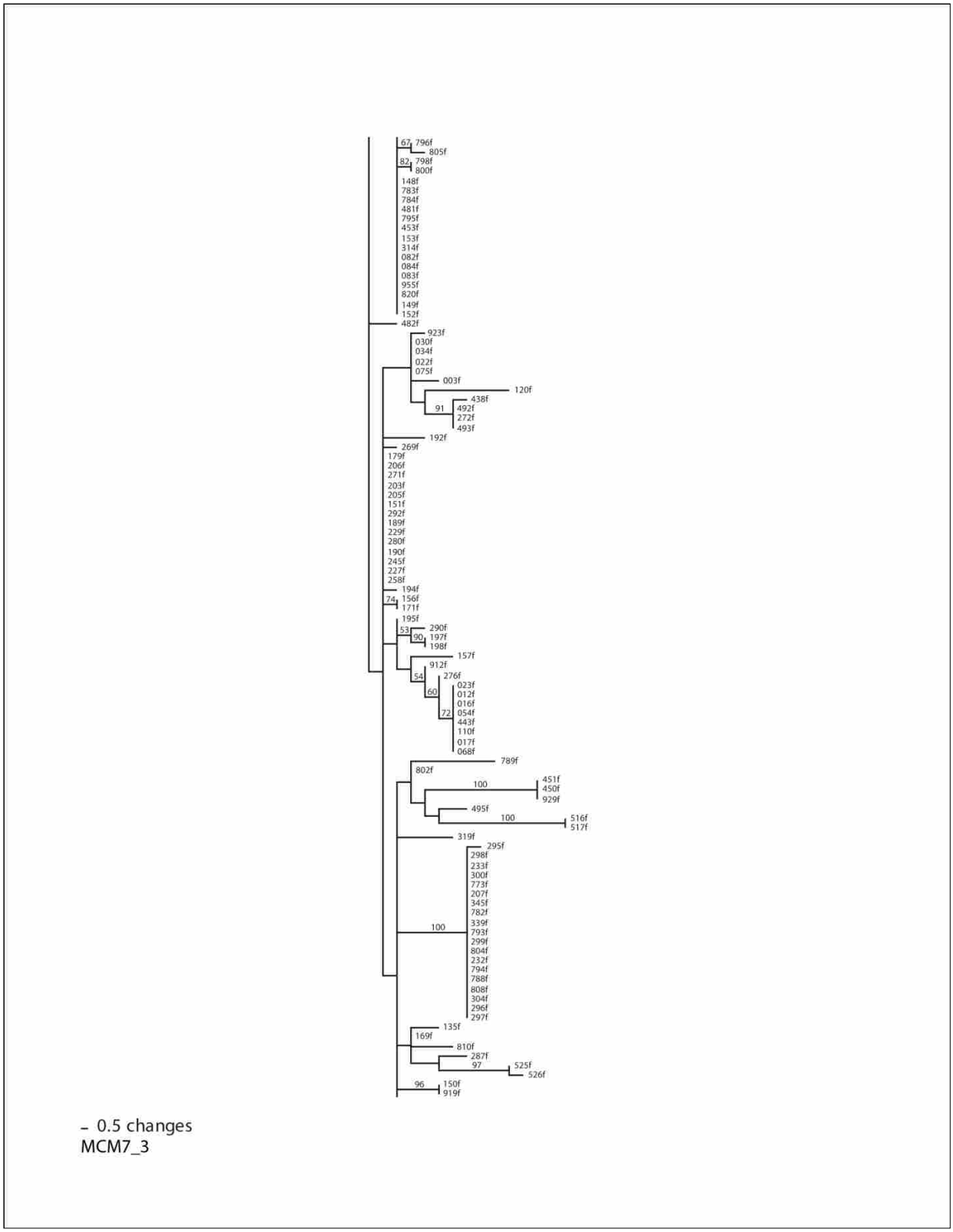
Supplementary Figure 2.3b-4. Maximum likelihood topology estimated from the β-tubulin fragment, with bootstrap support indicated at nodes.



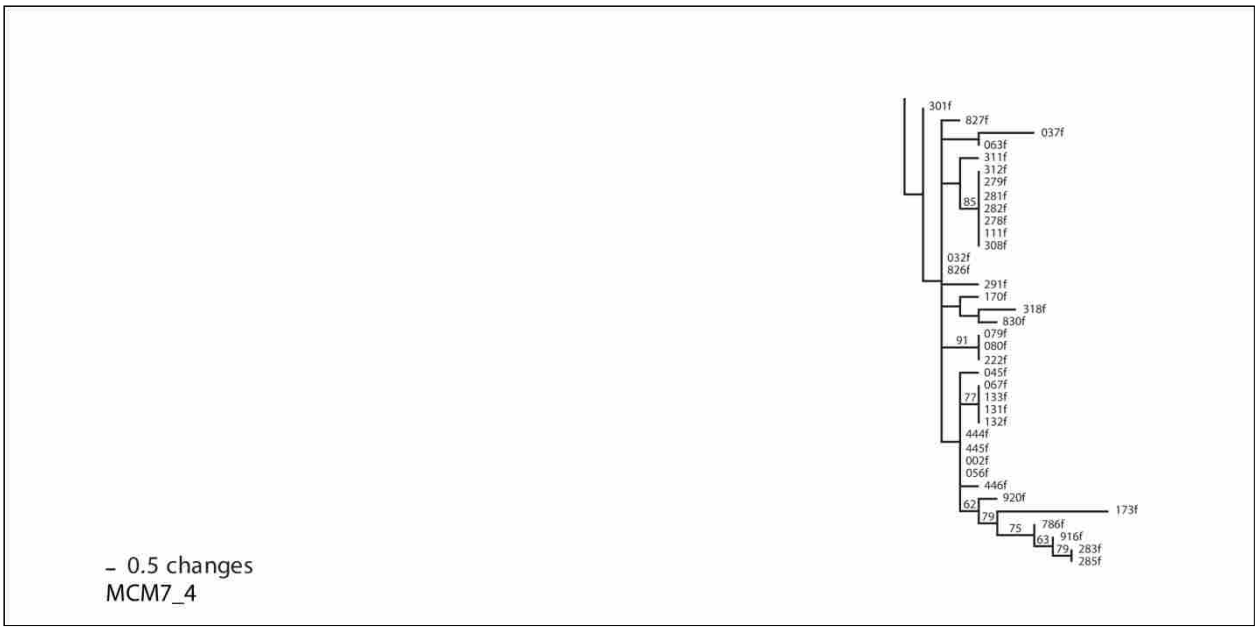
Supplementary Figure 2.3c-1. Maximum likelihood topology estimated from the *MCM7* fragment, with bootstrap support indicated at nodes.



Supplementary Figure 2.3c-2. Maximum likelihood topology estimated from the *MCM7* fragment, with bootstrap support indicated at nodes.

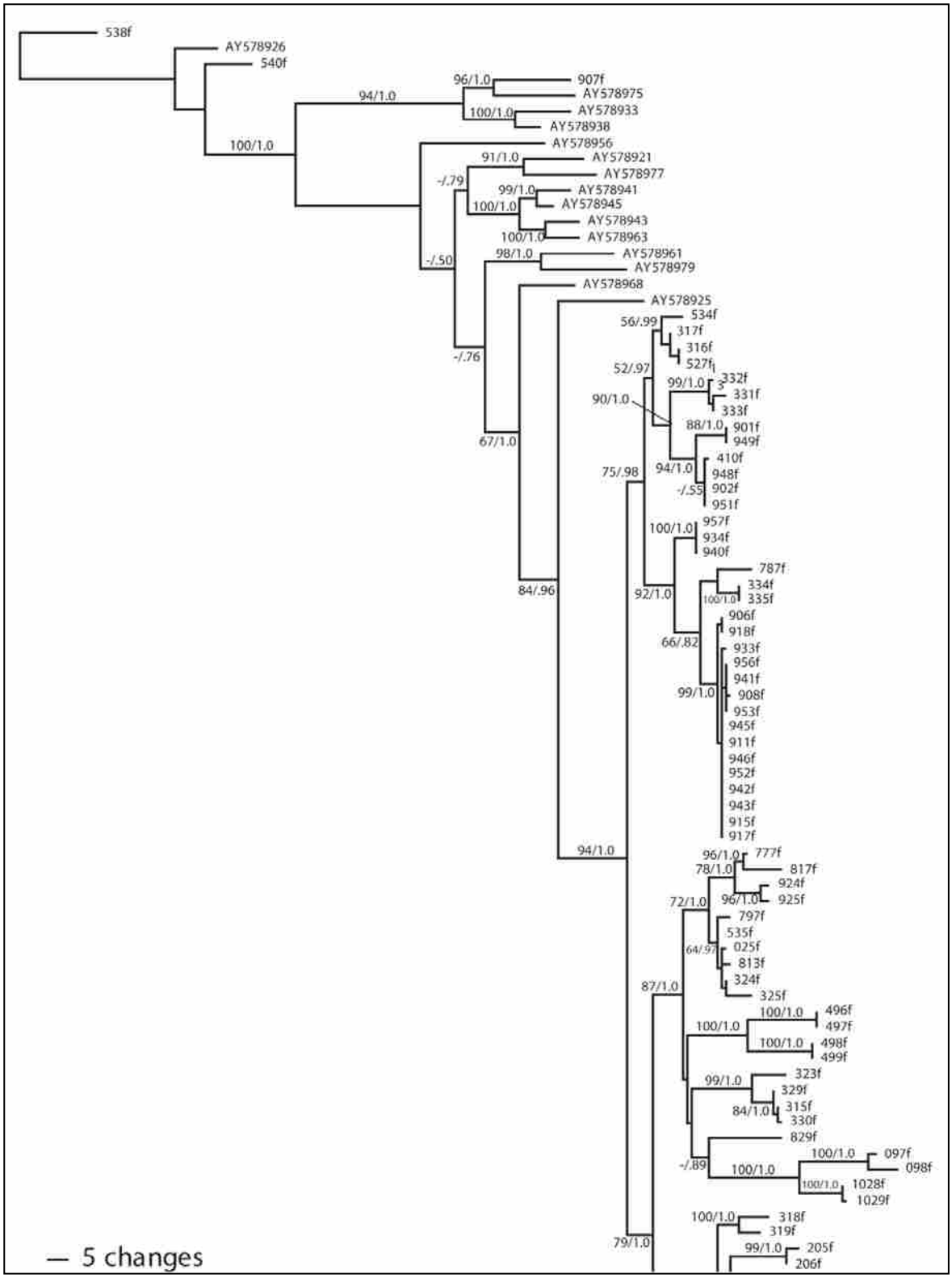


Supplementary Figure 2.3c-3. Maximum likelihood topology estimated from the *MCM7* fragment, with bootstrap support indicated at nodes.

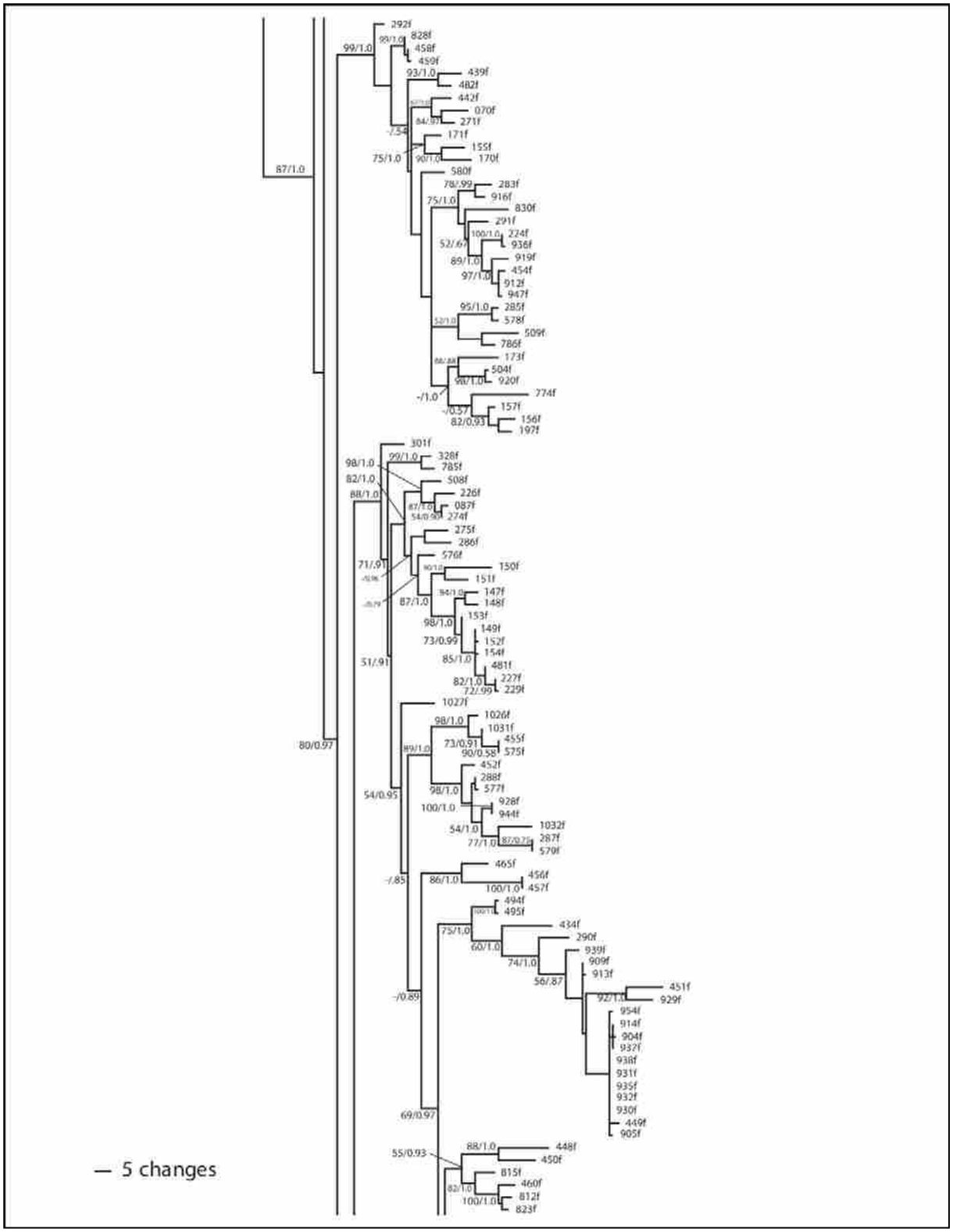


Supplementary Figure 2.3c-4. Maximum likelihood topology estimated from the *MCM7* fragment, with bootstrap support indicated at nodes.

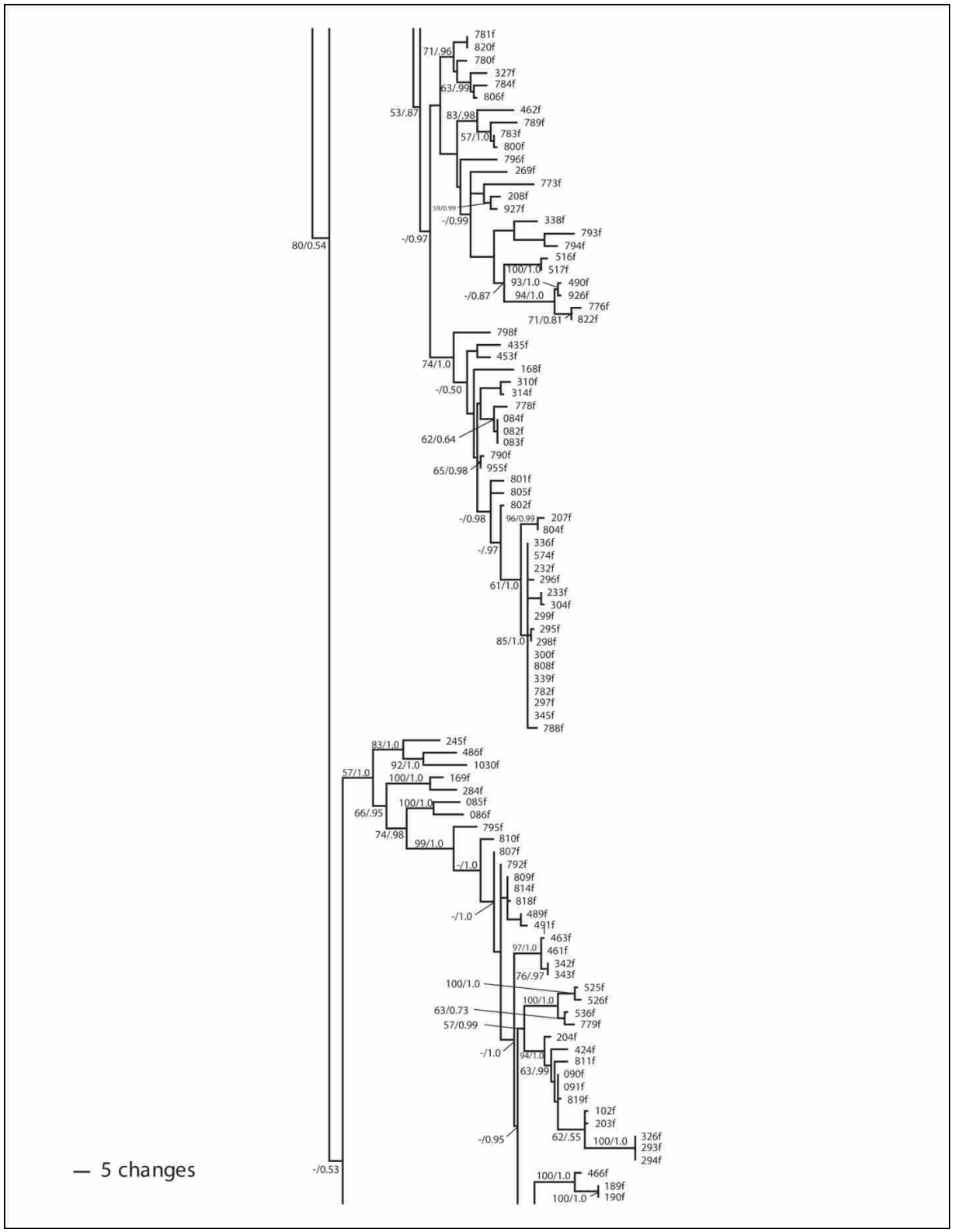
Supplementary data 2.4 (subsequent five pages). Full ML tree with Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values (BS) > 0.50/50 indicated at nodes.



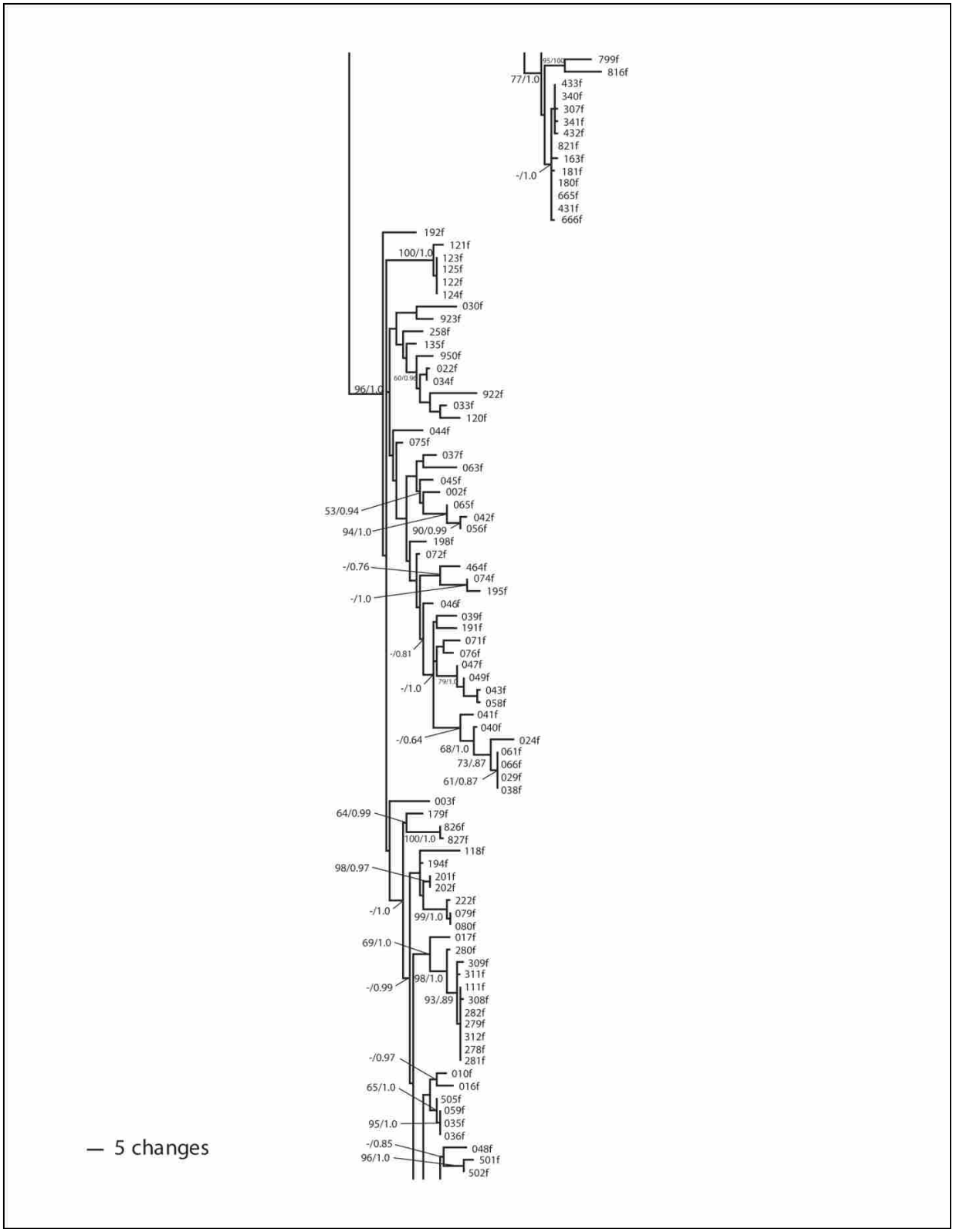
Supplementary data 2.4-1. Full ML tree with Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values (BS) > 0.50/50 indicated at nodes.



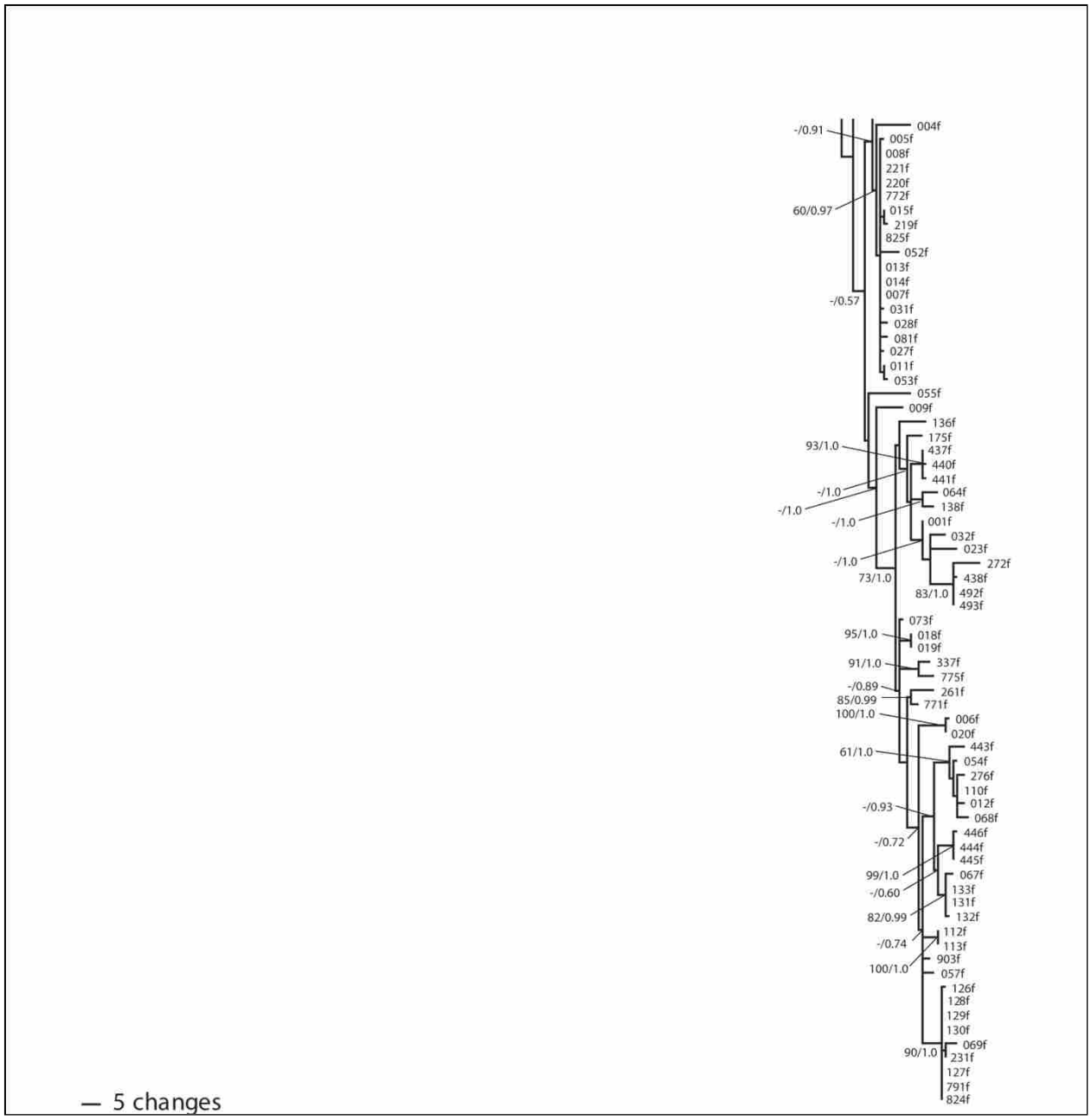
Supplementary Figure2.4-2. Full ML tree with Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values (BS) > 0.50/50 indicated at nodes.



Supplementary Figure 2.4-3. Full ML tree with Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values (BS) > 0.50/50 indicated at nodes.



Supplementary Figure 2.4-4. Full ML tree with Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values (BS) > 0.50/50 indicated at nodes.



Supplementary Figure 2.4-5. Full ML tree with Bayesian posterior probabilities (PP) and maximum likelihood bootstrap values (BS) > 0.50/50 indicated at nodes.

CHAPTER THREE

Species delimitation and evolution in morphologically and chemically diverse communities of the lichen-forming genus *Xanthoparmelia* (Parmeliaceae, Ascomycota) in western North America[1]

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Abstract

Premise of the study
Accurate species delimitation is important for understanding the factors that drive the diversification of biota and has critical implications for ecological and conservation studies. However, a growing body of evidence indicates that morphology-based species circumspection in lichenized fungi misrepresents fungal diversity. The foliose lichen genus *Xanthoparmelia* (Vainio) Hale. includes over 800 described species displaying a complex array of morphological and secondary metabolite diversity, and provides a model system to assess lichen species delimitation

Methods
In this study we used a multifaceted approach, applying phylogenetic, population genetic, and genealogical analyses to delimit species in a single well-supported monophyletic clade containing ten morphologically and chemically diverse *Xanthoparmelia* species in western North America. Specifically, sequence data from 4 ribosomal and 2 nuclear loci, along with chemical and morphological were used to assess species diversity.

Key results
We find that traditionally circumscribed species were not supported by molecular data. Rather, all sampled taxa were better represented by three polymorphic population clusters supported, in part, by multiple analytical approaches. Our results suggest that secondary metabolite variation may have limited utility in diagnosing lineages within this group, while identified populations clusters did not reflect major phylogeographic or ecological patterns.

Conclusions
In contrast to other studies revealing previously undiscovered fungal lineages masked within lichen species circumscribed by traditional morphological and chemical concepts, the present study suggests that species diversity has been overestimated in the species rich genus *Xanthoparmelia.* A concordance approach using multiple lines of evidence and analytical tools provides an effective approach to delimit lichenized fungi species in notoriously challenging groups.

Key words: character evolution; morphology, secondary metabolites; species delimitation, vagrant lichens; *Xanthoparmelia.*

Introduction

Lichens are stable, self-supporting, and self-reproducing obligate symbiotic associations consisting of an alga and/or cyanobacterium inhabiting the extracellular cavities within a fungal partner (DePriest, 2004). Evolutionarily and ecologically diverse, lichens involve one-fifth of all known extant fungal species globally distributed from tropic to the polar regions (Brodo, 2001; Lutzoni, Pagel, and Reeb, 2001). The co-evolution of lichen symbionts has resulted in the expression of a wide array of secondary metabolites and morphological structures not found in non-lichenized fungi that promote the overall success of the lichen association (Elix, 1996; Sanders, 2001). Morphological and chemical characters of the complete lichen structure have traditionally been used to delimit species boundaries in lichenized fungi. However, many of these characters provide little basis for inferring evolutionary histories, and the possibility of convergence poses a substantial problem for studies based solely on morphological and chemical data (Myllys, Lohtander, and Tehler, 2001; Gaya et al., 2003; Søchting and Lutzoni, 2003; Lumbsch et al., 2007; Amtoft, Lutzoni, and Miadlikowska, 2008). The widespread use of molecular data for testing current morphology- and chemistry-based species classifications in lichenized fungi has generally indicated that traditional taxonomic boundaries are in conflict with molecular reconstructions at all taxonomic levels (Crespo and Pérez-Ortega, 2009; Printzen, 2009). Here, we present a multifaceted approach, using multiple independent lines of data and various analytical methods to empirically delimit species within a common, conspicuous lichen-forming fungal genus in western North America. Diversification processes in lichenized fungi are not yet well understood, and these data provide important insights into challenges in assessing and delimiting lichen species boundaries.

Although the systematic value of morphological and chemical characters delimiting lichen-forming fungal species has been evaluated within a molecular context in only a limited number of cases, these studies suggest that lichen species diversity has been greatly misrepresented (Kroken and Taylor, 2001; Molina et al., 2004; Divakar et al., 2005; Buschbom and Mueller, 2006; Argüello et al., 2007; Wirtz, Printzen, and Lumbsch, 2008; O'Brien, Miadlikowska, and Lutzoni, 2009; Wedin et al., 2009). Incongruence between traditional lichen species boundaries and molecular phylogenetic reconstructions suggests that one of the greatest challenges in empirical species delimitation of lichenized fungi is finding and using the appropriate character sets and analytical tools (Crespo and Pérez-Ortega, 2009). In spite of the contentious efforts to conceptually define species, an apparent consensus has formed around the view that species are segments of separately evolving metapopulation lineages, termed the general lineage concept (GLC; de Queiroz, 1998, 1999, 2007). This approach allows investigators to delimit species using different operational criteria, data sets, and analytical methods (Sites and Marshall, 2004; de Queiroz, 2007). Under the GLC, the use of multiple operational criteria to delimit species can be used as lines of evidence to corroborate putative lineages (Sites and Marshall, 2004; de Queiroz, 2007). Furthermore, a rapidly growing interest in species delimitation methods has resulted in novel approaches to assess species boundaries (Knowles and Carstens, 2007; Groeneveld et al., 2009; Liu et al., 2009; O'Brien, Miadlikowska, and Lutzoni, 2009; Vieites et al., 2009; O'Meara, 2010; Weisrock et al., 2010; Yang and Rannala, 2010). An integrative approach to species delimitation using multiple independent data sets and analytical methods has been increasingly recognized as essential for rigorously testing species boundaries, particularly in the case of recent speciation events (Will, Mishler, and

Wheeler, 2005; Roe and Sperling, 2007; Groeneveld et al., 2009; Ruiz-Sanchez and Sosa, 2010; Weisrock et al., 2010).

Xanthoparmelia (Vainio) Hale is one of the best-studied and most species-rich genera in the Parmeliaceae (Ascomycota), including more than 800 described species worldwide (Crespo et al., 2007). The diversity of this genus is manifest in a wide array of morphological characters as well as the production of distinct secondary metabolite patterns, which traditionally have been used to diagnose species (Hale 1990). This approach has been problematic and many of the current groupings are disputed (Blanco et al., 2004a; Blanco, Crespo, and Elix, 2005; Blanco et al., 2006; Thell, Elix, and Søchting, 2009; Del-Prado et al., 2010). In recent years, systematic revisions within the Parmeliaceae have broadened the generic circumspection of *Xanthoparmelia*, and several major clades have been identified (Blanco et al., 2004a; Crespo et al., 2007; Del Prado et al., 2007). However, within this well-studied genus, α-level diversity and population-level dynamics remain relatively unexplored (Thell, Elix, and Søchting, 2009; Del-Prado et al., 2010; Hodkinson and Lendemer, 2010). Extensive species diversity within *Xanthoparmelia* provides a model system for evaluating current morphology and chemistry-based species boundaries in lichenized ascomycetes. In addition, many *Xanthoparmelia* species are broadly distributed both geographically and ecologically; and by defining population structure, identifying dispersal barriers, and characterizing ecological preference within these broadly distributed lineages will aid in identifying mechanisms that generated and maintain genetic diversity within the genus.

In this study we investigated α-level relationships in commonly occurring *Xanthoparmelia* species containing *β*-orcinol depsidone compounds in western North America as individuals with distinct chemistries and morphologies often co-occur in a wide range of

ecological settings, including, shrub-steppe, subalpine, and alpine communities (Hale, 1990; Rosentreter, 1993; Leavitt and St. Clair, 2008). Species within this complex differ markedly in vegetative morphology (Hale, 1990). The genus is generally characterized by various saxicolous species with some taxa showing some degree of attachment to soil surfaces, while other species are vagrant, or obligatory unattached. Vagrant taxa are commonly found in many deserts, steppes, and high plain areas of western North America. The relationship between vagrant and attached *Xanthoparmelia* species has long been in question (Mereschkowsky, 1918; Klement, 1950; Hale, 1990; Rosentreter, 1993). Recent studies indicate that the vagrant growth form has evolved multiple times independently in *Xanthoparmelia* (Leavitt, 2010). Although in some cases vegetative morphology provides important diagnostic characters, other species may be morphologically indistinguishable, and the expression of distinct secondary metabolites has traditionally been used to delimit both saxicolous and vagrant species within this group (Hale, 1990). Three major chemotypes are commonly used to delimit species within the *β*-orcinol depsidone containing complex in western North America: taxa containing stictic and accessory acids; taxa containing salazinic and accessory acids; and less commonly, taxa lacking both stictic and salazinc acid, but expressing norstictic acid. Chemical characters have also been shown to be highly homoplasious within *Xanthoparmelia* (Blanco et al., 2004a; Thell, Elix, and Søchting, 2009; Leavitt, 2010). However, reproductive barriers between different chemotypes in closely related *Xanthoparmelia* species have not been explicitly tested.

The primary focus of this study is on the delimitation of closely related lichen-forming fungal species, and here we present our analyses of species delimitation in the species-rich genus *Xanthoparmelia* as a working example typifying some of the inherent challenges related to the process of speciation in a complex and problematic taxonomic group. The current study

involves evaluating current species boundaries within the lichen genus *Xanthoparmelia*, while ultimately providing a basic knowledge about the evolution of those morphological and chemical characters commonly used to delimit species. Specifically we investigate the relationship between ten chemically and morphologically diverse *Xanthoparmelia* species from a single, well-supported clade (Leavitt, 2010). We are particularly interested in: 1) empirically delimiting species within this diverse clade using multiple analytical methods; 2) evaluating character evolution and the utility of morphological and chemical characters for delimiting species; 3) inferring distribution patterns, dispersal barriers, and ecological preferences within this group; and 4) providing insights into the origins of the vagrant life form at a local scale. Using the general metapopulation lineage concept (de Queiroz, 1998; Mayden, 1999; de Queiroz, 2007) and multiple sources of data, we apply multiple analytical methods to empirically assess species boundaries and evolution of major diagnostic characters within the focal group. We evaluate putative lineages, including currently accepted *Xanthoparmelia* species and two alternative classifications, within a population-level framework designed to assess gene flow and genetic differentiation (O'Brien, Miadlikowska, and Lutzoni, 2009). We also analyze molecular data within a phylogenetic framework to assess monophyly of currently accepted taxa; assess putative lineages across gene haplotype networks to identify groups that exhibitgenealogical exclusivity (an expected pattern for divergent lineages; (Avise and Ball, 1990; Baum and Shaw, 1995; Hudson and Coyne, 2002a). Furthermore, we use multi-locus sequence data to identify genetic clusters without a priori assignment of individuals (Groeneveld et al., 2009; Weisrock et al., 2010). The use of multiple datasets, along with the specified combination of analytical methods, provides a robust approach for assessing putative lineages and delimiting species within closely related *Xanthoparmelia* lineages.

Materials and Methods

Taxon sampling—We investigated the relationship between a total of 146 morphologically, chemically, and ecologically diverse *Xanthoparmelia* accessions collected from 47 populations in the Intermountain western United States. Samples were limited to a single, well-supported lineage identified in Leavitt (2010). To more specifically assess potential gene flow between sympatric congeners, and infer distribution patterns and dispersal barriers between populations, we sampled individuals from six sites distributed across the summit of Boulder Mountain Plateau, Garfield and Wayne Counties, and eight locations in the Uinta Mountain Range in Duchesne and Summit Counties, Utah, USA. A total of 1528 specimens were collected from these sites for initial morphological, chemical, and molecular analyses. Fifty-nine individuals from Boulder Mountain Plateau and 30 from the Uintah Mountain Range were selected to represent the overall chemical and morphological diversity of the baseline sample. In addition, 57 accessions recovered in the same monophyletic lineage in Leavitt (2010), were also included in this study. The geographic distribution of the ingroup accessions is shown in Fig. 1. Eleven closely related individuals indentified in Leavitt (2010), were chosen as outgroups, and detailed collection information for all accessions included in the present study are listed in Appendix S1. Voucher material used for this study is housed at the Brigham Young University Herbarium of Nonvascular Cryptogams, Brigham Young University, Provo, Utah.

Secondary metabolite data were generated for all vouchers using thin layer chromatography (TLC). Lichen compounds were extracted in acetone using 0.02 grams of thallus material; an acetone wash was subsequently used for chromatography in solvents C and G (Orange, James, and White, 2001). Taxonomic assignments were based on morphological and chemical data following Hale (1990) and Nash and Elix (2004) and are summarized in Table 1.

Based on current taxonomy, these individuals represent ten described taxa, including five vagrant taxa: *X. chlorochroa* (Tuck.) Hale (51 individuals), *X. lipochlorochroa* Hale & Elix (3), *X. neochlorochroa* Hale (4), *X. norcholorochroa* Hale (3), *and X. vagans* (Nyl.) Hale (4); and five saxicolous (or terricolous) taxa: *X. californica* Hale (2), *X. coloradoënsis* (Gyelnik) Hale (28), *X. cumberlandia* (Gyelnik) Hale (40), *X. neowyomingica* Hale (7), and *X. wyomingica* (Gyelnik) Hale (6). However, confusion surrounding the *diagnosability* and significance of most vegetative morphological characters has been reported (Blanco et al., 2004a; Thell, Elix, and Søchting, 2009; Del-Prado et al., 2010; Leavitt, 2010), and we therefore chose to represent all taxonomic assignments sensu lato (s. l.).

Molecular data—Total genomic DNA was extracted using either the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) according to the manufacturer's instructions, or the Prepease DNA Isolation Kit (USB, Cleveland, Ohio, USA), following the plant leaf extraction protocol. Fungal specific primers were used to amplify six nuclear markers, including four nuclear ribosomal loci: the entire internal transcribed spacer (ITS: ITS1, 5.8S, ITS2), a fragment of the intergenic spacer (IGS), a fragment of the large subunit (LSU), and a group I intron located in the small subunit (Gutiérrez et al., 2007). In addition, fragments from two low-copy protein coding loci, β-tubulin and *MCM7* were amplified. While low levels of intragenomic variation in fungal rDNA repeats suggests convergent evolution in which homogenization effectively maintaining highly similar repeat arrays (Ganley and Kobayashi, 2007), previous studies have confirmed the utility of the sampled ribosomal loci for species and population-level studies in lichenized ascomycetes (Thell, 1999; Kroken and Taylor, 2001; Blanco et al., 2004a; Blanco O and et al., 2004; Buschbom and Mueller, 2006; Lindblom and Ekman, 2006; Brunauer et al., 2007; Gutiérrez et al., 2007; Wirtz, Printzen, and Lumbsch, 2008; O'Brien, Miadlikowska,

and Lutzoni, 2009; Wedin et al., 2009). Although a duplication of the β-tubulin gene has occurred within Ascomycota, the paralogs are easily distinguishable within the analyzed group and the marker has been successfully used to investigate α-level relationships in lichenized ascoymycetes (Buschbom and Mueller, 2006; O'Brien, Miadlikowska, and Lutzoni, 2009; Wedin et al., 2009).

Standard polymerase chain reactions (PCR) were used to amplify targeted loci. Fungal-specific primers used in PCR amplifications and in the cycle sequencing reactions are shown in Table 2. PCR cycling parameters used for amplifying the ITS, group I intron, LSU, and β-tubulin loci followed the methods of Blanco et al. (2004a); while cycling parameters for amplifying the IGS followed the 66-56° touchdown reaction described in (Lindblom and Ekman, 2006). PCR cycling parameters for amplifying the *MCM7* fragment followed (Schmitt et al., 2009). PCR products were quantified on 1% agrose gel and stained with ethidium bromide. In those cases where no PCR products were visualized for the β-tubulin, *MCM7*, and IGS fragments, internally nested PCR reactions were performed using 0.3 ul of the PCR product from the original reaction with newly designed primers; namely, 'BT-RhizoF' and 'BT-RhizoR' for the β-tubulin fragment, 'X*MCM7*f' and 'X*MCM7*r' for the *MCM7* fragment, and IGS rDNA: IGS12a-5' (Carbone and Kohn, 1999) and 'XIGSr' for the IGS fragment, using the touchdown PCR cycling parameters described above used to amplify the IGS marker. PCR fragments were cleaned using the PrepEase PCR Purification Kit, following the manufacturer's protocol (USB, Cleveland, OH), and complementary strands were sequenced using the same primers used for amplification. Sequencing reactions were performed using the Big Dye3 Termination Sequencing Kit (Applied Biosystems, Foster City, California) at 1/8 the standard reaction

volume. Products were run on an AB 3730xl automated sequencer at the DNA Sequencing Center at Brigham Young University, Provo, Utah, USA.

Sequences were assembled and edited using Sequencher version 4.2 (Gene Codes Corporation, Ann Arbor, Michigan) and Se-Al v2.0a11 (Rambault, 1996). Sequence identity was checked using the 'megablast' search option in GenBank (Wheeler et al., 2006). All sequences were aligned with outgroup taxa identified in preliminary phylogenetic analyses using defaults settings in Muscle v3.7 because of the improved speed and alignment accuracy as compared with other currently available programs (Edgar, 2004).

Nucleotide diversity and gene-flow estimation—Basic nucleotide polymorphism statistics, including number of polymorphic sites and estimates of θ (Watterson, 1975) and average pairwise differences (π; Nei, 1987) were calculated using DnaSP version 5.10.01 (Librado and Rozas, 2009) for each putative species, three major chemotypes (norstictic, salazinic, and stictic), and populations clusters recovered in the STRUCTURE analyses (see below). Genetic differentiation between putative species, chemotypes, and population clusters was assessed by counting the number of fixed nucleotide differences (O'Brien, Miadlikowska, and Lutzoni, 2009) and calculating F_{ST} values using Arelequin v 3.11 (Laurent, Guillaume, and Stefan, 2005), with 10,000 permutations to determine significance. Pairwise species comparisons were limited to the seven most common putative species recovered in this clade, *X. chlorochroa* (51 individuals), *X. coloradöensis* (28), *X. cumberlandia* (40), *X. neochlrochroa* (4), *X. neowyomingica* Hale (7), *X. vagans* (4), and *X. wyomingica* (6).

Phylogenetic analysis—Preliminary phylogenetic reconstructions were performed independently for each sampled marker. However, a weak phylogenetic signal was generally identified across all markers, and we opted to concatenate all markers for phylogenetic

reconstructions to resolve important relationships and improve nodal support (Wiens, 1998; Rokas and Carroll, 2005). Heterogeneity in phylogenetic signal between sampled markers was assessed before combining the six datasets (Lutzoni et al., 2004). Maximum likelihood (ML) analyses were performed for the concatenated ribosomal dataset (ITS, IGS, LSU, and group I intron), while β-tubulin, and *MCM7* markers separately using the program RAxML 7.0.4 (Stamatakis, 2006). Support was assessed using 1000 "fastbootstrap" replicates implemented in the CIPRES Web Portal (Stamatakis, 2006; Stamatakis, Hoover, and Rougemont, 2008). RAxML allows partitioned analyses implementing the general time reversible (GTR) substitution model for all partitions (Stamatakis, 2006). We compared two partition strategies for protein-coding gene fragments. First, we treated the entire marker as a single partition. Second, we used a 3-partition approach using the first, second, and third codon positions as separate model partitions for the *MCM7* marker, and a 4-partition strategy for the β-tubulin marker using the first, second, and third codon positions and a 55 base pair intron located within the fragment as separate model partitions. We assumed that partitions within genes had the same overall model as the entire gene, as simulations show there may be frequent errors in supporting complex models from a sample of limited characters (Posada and Crandall, 2001a). We implemented the GTRGAMMA model, which includes a parameter (Γ) for rate heterogeneity among sites, but chose not to include a parameter for estimating the proportion of invariable sites following the recommendations of Stamatakis (2006). Support values for the ribosomal, β-tubulin, and *MCM7* phylogenies were examined for well-supported (≥70% bootstrap values) conflict between datasets (Lutzoni et al., 2004). Given no conflict was identified; we combined all datasets for subsequent phylogenetic analyses.

Phylogenetic relationships were estimated from the combined dataset using mixed-model Bayesian Inference (BI) as implemented in Mr.Bayes ver. 3.1.2 (Huelsenbeck and Ronquist, 2001). We used MrModeltest2 version 2.3 (Nylander et al., 2004) to identify the appropriate model of evolution for each marker using the Akaike Information Criterion (AIC) see (Posada and Buckley, 2004). The combined dataset was analyzed using locus-specific model partitions. Exploratory analyses indicated that nodal support was generally improved across the topology (comparisons not shown), and each ribosomal marker was treated as a separate partition, and protein-coding markers were partitioned using the 3-partition strategy for the *MCM7* marker, and the 4-partition strategy for the β-tubulin marker as described above. Four independent replicate searches were executed with eight chains; each run started from randomly generated trees and involved sampling every 1000 generations for 20,000,000 generations. To evaluate stationarity and convergence between runs, log-likelihood scores were plotted using TRACER version. 1.5 (Drummond et al., 2003), effective sample size (ESS) statistics were evaluated, and the average standard deviation in split frequencies was assessed at the end of the run. Trees generated prior to stationarity were discarded as burn-in (Huelsenbeck et al., 2001), and results were summarized with a majority-rule consensus tree from the remaining trees from the four independent runs. Bayesian posterior probabilities (PP) were assessed at all nodes and clades with PP $\geq$ 95 were considered strongly supported (Huelsenbeck and Rannala, 2004).

Because BI may resolve bifurcations with strong support when relationships are really unresolved (Kolaczkowski and Thornton, 2007), we conducted a ML analysis implemented RAxML 7.0.4 using the concatenated data set (ITS, LSU, group I intron, IGS, *MCM7* and β-tubulin loci). Data were partitioned as described for the BI analysis. We used the GTRGAMMA model, which includes a parameter (Γ) for rate heterogeneity among sites. Following the

recommendations of Stamatakis (2008) we did not include a parameter for the proportion of invariable sites because Γ accounts for this source of rate heterogeneity by using 25 rate categories. Analyses proceeded by combining 200 separate maximum likelihood searches (to find the optimal tree) and 1000 bootstrap pseudoreplicates to evaluate support for each node was conducted.

Testing alternative hypotheses—We compared three alternative topologies to the best ML hypothesis generated in this study; specifically: 1) constraining the tree search to recover each putative species as monophyletic; 2) constraining the search to recover the three diagnostic chemotypes recovered in this lineage, norstictic, salazinic, and stictic acids respectively, as monophyletic; and 3) constraining the search to recover each population detected in the STRUCTURE analysis (described below) as monophyletic. In the second alternative topology we left the relationship of *X. lipochlorochroa* unresolved because this taxon does not contain any of the three diagnostic chemotypes, but rather is characterized by the occurrence of fatty acids. In the third alternative topology we left relationship of individuals assigned to a population cluster with < 0.70 probability unresolved. Alternative hypotheses were constructed in Mesquite version 4.03 (Maddison and Maddison, 2007). Constrained topologies were estimated in RAxML using the partitioning strategies described above. We used the Shimodaira and Hasegawa (SH; 1999) likelihood comparison test as implemented in RAxML to test our best-scoring ML topology against the three alternative topologies.

Haplotype networks—Phylogenetic reconstruction methods, such as maximum likelihood (ML), maximum parsimony (MP), and Bayesian inference (BI), estimate interspecific relationships and often lead to poor resolution or inadequate portrayals of genealogical relationships in cases of low divergence, extant ancestral nodes, multifurcations, and

reticulations (Templeton, Crandall, and Sing, 1992; Posada and Crandall, 2001b). Therefore, we used statistical parsimony to assess the genealogical relationships of every individual and to compare the relationships of putative lineages between genes. Because recombination within nuclear genes can lead to errors in the estimated topology (Posada, Crandall, and Holmes, 2002), we tested for recombination events in the low-copy protein-coding markers using methods implemented in Recombination Detection Program (RPD3; (Martin, Williamson, and Posada, 2005; Heath et al., 2006). Networks were constructed from the concatenated ribosomal sequences (ITS, LSU, IGS, intron), as well as the β-tubulin and the *MCM7* fragments under a 95% statistical parsimony criteria using the program TCS version 1.21 (Clement, Posada, and Crandall, 2000). In order to reduce network uncertainties due to missing data, individuals missing one of the four ribosomal markers were removed, and gaps within markers were treated as missing data for the ribosomal network reconstruction. All protein-coding sequences were trimmed to the length of the fragment generated by the nested PCR reactions in the network calculations. Network uncertainties (i.e., closed loops) were treated following Templeton and Sing (1993). Relationships of putative species, chemotypes, and population clusters were evaluated within and between individual gene trees to identify lineages that exhibited genealogical exclusivity across multiple loci (Avise and Ball, 1990; Hudson and Coyne, 2002b). The presence of the same groups in the majority of single-locus genealogies can be taken as evidence that the groups represent reproductively isolated lineages (Dettman et al., 2003; Pringle et al., 2005).

Population genetic clustering—Individual-based approaches provide an alternative for identifying genetic structure and barriers to gene flow, as analyses based on predefined delineations of groups can obscure patterns of differentiation (Latch et al., 2006; Rowe and

Beebee, 2007). We used a multilocus Bayesian population assignment test implemented in STRUCTURE 2.32 (Pritchard, Stephens, and Donnelly, 2000; Falush, Stephens, and Pritchard, 2003) to determine the most likely number of population clusters within the focal group. Studies suggest that STRUCTURE can provide an accurate portrayal of the uppermost level of hierarchical structure in a wide array of scenarios, and 'populations' inferred by STRUCTURE should be viewed as networks of local populations connected by patterns of gene flow over long timescales (Evanno, Regnaut, and Goudet, 2005). This approach had been useful in identifying lineages in the early stages of species divergence (Weisrock et al., 2010). The six sampled loci in our study were estimated to be sufficient to provide an overview of the highly differentiated groups (Saisho and Purugganan, 2007; Groeneveld et al., 2009; Weisrock et al., 2010). Based on our exploratory studies, we implemented ten replicate runs for each K value, from 1-12, with burn-in generations set to 15,000, followed by 30,000 iterations for each run using the admixture options. The median log likelihood of each *K* value was calculated from the 10 runs. Following the procedure outlined by Evanno et al. (2005), the modal value (ΔK) based on the second order rate of change of the likelihood function, with respect to *K*, was used to estimate the most likely number of clusters within the sample. We classified individuals with posterior probabilities < 0.70 to any cluster into an "admixed" group.

Results

Molecular data—Over the course of this study we obtained 885 new sequences from six loci. Variation in the six sampled loci consist of 3503 aligned nucleotide positions in the combined analyses representing 157 individuals is summarized in Table 3. All representative haplotypes of the six gene fragments were submitted to GenBank (Appendix 1).

Nucleotide diversity and gene-flow estimation—Nucleotide diversity statistics for putative lineages are reported in Table 4. Pairwise F_{ST} comparisons indicate that generally population structure is not maintained between putative species, although statistically significant F_{ST} values were estimated between *X. chlorochroa* and *X. cumberlandia*; *X. neochlorochroa* and *X. neowyomingica*; *X. neowyomingica* and *X. vagans*; and *X. neowyomingica* and *X. wyomingica* (Table 5). Significant F_{ST} values reveal genetic differentiation between the two most common major chemotypes (i.e. salazinic and stictic acids) and also between population clusters inferred in the STRUCTURE analyses (Table 6). However, fixed nucleotide differences were not identified between putative species, chemotypes, or population clusters.

Phylogenetic analyses—Individual gene trees generally showed only weak genetic structure, particularly for the protein-coding and the group I intron topologies (see Appendix S2). Preliminary analyses indicated that nodal support generally improved across the topology when the data set was considered with additional partitioning of the protein-coding fragments. We opted to use the more complex partitioning strategy in subsequent analyses to provide a better estimate of the phylogeny (Ronquist and Deans, 2009). No incongruence was identified between loci using the ≥ 70 ML support incongruence test; therefore all loci were combined for phylogenetic analyses.

The partitioned Bayesian analyses, summed from four independent runs, yielded a negative harmonic mean ln likelihood=11 517.6284. All parameters converged within the first 25% of sampled generations, leaving a posterior distribution estimated from 15 000 trees per run (60 000 total post-burn-in sampled trees). Partitioned ML analyses yielded a single best-score tree –ln likelihood=11 156.9153. The ML and BI topologies from the combined datset of six gene regions were highly similar, exceptions being restricted to minor differences in the

arrangement of some terminals, but relationships at all deeper nodes and well-supported clades were identical. We chose to present the ML topology (Fig. 2). A single well-supported clade (bootstrap support BS=99, Bayesian posterior probability PP=1.00) with 146 individuals, representing ten taxa was identified as the focal group for this study, called hereafter the Intermountain *Xanthoparmelia* group. Species assigned to this group include five described vagrant taxa, *X. chlorochroa*, *X. lipochlorochroa*, *X. neochlorochroa*, *X. norchlorochroa*, and *X. vagans*; and five saxicolous taxa *X. californica*, *X. coloradoënsis*, *X. cumberlandia*, *X. neowyomigica*, and *X. wyomingica*. A well-supported lineage (BS=93, PP=1.00), comprised of geographically broadly distributed representatives of *X. cumberlandia*, *X. mexicana*, and *X. wyomingica*, was recovered as sister to the focal group with weak support (BS=50, PP=0.73). Within the Intermountain *Xanthoparmelia* group, *X. coloradoënsis* 030f was supported as sister to the remaining group with a high PP value (1.00), although BS support was < 50. Many relationships within this group lacked strong statistical support and were unresolved, and all putative species were found to be poly- or paraphyletic.

Table 7 shows the results of the SH tests comparing our best topology to three potential alternative classifications. Both constrained topologies representing currently accepted species and chemotypes represented significantly worse alternatives to our best tree. However, the constrained topology representing population clusters identified in the STRUCTURE analysis was not significantly different from the best unconstrained topology recovered in this study. Therefore, we determined that the population clusters defined in this study a serve as a reasonable working hypothesis of relationships among the sampled individuals representing the Intermountain *Xanthoparmelia* group.

Haplotype network analyses—Evidence of recombination was not detected in the nuclear genes and genealogical relationships inferred by statistical parsimony are shown in Fig. 3. Thirty-one individuals missing at least one of the ribosomal markers were removed from the dataset and the ribosomal network with the remaining 114 *Xanthoparmelia* individuals grouped in 74 unique haplotypes within a single network. The β-tubulin network with 137 individuals was grouped in 22 unique haplotypes within a single network, while the *MCM7* network including 138 individuals was grouped in 58 unique haplotypes within a single network. The most common haplotypes for all sampled loci were found in the most commonly represented taxa, *X. chlorochroa*, *X. coloradöensis*, and *X. cumberlandia*. Individuals representing *X. californica*, *X. lipochlorochroa*, *X. neochlorochroa*, and *X. vagans* shared haplotypes with representatives of the more common taxa or were separated by a single mutation event in all haplotype networks. Individuals (0-3 individuals/locus) beyond the 95% statistical parsimony confidence limit were not identical across loci and were not represented in haplotype networks. The genealogical concordance criterion was not fulfilled for putative species, chemotypes, or population clusters. However, apart from a single individual in the ribosomal haplotype network, population cluster No. 1 exhibited genealogical exclusivity in both the ribosomal and β-tubulin haplotype networks, and general concordance was found between the ribosomal haplotype network and the population clusters inferred from the STRUCTURE analysis.

Population genetic clustering—The median ML values of the Bayesian clustering analysis using STRUCTURE with estimates of K=1-12 are shown in Fig. 4a, and the ΔK method (Evanno et al. 2005) indicates that a K=3 model best fits the data (ΔK=30.00 for K=3; ΔK=<12.0 for all other K values; Fig. 4b). STRUCTURE plots for K>3 generally did not yield additional population clusters with high membership coefficients for more exclusive sets of populations or

clusters. Therefore, we examined the phenotypic expressions and geographic distributions of population clusters within the *K*=3 model. The identified groupings were not consistent with any of the putative species, nor is there clear phylogeographic pattern in the distribution of the inferred population cluster. The assignment of current species to inferred population clusters and the geographic distributions of individual assignments are shown in Fig. 1. In the *K*=3 model, individuals assigned to population cluster No. 1 generally expressed the stictic acid chemotype, although a few individuals representing salazinic acid chemotypes were also assigned to this cluster. However, none of the vagrant taxa were assigned to this group. Individual accessions containing salazinic acid chemotypes (*X. chlorochroa, X. coloradoënsis,* and *X. wyomingica*) were primarily assigned to population clusters No. 2 and 3; although multiple representatives of the most common species, *X. chlorochroa, X. coloradoënsis,* and *X. cumberlandia,* were recovered within both population clusters No. 2 and 3. Vagrant specimens representing *X. chlorochroa* with membership in cluster No. 2 were generally collected in the vicinity of the Uinta Mountain Range in northeastern Utah, including both the northern slopes in southwestern Wyoming and the south slopes in Duchesne County, Utah. However, *X. chlorochroa* from western Idaho (Owyhee County), and two locations in Colorado (Moffat and Summit Counties) were also included in this cluster. Individuals representing *X. neochlorochroa, X. norchlorochroa, X. vagans,* and *X. wyomingica* were also assigned to population cluster No. 2 with posterior probabilities $\geq$0.95. The majority of individuals assigned to population cluster No. 3 represent vagrant taxa, including individuals of *X. chlorochroa, X. lipochlorochroa, X. norchlorochroa, X. neochlorochroa, X. vagans,* and *X. wyomingica*. Although all vagrant taxa sampled on Boulder Mountain, Utah were assigned to population cluster No. 3, this group showed the greatest geographic distribution of vagrant taxa with individuals collected from

Colorado, Montana, Utah, Washington, and Wyoming. Relatively few saxicolous individuals (7 of 38) were assigned membership to this group. Individuals from all inferred population clusters were found across the geographic distribution of the Intermountain *Xanthoparmelia* group; although those assigned to population cluster No. 2 generally occurred in areas with geographic proximity to the Uinta Mountain Range in northeastern Utah (Fig. 1). Admixed individuals included *X. chlorochroa* (004f and 009f), *X. coloradoënsis* (055f and 118f), and *X. wyomingica* collected from the type locality in the Bighorn Mountains, Wyoming, USA (826f and 827f).

Discussion

In contrast to recent molecular studies showing previously undiscovered fungal lineages masked within lichen species circumscribed by traditional morphological and chemical concepts (Kroken and Taylor, 2001; Goffinet, Miadlikowska, and Goward, 2003; Blanco et al., 2004b; Molina et al., 2004; Argüello et al., 2007; Wirtz, Printzen, and Lumbsch, 2008; O'Brien, Miadlikowska, and Lutzoni, 2009; Vondrák et al., 2009; Wedin et al., 2009), the present study suggests that species diversity has been overestimated in the large and species diverse lichen genus *Xanthoparmelia*. Our analysis of 146 morphologically and chemically diverse *Xanthoparmelia* specimens using six nuclear loci did not support any of the currently described species reported for western North America. The application of species delimitation criteria to identify lineages in the early stages of divergence suggests that the Intermountain *Xanthoparmelia* species complex may be more appropriately represented by three polymorphic lineages. Although previous studies have indicated that *Xanthoparmelia* species diversity has been misrepresented (Blanco et al., 2004a; Thell, Elix, and Søchting, 2009; Del-Prado et al.,

2010; Hodkinson and Lendemer, 2010), our results provide one of the first empirical investigations into species delimitation in closely related species complexes in the genus.

Species delimitation—We used a multifaceted approach, combining molecular systematics with methods derived from population genetics to identify lineages in the early stages of divergence (Groeneveld et al., 2009; O'Brien, Miadlikowska, and Lutzoni, 2009; Weisrock et al., 2010). By examining populations in the earlier stages of speciation mechanisms driving divergence become more evident and informative (Wiens, 2004; Knowles and Carstens, 2007; Weisrock et al., 2010).

Although the results of this study did not support currently described *Xanthoparmelia* species, our data do show strong partitioning into three differentiated population clusters inferred from the STRUCTURE analysis. These three groups were supported, in part, from other lines of evidence assembled from the analysis of multi-locus sequence data and chemical and morphological characters. Generally, basic polymorphisms statistics, including number of polymorphic sites and estimates of θ and π, show that the population clusters inferred in this showed similar or less nucleotide diversity within groups, compared to values calculated from the ten putative species. This pattern suggests that the more inclusive population clusters may more accurately portray natural groupings with less taxonomic subdivision. Population cluster No. 2 was concordant with a well-supported, monophyletic lineage recovered in the both the ML and BI phylogenetic reconstructions (Fig. 2B), while clusters No. 1 and 3 did not correspond to monophyletic lineages recovered in either topology. However, SH tests of alternative hypotheses indicate that population clusters inferred from STRUCTURE provide a reasonable working hypothesis of relationships within the Intermountain *Xanthoparmelia* group, relative to the best-scoring ML topology. In contrast, currently accepted species boundaries or a simple

subdivision of chemotypes provided significantly weaker alternative hypotheses of relationships, and were therefore not considered as reasonable alternatives. Generally, population clusters were concordant with the ribosomal haplotype network (Fig. 3), and general concordance was identified between the ribosomal and β-tubulin haplotype networks for population cluster No. 1.

Although boundaries between these population clusters are often 'fuzzy', lacking distinct discordance between characters sets (Sites and Marshall, 2004; Cardoso and Vogler, 2005), some level of concordance between methods and independent datasets indicates these clusters represent species-level lineages in the early stages of divergence. The assignment of taxonomic rank to a given lineage is not straightforward, particularly in cases where diagnostic morphological or chemical characters and phylogeographic patterns are ambiguous. In our study, traditional diagnostic characters were somewhat variable within population clusters, and the concordance approach did not unambiguously support any of the putative lineages. A potential criticism is that these methods excessively subdivide a single lineage, or, in contrast, it may be argued that molecular taxonomic approaches may fail to uncover genetic variation that correlates with the phenotypic variation used to diagnose species, particularly when closely related species co-occur or have diverged only recently (Wood and Nakazato, 2009). We contend that based on the general metapopulation lineage concept and multiple sources of data, this approach exhibits at least one layer of evidence for lineage divergence within the Intermaountain *Xanthoparmelia* group (Sites and Marshall, 2004; de Queiroz, 2007; Weisrock et al., 2010).

Importance of biochemical characters—Morphological and secondary chemical patterns offered limited supported for inferred lineages, and these characters were polymorphic within each of the inferred population clusters. However, general trends in the expression of secondary

metabolites suggest at least some level of reproductive isolation between salazinic and stictic acid chemotypes. Population cluster No. 1 was primarily characterized by specimens expressing stictic acid, while clusters No. 2 and 3 were characterized by specimens expressing salazinic acid. However, each population cluster also contained some accessions expressing the opposing chemotype. Average individual cluster memberships coefficients for conflicting chemotypes in each population cluster were relatively high (>0.90), showing limited signs of admixture. Whether polymorphic accessions in the inferred population clusters indicate ongoing or recent gene flow rather than incomplete lineage sorting remains unclear.

Chemically variable *Xanthoparmelia* species complexes have shown a strong correlation of chemotypes with ecological preferences (Nash and Zavada, 1977; Benedict and Nash, 1990). However, a chemically distinct group of *Xanthoparmelia* specimens collected across a relatively homogenous environment on Boulder Mountain, Utah demonstrated a level of reproductive isolation, suggesting microhabitat variation may be an important factor driving divergence rather than broad ecological preferences (Beard and Depriest, 1996; Chunco et al., 2009). Various functions for these secondary compounds have been suggested, including protection from UV-B radiation, herbivory defense, and antifungal and antibiotic activity (Huneck, 1999; Gauslaa et al., 2006; Solhaug et al., 2009). Furthermore, carbon source and photobiont have been shown to influence the secondary metabolism of the mycobiont (Brunauer et al., 2007). In spite of some uncertainty, our data suggests that species delimitation based on the expression of stictic acid within the Intermountain *Xanthoparmelia* clade may be warranted.

Ecological and geographic distributions—Inferred population clusters and identical haplotypes were often found distributed across relatively broad geographical and ecological landscapes, indicating wide ecological amplitude for these lineages. Individuals containing

salazinic acid sampled from the Uinta Mountain Range and vicinity were generally inferred to belong to a single population cluster (cluster No. 2 of the $K = 3$ model) regardless of putative species assignment, while individuals collected from the more geographically and ecologically restricted Aquarius Plateau were generally equally distributed between the three population clusters. The geographic and ecological distributions of saxicolous forms within all inferred population clusters suggests that geographic or ecological constraints do not effectively maintain barriers to gene flow at this scale. Sexual reproductive structures (apothecia) were observed in only 7 of the 146 individuals assigned to the Intermountain *Xanthoparmelia* group, and specialized asexual diaspores (isidia) were not observed. Fertile individuals were found in each of the three population clusters, and reproductive strategies within this group remain unclear. More extensive sampling and analysis will be essential in order to more fully characterize saxicolous population structure and distribution as a function of sexual reproduction.

Evolution of vagrancy at a local level—Representatives of vagrant taxa were identified in multiple well-supported lineages in ML and Bayesian topologies (although relationships between these lineages generally were not supported), suggesting multiple independent origins of the vagrant condition. Additionally, statistical parsimony networks suggest multiple independent origins of the vagrant habit as haplotypes representing vagrant specimens are generally found throughout all haplotype networks. The $K = 3$ STRUCUTRE model suggests two distinct groups containing vagrant *Xanthoparmelia*. Vagrant accessions did not occur in population No. 1, while membership in population cluster No. 3 was dominated by vagrant specimens, and population cluster No. 2 contained a mixture of both saxicolous and vagrant specimens. Vagrant individuals in population cluster No. 2 are generally limited to northeastern Utah and southwestern Wyoming. Relatively few individuals beyond this limited distribution were assigned to

population cluster No. 2; this group included individuals from western Idaho (*X. chlorochroa* 112f and 113f), northwestern Colorado (*X. chlorochroa* 775f, 824f; and *X. norchlorochroa* 771f), southeastern Wyoming (*X. neochlorochroa* 337f), and southern Utah (*X. neochlorochroa* 231f). In contrast, vagrant individuals with membership in population cluster No. 3 showed a much broader geographic distribution. Unspecialized vegetative fragments have been proposed as the major, if not exclusive, method of reproduction for most vagrant *Xanthoparmelia* species, limiting dispersal and genetic exchange between populations (Bailey, 1976; Rosentreter, 1993). It has been proposed that some long distance dispersal may be accomplished by migrating pronghorn antelope and other wild and domesticated ungulates (Thomas and Rosentreter, 1992; Rosentreter, 1993; St. Clair et al., 2007). The occurrence of similar haplotypes across a broad geographic range supports the grazing ungulate-mediated dispersal of vagrant forms. However, they may have also been independently derived from a common widespread attached haplotype. In spite of the inherent reproductive limitations of unspecialized vegetative fragments, vagrant accessions exhibited high haplotype diversity, and two of the admixed individuals identified in the STRUCTURE analysis were vagrant forms. These results suggest that sexual reproduction may be more common in vagrant *Xanthoparmelia* than previously thought.

Speciation in Xanthoparmelia—Accurate species delimitation is essential, as species are fundamental units for various sub-disciplines of biology. Following the GLC using multiple datasets and analytical tools we have been able to show that species diversity in *Xanthoparmelia* has been greatly misrepresented. These results emphasize the need to re-evaluate species boundaries in the large and diverse genus *Xanthoparmelia*. We conclude that that the concordance-based approach presented in this study is well-suited for species delimitation in lichenized ascomycetes where traditional morphological and chemical characters are apparently

misleading with respect to species diversity. However, at this point we are hesitant to make any taxonomic revisions in order to avoid unwarranted and confounding taxonomic changes until we have sampled and analyzed specimens from the type localities of the currently accepted species identified within this group. The next phase in our research will include analysis of molecular data, as well as additional morphological and chemical characters. At present, it remains unclear whether an accurate and consistent definition based on morphological characters can be found for the three population clusters. Furthermore, lichenized fungi typically display few taxonomically useful morphological characters, when compared to vascular plants and vertebrates. Furthermore, the general absence of reproductive characters in specimens collected as part of the Intermountain *Xanthoparmelia* complex pose a significant limitation in identifying putatively diagnostic morphological traits. Due to these challenges, a molecular taxonomy may provide the most practical approach to a consistent treatment of species within this group.

Conclusions

This study also suggests several avenues for ongoing investigation: 1) what are the barriers to reproduction that would maintain divergent lineages occurring in sympatry? 2) How are these sympatric populations partitioning resources? 3) What events may have led to the diversification, dispersal, and establishment of recently diverged lineages? 4) Is there a role for sexual reproduction in vagrant forms? Given these questions are tractable, we suggest *Xanthoparmelia* provides a model system for investigating the processes of speciation in lichenized ascomycetes.

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Literature Cited

AMTOFT, A., F. LUTZONI, and J. MIADLIKOWSKA. 2008. Dermatocarpon (Verrucariaceae) in the Ozark Highlands, North America. *The Bryologist* 111: 1-40.

ARGÜELLO, A., R. DEL PRADO, P. CUBAS, and A. CRESPO. 2007. *Parmelina quercina* (Parmeliaceae, Lecanorales) includes four phylogenetically supported morphospecies. *Biological Journal of the Linnean Society* 91: 455-467.

AVISE, J. C., and A. S. BALL. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. *In* D. Futuyma AND Antonovics [eds.], Oxford Surveys in Evolutionary Biology. Oxford University Press, Oxford, UK.

BAILEY, R. H. 1976. Ecological aspects of dispersal and establishment in lichens. *In* D. H. Brown, D. L. Hawksworth, AND R. H. Bailey [eds.], Lichenology: Progress and Problems, 215-247. Academic Press, New York, New York, USA.

BAUM, D. A., and K. L. SHAW. 1995. Genealogical perspectives on the species problem. *In* P. C. Hoch AND A. G. Stephenson [eds.], Experimental and Molecular Approaches to Plant Biosystematics, 289-303. Missouri Botanical Garden, St. Louis, Missouri, USA.

BEARD, K. H., and P. T. DEPRIEST. 1996. Genetic Variation Within and Among Mats of the Reindeer Lichen, *Cladina subtenuis*. *The Lichenologist* 28: 171-182.

BENEDICT, J. B., and T. H. NASH III. 1990. Radial Growth and Habitat Selection by Morphologically Similar Chemotypes of *Xanthoparmelia*. *The Bryologist* 93: 319-327.

BLANCO, O., A. CRESPO, and J. A. ELIX. 2005. Two new species of *Xanthoparmelia* (Ascomycota: Parmeliaceae) from Spain. *The Lichenologist* 37: 97-100.

BLANCO, O., A. CRESPO, R. H. REE, and H. T. LUMBSCH. 2006. Major clades of parmelioid lichens (Parmeliaceae, Ascomycota) and the evolution of their morphological and chemical diversity. *Molecular Phylogenetics and Evolution* 39: 52-69.

BLANCO, O., A. CRESPO, J. A. ELIX, D. L. HAWKSWORTH, and H. T. LUMBSCH. 2004a. A Molecular Phylogeny and a New Classification of Parmelioid Lichens Containing *Xanthoparmelia*-Type Lichenan (Ascomycota: Lecanorales). *Taxon* 53: 959-975.

BLANCO, O., A. CRESPO, P. K. DIVAKAR, T. L. ESSLINGER, D. L. HAWKSWORTH, and H. THORSTEN LUMBSCH. 2004b. *Melanelixia* and *Melanohalea*, two new genera segregated from *Melanelia* (Parmeliaceae) based on molecular and morphological data. *Mycological Research* 108: 873-884.

BRODO, I. M. 2001. Lichens of North America. Yale University Press, New Haven, Connetecuit, USA.

BRUNAUER, G., A. HAGER, M. GRUBE, R. TÜRK, and E. STOCKER-WÖRGÖTTER. 2007. Alterations in secondary metabolism of aposymbiotically grown mycobionts of *Xanthoria elegans* and cultured resynthesis stages. *Plant Physiology and Biochemistry* 45: 146-151.

BUSCHBOM, J., and G. M. MUELLER. 2006. Testing "Species Pair" Hypotheses: Evolutionary Processes in the Lichen-Forming Species Complex *Porpidia flavocoerulescens* and *Porpidia melinodes*. *Molecular Biology and Evolution* 23: 574-586.

CARBONE, I., and L. M. KOHN. 1999. A Method for Designing Primer Sets for Speciation Studies in Filamentous Ascomycetes. *Mycologia* 91: 553-556.

CARDOSO, A., and A. P. VOGLER. 2005. DNA taxonomy, phylogeny and Pleistocene diversification of the *Cicindela hybrida* species group (Coleoptera: Cicindelidae). *Molecular Ecology* 14: 3531-3546.

CHUNCO, A. J., J. S. MCKINNON, M. R. SERVEDIO, and T. HANSEN. 2009. Microhabitat variation and sexual selection can maintain male color polymorphisms. *Evolution* 61: 2504-2515.

CLEMENT, M., D. POSADA, and K. A. CRANDALL. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1659.

CRESPO, A., and S. PÉREZ-ORTEGA. 2009. Cryptic species and species pairs in lichens: A discussion on the relationship between molecular phylogenies and morphological characters. *Anales del Jardin Botanico de Madrid* 66: 71-81.

CRESPO, A., H. T. LUMBSCH, J.-E. MATTSSON, O. BLANCO, P. K. DIVAKAR, K. ARTICUS, E. WIKLUND, et al. 2007. Testing morphology-based hypotheses of phylogenetic relationships in Parmeliaceae (Ascomycota) using three ribosomal markers and the nuclear RPB1 gene. *Molecular Phylogenetics and Evolution* 44: 812-824.

DE QUEIROZ, K. 1998. The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations, 57-75. Oxford University Press, Oxford, UK.

_____. 1999. The general lineage concept of species and the defining properties of the species category. *Species, New Interdisciplinary Essays*: 49-89.

_____. 2007. Species Concepts and Species Delimitation. *Systematic Biology* 56: 879-886.

DEL-PRADO, R., P. CUBAS, H. T. LUMBSCH, P. K. DIVAKAR, O. BLANCO, G. A. DE PAZ, M. C. MOLINA, et al. 2010. Genetic distances within and among species in monophyletic lineages of Parmeliaceae (Ascomycota) as a tool for taxon delimitation. *Molecular Phylogenetics and Evolution* 56: 125-133.

DEL PRADO, R., Z. FERENCOVÁ, V. ARMAS-CRESPO, G. AMO DE PAZ, P. CUBAS, and A. CRESPO. 2007. The arachiform vacuolar body: an overlooked shared character in the ascospores of a large monophyletic group within Parmeliaceae (*Xanthoparmelia* clade, Lecanorales). *Mycological Research* 111: 685-692.

DEPRIEST, P. T. 2004. Early Molecular Investigations of Lichen-Forming Symbionts: 1986–2001*. *Annual Review of Microbiology* 58: 273-301.

DETTMAN, J. R., D. J. JACOBSON, E. TURNER, A. PRINGLE, and J. W. TAYLOR. 2003. Reproductive isolation and phylogenetic divergence in *Neurospora*: comparing methods of species recognition in a model eukaryote. *Evolution* 57: 2721.

DIVAKAR, P. K., O. BLANCO, D. L. HAWKSWORTH, and A. CRESPO. 2005. Molecular phylogenetic studies on the *Parmotrema reticulatum* (syn. *Rimelia reticulata*) complex, including the confirmation of *P. pseudoreticulatum* as a distinct species. *The Lichenologist* 37: 55-65.

DRUMMOND, A., O. PYBUS, A. RAMBAUT, R. FORSBERG, and A. RODRIGO. 2003. Measurably evolving populations. *Trends in Ecology & Evolution* 18: 481 - 488.

EDGAR, R. C. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: 1-19.

ELIX, J. A. 1996. Biochemistry and secondary metabolites. *In* T. H. Nash III [ed.], Lichen Biolgy, 154-180. Cambridge University Press, Cambridge, Massachusetts, USA

EVANNO, G., S. REGNAUT, and J. GOUDET. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611-2620.

FALUSH, D., M. STEPHENS, and J. K. PRITCHARD. 2003. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics* 164: 1567-1587.

GANLEY, A. R. D., and T. KOBAYASHI. 2007. Highly efficient concerted evolution in the ribosomal DNA repeats: Total rDNA repeat variation revealed by whole-genome shotgun sequence data. *Genome Research* 17: 184-191.

GARDES, M., and T. D. BRUNS. 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology Notes* 2: 113-118.

GAUSLAA, Y., H. HOLIEN, M. OHLSON, and T. SOLHØY. 2006. Does snail grazing affect growth of the old forest lichen Lobaria pulmonaria? *The Lichenologist* 38: 587-593.

GAYA, E., F. LUTZONI, S. ZOLLER, and P. NAVARRO-ROSINES. 2003. Phylogenetic study of *Fulgensia* and allied *Caloplaca* and *Xanthoria* species (Teloschistaceae, lichen-forming ascomycota). *American Journal of Botany* 90: 1095-1103.

GOFFINET, B., J. MIADLIKOWSKA, and T. GOWARD. 2003. Phylogenetic Inferences Based on nrDNA Sequences Support Five Morphospecies within the *Peltigera didactyla* Complex (Lichenized Ascomycota). *The Bryologist* 106: 349-364.

GROENEVELD, L., D. WEISROCK, R. RASOLOARISON, A. YODER, and P. KAPPELER. 2009. Species delimitation in lemurs: multiple genetic loci reveal low levels of species diversity in the genus Cheirogaleus. *BMC Evolutionary Biology* 9: 30.

GUTIÉRREZ, G., O. BLANCO, P. DIVAKAR, H. LUMBSCH, and A. CRESPO. 2007. Patterns of Group I Intron Presence in Nuclear SSU rDNA of the Lichen Family Parmeliaceae. *Journal of Molecular Evolution* 64: 181-195.

HALE, M. E. 1990. A synopsis of the lichen genus *Xanthoparmelia* (Vainio) Hale (Ascomycotina, Parmeliaceae), vol. Book, Whole. Smithsonian Institution Press, Washington D.C., USA.

HEATH, L., E. VAN DER WALT, A. VARSANI, and D. P. MARTIN. 2006. Recombination Patterns in Aphthoviruses Mirror Those Found in Other Picornaviruses. *Journal of Virology.* 80: 11827-11832.

HODKINSON, B., and J. C. LENDEMER. 2010. Molecular analyses reveal semi-cryptic species in *Xanthoparmelia tasmanica*. *Bibliotheca Lichenologica*: in press.

HUDSON, R. R., and J. A. COYNE. 2002a. Mathematical Consequences of the Genealogical Species Concept. *Evolution* 56: 1557-1565.

_____. 2002b. Mathematical consequences of the genealogical species concept. *Evolution* 56: 1557.

HUELSENBECK, J. P., and F. RONQUIST. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754 - 755.

HUELSENBECK, J. P., and B. RANNALA. 2004. Frequentist Properties of Bayesian Posterior Probabilities of Phylogenetic Trees Under Simple and Complex Substitution Models. *Systematic Biology* 53: 904 - 913.

HUELSENBECK, J. P., F. RONQUIST, R. NIELSEN, and J. P. BOLLBACK. 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* 294: 2310-2314.

HUNECK, S. 1999. The Significance of Lichens and Their Metabolites. *Naturwissenschaften* 86: 559-570.

KLEMENT, O. 1950. Über die Artberechtigung einiger *Parmelien*. *Berichten der Deutschen Botanischen Gesellschaft, Jahrgang* 63: 47-52.

KNOWLES, L. L., and B. C. CARSTENS. 2007. Delimiting Species without Monophyletic Gene Trees. *Systematic Biology* 56: 887-895.

KOLACZKOWSKI, B., and J. W. THORNTON. 2007. Effects of Branch Length Uncertainty on Bayesian Posterior Probabilities for Phylogenetic Hypotheses. *Molecular Biology and Evolution* 24: 2108-2118.

KROKEN, S., and J. W. TAYLOR. 2001. A Gene Genealogical Approach to Recognize Phylogenetic Species Boundaries in the Lichenized Fungus *Letharia*. *Mycologia* 93: 38-53.

LATCH, E., G. DHARMARAJAN, J. GLAUBITZ, and O. RHODES. 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics* 7: 295-302.

LAURENT, E., L. GUILLAUME, and S. STEFAN. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1: 47-50.

LEAVITT, S. D. 2010. Assessing traditional morphology- and chemistry-based species circumspections in lichenized ascomycetes: character evolution and species delimitation in common western North American lichens. Ph.D. dissertation, Brigham Young University, Provo, Utah, USA.

LEAVITT, S. D., and L. L. ST. CLAIR. 2008. Lichens of Boulder Mountain Plateau, Wayne, County, Utah, USA. *Evansia* 26: 85-89.

LIBRADO, P., and J. ROZAS. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451-1452.

LINDBLOM, L., and S. EKMAN. 2006. Genetic variation and population differentiation in the lichen-forming ascomycete *Xanthoria parietina* on the island Storfosna, central Norway. *Molecular ecology* 15: 1545-1559.

LIU, L., L. YU, L. KUBATKO, D. K. PEARL, and S. V. EDWARDS. 2009. Coalescent methods for estimating phylogenetic trees. *Molecular Phylogenetics and Evolution* 53: 320-328.

LUMBSCH, H. T., I. SCHMITT, A. MANGOLD, and M. WEDIN. 2007. Ascus types are phylogenetically misleading in Trapeliaceae and Agyriaceae (Ostropomycetidae, Ascomycota). *Mycological Research* 111: 1133-1141.

LUTZONI, F., M. PAGEL, and V. REEB. 2001. Major fungal lineages are derived from lichen symbiotic ancestors. *Nature* 411: 937-940.

LUTZONI, F., F. KAUFF, C. J. COX, D. MCLAUGHLIN, G. CELIO, B. DENTINGER, M. PADAMSEE, et al. 2004. Assembling the fungal tree of life: progress, classification, and evolution of subcellular traits. *American Journal of Botany* 91: 1446-1480.

MADDISON, W. P., and D. R. MADDISON. 2007. Mesquite: A modular system for evolutionary analysis, version 2.01. Website http://mesquiteproject.org.

MARTIN, D. P., C. WILLIAMSON, and D. POSADA. 2005. RDP2: recombination detection and analysis from sequence alignments. *Bioinformatics* 21: 260-262.

MAYDEN, R. L. 1999. Consilience and a hierarchy of species concepts: Advances towards closure on the species puzzle. *The Journal of Nematology* 31: 95-116.

MERESCHKOWSKY, C. 1918. Note sur une nouvelle forme de *Parmelia* vivant à l'état libre. *Bulletin de la Société Botanique de Genève, series 2* 10.

MOLINA, M. D. C., A. CRESPO, O. BLANCO, H. T. LUMBSCH, and D. L. HAWKSWORTH. 2004. Phylogenetic relationships and species concepts in *Parmelia* s. str. (Parmeliaceae) inferred from nuclear ITS rDNA and β;-tubulin sequences. *The Lichenologist* 36: 37-54.

MYLLYS, L., K. LOHTANDER, and A. TEHLER. 2001. β-Tubulin, ITS and Group I Intron Sequences Challenge the Species Pair Concept in Physcia aipolia and P. caesia. *Mycologia* 93: 335-343.

NASH III, T. H., and M. ZAVADA. 1977. Population Studies Among Sonoran Desert Species of Parmelia subg. *Xanthoparmelia* (Parmeliaceae). *American Journal of Botany* 64: 664-669.

NASH III, T. H., and J. A. ELIX. 2004. *Xanthoparmelia*. *In* T. H. Nash III, B. D. Ryan, P. Diederich, C. Gries, AND F. BUNGARTZ [eds.], Lichen Flora of the Greater Sonoran Desert Region, vol. 2. Lichens Unlilmited, Tempe.

NEI, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York, New York, USA.

NYLANDER, J. A. A., F. RONQUIST, J. P. HUELSENBECK, and J. NIEVES-ALDREY. 2004. Bayesian Phylogenetic Analysis of Combined Data. *Systematic Biology* 53: 47-67.

O'BRIEN, H. E., J. MIADLIKOWSKA, and F. LUTZONI. 2009. Assessing reproductive isolation in highly diverse communities of the lichen-forming funal genus *Peltigera*. *Evolution* 63: 2076-2086.

O'MEARA, B. C. 2010. New Heuristic Methods for Joint Species Delimitation and Species Tree Inference. *Systematic Biology* 59: 59-73.

ORANGE, A., P. W. JAMES, and F. J. WHITE. 2001. Microchemical methods for the identification of lichens, 1-101. British Lichen Society, London.

POSADA, D., and K. A. CRANDALL. 2001a. Selecting the Best-Fit Model of Nucleotide Substitution. *Systematic Biology* 50: 580-601.

_____. 2001b. Evaluation of methods for detecting recombination from DNA sequences: Computer simulations. *Proceedings of the National Academy of Sciences of the United States of America* 98: 13757-13762.

POSADA, D., and T. R. BUCKLEY. 2004. Model Selection and Model Averaging in Phylogenetics: Advantages of Akaike Information Criterion and Bayesian Approaches Over Likelihood Ratio Tests. *Systematic Biology* 53: 793-808.

POSADA, D., K. A. CRANDALL, and E. C. HOLMES. 2002. Recombination in evolutionary genomics. *Annual Review of Genetics* 36: 75-97.

PRINGLE, A., D. M. BAKER, J. L. PLATT, J. P. WARES, J. P. LATGÉ, and J. W. TAYLOR. 2005. Cryptic Speciation in the Cosmopolitan and Clonal Human Pathogenic Fungus *Aspergillus fumigatus*. *Evolution* 59: 1886-1899.

PRINTZEN, C. 2009. Lichen Systematics: The Role of Morphological and Molecular Data to Reconstruct Phylogenetic Relationships, Progress in Botany 71: 233-275. Springer Berlin Heidelberg, Berlin, Germany.

PRITCHARD, J. K., M. STEPHENS, and P. DONNELLY. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

RAMBAULT, A. 1996. Sequence Alignment Editor *Available from:* <*http://tree.bio.ed.ac.uk/software/seal/*>.

ROE, A. D., and F. A. H. SPERLING. 2007. Population structure and species boundary delimitation of cryptic *Dioryctria* moths: an integrative approach. *Molecular Ecology* 16: 3617-3633.

ROKAS, A., and S. B. CARROLL. 2005. More Genes or More Taxa? The Relative Contribution of Gene Number and Taxon Number to Phylogenetic Accuracy. *Molecular Biology and Evolution* 22: 1337-1344.

RONQUIST, F., and A. R. DEANS. 2009. Bayesian Phylogenetics and Its Influence on Insect Systematics. *Annual Review of Entomology* 55: 189-206.

ROSENTRETER, R. 1993. Vagrant Lichens in North America. *The Bryologist* 96: 333-338.

ROWE, G., and T. J. C. BEEBEE. 2007. Defining population boundaries: use of three Bayesian approaches with microsatellite data from British natterjack toads (*Bufo calamita*). *Molecular Ecology* 16: 785-796.

RUIZ-SANCHEZ, E., and V. SOSA. 2010. Delimiting species boundaries within the Neotropical bamboo Otatea (Poaceae: Bambusoideae) using molecular, morphological and ecological data. *Molecular Phylogenetics and Evolution* 54: 344-356.

SAISHO, D., and M. D. PURUGGANAN. 2007. Molecular Phylogeography of Domesticated Barley Traces Expansion of Agriculture in the Old World. *Genetics* 177: 1765-1776.

SANDERS, W. B. 2001. Lichens: The Interface between Mycology and Plant Morphology. *Bioscience* 51: 1025-1035.

SCHMITT, I., A. CRESPO, P. K. DIVAKAR, J. D. FANKHAUSER, E. HERMAN-SACKETT, K. KALB, M. P. NELSEN, et al. 2009. New primers for promising single-copy genes in fungal phylogenies and systematics. *Persoonia* 23: 35-40.

SHIMODAIRA, H., and M. HASEGAWA. 1999. Multiple Comparisons of Log-Likelihoods with Applications to Phylogenetic Inference. *Molecular Biology and Evolution* 16: 1114-1116

SITES, J. W., and J. C. MARSHALL. 2004. Operational criteria for delimiting species. *Annual Review of Ecology, Evolution, and Systematics* 35: 199-227.

SØCHTING, U., and F. LUTZONI. 2003. Molecular phylogenetic study at the generic boundary between the lichen-forming fungi Caloplaca and *Xanthoria* (Ascomycota, Teloschistaceae). *Mycological Research* 107: 1266-1276.

SOLHAUG, K. A., M. LIND, L. NYBAKKEN, and Y. GAUSLAA. 2009. Possible functional roles of cortical depsides and medullary depsidones in the foliose lichen Hypogymnia physodes. *Flora - Morphology, Distribution, Functional Ecology of Plants* 204: 40-48.

ST. CLAIR, L., J. JOHANSEN, S. ST. CLAIR, and K. KNIGHT. 2007. The Influence of Grazing and Other Environmental Factors on Lichen Community Structure along an Alpine Tundra Ridge in the Uinta Mountains, Utah, U.S.A. *Arctic, Antarctic, and Alpine Research* 39: 603-613.

STAMATAKIS, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688-2690.

STAMATAKIS, A., P. HOOVER, and J. ROUGEMONT. 2008. A Rapid Bootstrap Algorithm for the RAxML Web Servers. *Systematic Biology* 57: 758-771.

TEMPLETON, A. R., and C. F. SING. 1993. A Cladistic Analysis of Phenotypic Associations with Haplotypes Inferred from Restriction Endonuclease Mapping. IV. Nested Analyses with Cladogram Uncertainty and Recombination. *Genetics* 134: 659-669.

TEMPLETON, A. R., K. A. CRANDALL, and C. F. SING. 1992. A Cladistic Analysis of Phenotypic Associations With Haplotypes Inferred From Restriction Endonuclease Mapping and DNA Sequence Data. III. Cladogram Estimation. *Genetics* 132: 619-633.

THELL, A. 1999. Group I Intron Versus its Sequences in Phylogeny of Cetrarioid Lichens. *The Lichenologist* 31: 441-449.

THELL, A., J. A. ELIX, and U. SØCHTING. 2009. *Xanthoparmelia lineola* s. l. in Australia and North America. *Bibliothecia Lichenologica* 99: 393-404.

THOMAS, A., and R. ROSENTRETER. 1992. Antelope utilization of lichen in the Birch Creek Valley of Idaho. *In* E. Raper [ed.], Proceedings - Symposium of the 15th Biennial Pronghorn Antelope Workshop, Rocksprings Wyoming, June 9-11, 1992., 6-12. Wyoming Game and Fish Department, Rock Springs, Wyoming, USA.

VIEITES, D. R., K. C. WOLLENBERG, F. ANDREONE, J. KÖHLER, F. GLAW, and M. VENCES. 2009. Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. *Proceedings of the National Academy of Sciences* 106: 8267-8272.

VONDRÁK, J., P. ŘÍHA, U. ARUP, and U. SØCHTING. 2009. The taxonomy of the Caloplaca citrina group (Teloschistaceae) in the Black Sea region; with contributions to the cryptic species concept in lichenology. *The Lichenologist* 41: 571-604.

WATTERSON, G. A. 1975. On the number of segregating sites in genetical models without recombination. *Theoretical Population Biology* 7: 256-276.

WEDIN, M., M. WESTBERG, A. T. CREWE, A. TEHLER, and O. W. PURVIS. 2009. Species delimitation and evolution of metal bioaccumulation in the lichenized *Acarospora smaragdula* (Ascomycota, Fungi) complex. *Cladistics* 25: 161-172.

WEISROCK, D. W., R. M. RASOLOARISON, I. FIORENTINO, J. M. RALISON, S. M. GOODMAN, P. M. KAPPELER, and A. D. YODER. 2010. Delimiting Species without Nuclear Monophyly in Madagascar's Mouse Lemurs. *PLoS ONE* 5: e9883.

WHEELER, D. L., T. BARRETT, D. A. BENSON, S. H. BRYANT, K. CANESE, V. CHETVERNIN, D. M. CHURCH, et al. 2006. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research*: 33: D39-45.

WHITE, T. J., T. D. BRUNS, S. LEE, and J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Academic Press, San Diego, California, USA.

WIENS, J. J. 1998. Combining Data Sets with Different Phylogenetic Histories. *Systematic Biology* 47: 568-581.

WIENS, JOHN J. 2004. What Is Speciation and How Should We Study It? *The American Naturalist* 163: 914-923.

WILL, K. W., B. D. MISHLER, and Q. D. WHEELER. 2005. The Perils of DNA Barcoding and the Need for Integrative Taxonomy. *Systematic Biology* 54: 844-851.

WIRTZ, N., C. PRINTZEN, and H. T. LUMBSCH. 2008. The delimitation of Antarctic and bipolar species of neuropogonoid *Usnea* (Ascomycota, Lecanorales): a cohesion approach of species recognition for the *Usnea perpusilla* complex. *Mycological Research* 112: 472-484.

WOOD, T. E., and T. NAKAZATO. 2009. Investigating species boundaries in the *Giliopsis* group of *Ipomopsis* (Polemoniaceae): Strong discordance among molecular and morphological markers. *Ameriucan Journal of Botany* 96: 853-861.

YANG, Z., and B. RANNALA. 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences* 107: 9264-9269.

Table 3.1. Summary of diagnostic morphological and chemical characteristics for ingroup taxa; "*" indicate that erratic, unattached forms were identified in the present study; "§" indicate specimens identified without sexual reproductive characters were included in nominal taxon.

Species	Form	Diagnostic chemistry	Mode of reproduction	Picnidia	Rhizines	Undersurface color	Degree of adnation
X. californica	saxicolous	norstictic	apothecia§	present	simple	pale brown	adnate
X. chlorochroa	vagrant	salazinic	fragmentation	rare	simple to furcate	pale-dark brown	free growing
X. coloradoësis	saxicolous*	salazinic	apothecia§	present	simple	pale brown	adnate to loosley adnate
X. cumberlandia	saxicolous*	stictic	apothecia§	present	simple	pale brown or brown	adnate
X. lipochlorochroa	vagrant	fatty acids	fragmentation	absent	simple	pale brown	free growing
X. neochlorochroa	vagrant	norstictic	fragmentation	absent	simple to furcate	pale brown	free growing
X. neowyomingica	terricolous	stictic	apothecia§	present	simple to tufted	pale to dark brown	loosely adnate to free growing
X. norchlorochroa	vagrant	salazinic	fragmentation	absent	absent	dark brown to black	free growing
X. vagans	vagrant	stictic	fragmentation	absent	simple	pale brown to dark brown	free growing
X. wyomingica	terricolous	salazinic	apothecia§	present	simple	pale to dark brown	loosely adnate to free growing

Table 3.2. Primers used for PCR amplification and sequencing of the nuclear ribosomal IGS, ITS, and group I intron markers and low-copy protein-coding markers β-tubulin and *MCM7* in sampled *Xanthoparmelia* taxa.

Marker	Primer name	Forward primer sequence	Annealing temperature (°C)	Reference
IGS	IGS12	5'-AGTCTGTGGATTAGTGGCCG-3'	66- 56 (touchdown)	Carbone & Kohn, 1999
	NS1R	5'-GAGACAAGCATATGACTAC-3'		Carbone & Kohn, 1999
	XIGS_R	5'-TAC TGG CAG AAT CAR CCA GG-3'		Leavitt, 2010
ITS/group I intron	ITS1F	5'-CTT GGT CAT TTA GAG GAA GTA A-3'	55-60	(Gardes and Bruns, 1993)
	ITS4	5'- TCC TCC GCT TAT TGA TAT GC-3'		(White et al., 1990)
LSU	LROR	5'-ACC CGC TGA ACT TAA GC-3'	55-60	Vilgalys unpublished
	LR5	5'-ATC CTG AGG GAA ACT TC-3'		Vilgalys unpublished
β-tubulin	Bt3-LM	5'-GAACGTCTACTTCAACGAG-3'	55-60	(Myllys, Lohtander, and Tehler, 2001)
	Bt10-LM	5'-TCGGAAGCAGCCATCATGTTCTT-3'		(Myllys, Lohtander, and Tehler, 2001)
	BT_rhizo_F	5'-GCA ACA AGT ATG TTC CTC GTG C-3'	66- 56 (touchdown)	Leavitt, 2010
	BT_rhizo_R	5'-GTAAGAGGTGCGAAGCCAACC-3'		Leavitt, 2010
MCM7	Mcm7-709for	5'-ACI MGI GTI TCV GAY GTH AARCC-3'	56	Schmitt et al., 2009a
	Mcm7-1348rev	5'-GAY TTD GCI ACI CCI GGR TCW CCC AT-3'		Schmitt et al., 2009a
	X_Mcm7_F	5'- CGT ACA CYT GTG ATC GAT GTG -3'	66- 56 (touchdown)	Leavitt, 2010
	X_Mcm7_R	5'- GTC TCC ACG TAT TCG CAT TCC-3'		Leavitt, 2010

Table 3.3. Genetic variability of sampled loci, including alignment length and parsimony informative (PI) sites for each sampled; numbers in parentheses indicate the number of variable and parsimony-informative sites for the Intermountain *Xanthoparmelia* group only.

Locus	**N**	**Aligned bp**	**# of variable sites**	**# PI sites**	**Model selected**
ITS	158 (145)	543 (535)	108 (68)	67 (41)	SYM+I+G
LSU	155 (142)	843 (843)	57 (25)	20 (13)	GTR+I
IGS	144 (131)	380 (380)	80 (46)	39 (22)	GTR+I+G
group I intron	135 (125)	387 (385)	64 (51)	35 (29)	SYM+G
β-tubulin	147 (135)	809 (809)	74 (42)	27 (17)	GTR+I
MCM7	146 (136)	541 (541)	89 (63)	48 (36)	GTR+I+G
Total	158 (145)	3503 (3493)	462 (295)	236 (158)	na

Table 3.4. Polymorphism statistic for *Xanthoparmelia* species examined. Species sampled; *N* total, number of individuals sampled; and loci sampled. Within each locus *N*, number of individuals sampled for that loci/ N_{poly}, number of polymorphic sites/ *H*, number of unique haplotypes; π, estimate of $4N\mu$ per base pair using average pairwise differences / θ, estimates of haplotype diversity using the number of pairwise differences.

	N total	ITS *N*/ N_{poly} /*H*	π /θ	LSU *N*/ N_{poly} /*H*	π/ θ	IGS *N*/ N_{poly} /*H*	π /θ	Intron *N*/ N_{poly} /*H*	π /θ	β-tubulin *N*/ N_{poly} /*H*	π /θ	*MCM7* *N*/ N_{poly} /*H*	π/ θ
Species													
X. californica	2	2/4/2	0.00800/ 0.00800	2/2/2	0.00238/ 0.00238	1/0/1	na/na	2/5/2	0.01348/ 0.01348	2/7/2	0.00950/ 0.00950	2/1/2	0.02033/ 0.02033
X. chlorochroa	51	51/15/15	0.00736/ 0.00929	50/10/8	0.00117/ 0.00266	51/16/14	0.00603/ 0.00959	48/23/17	0.01257/ 0.01417	51/5/5	0.00116/ 0.00285	50/28/18	0.01161/ 0.01202
X. coloradoënsis	29	29/25/17	0.00840/ 0.01335	29/8/7	0.00116/ 0.00273	27/14/13	0.00709/ 0.00987	28/18/11	0.01283/ 0.01450	28/9/7	0.00316/ 0.00348	28/30/20	0.01258/ 0.01487
X. cumberlandia	36	36/36/20	0.00816/ 0.01743	37/10/12	0.00188/ 0.00292	25/20/15	0.00943/ 0.01424	26/21/16	0.00744/ 0.01491	27/9/	0.00569/ 0.00569	32/34/22	0.01319/ 0.01698
X. lipochlorochroa	3	3/0/1	0.0000/ 0.0000	3/0/1	0.00000/ 0.00000	1/0/1	0/0	1/0/1	0.00000/ 0.00000	3/0/1	0.00000/ 0.00000	3/5/2	0.00616/ 0.00616
X. neochlorochroa	4	4/7/2	0.00889/ 0.00727	4/2/3	0.00139/ 0.00139	4/4/2	0.00705/ 0.00705	4/11/3	0.01932/ 0.01617	4/0/1	0.00000/ 0.00000	4/11/3	0.01109/ 0.01109
X. neowyomingica	7	7/3/3	0.0021/ 0.00245	7/5/4	0.00193/ 0.00243	7/4/3	0.00307/ 0.00307	6/2/3	0.00234/ 0.00236	7/7/2	0.00452/ 0.00388	7/15/3	0.01074/ 0.01132
X. norchlorochroa	3	3/7/2	0.00933/ 0.00933	2/0/1	0.00000/ 0.00000	3/1/2	0.00179/ 0.00179	3/5/1	0.00898/ 0.00898	3/0/1	0.00000/ 0.00000	0	0.00616/ 0.00616
X. vagans	4	4/11/3	0.01133/ 0.01200	4/0/1	0.00000/ 0.00000	4/5/3	0.00672/ 0.00733	0	na/na	4/1/2	0.00090/ 0.00074	4/8/2	0.00739/ 0.00807
X. wyomingica	6	6/11/4	0.00906/ 0.00977	6/5/4	0.00261/ 0.00302	5/8/5	0.00968/ 0.01032	5/7/3	0.00916/ 0.00906	6/6/4	0.00301/ 0.00396	4/14/4	0.01571/ 0.01412
Chemotype													
Norstictic	6	6/8/4	0.00853/ 0.00863	6/4/4	0.00183/ 0.00183	5/5/3	0.00753/ 0.00760	4/11/3	0.01932/ 0.01983	6/8/3	0.00389/ 0.00391	6/18/5	0.01368/ 0.01393
Salazinic	86	86/26/20	0.00657/ 0.00663	85/20/15	0.00137/ 0.00137	84/24/23	0.00670/ 0.00676	81/27/19	0.01352/ 0.01377	85/10/10	0.00196/ 0.00197	81/41/35	0.01225/ 0.01245
Stictic	50	50/42/26	0.00883/ 0.00893	48/12/13	0.00197/ 0.00197	39/27/20	0.00824/ 0.00833	35/24/20	0.00783/ 0.00792	41/12/10	0.00578/ 0.00582	46/45/28	0.01493/ 0.01523
Population cluster													
1	47	47/42/24	0.00754/ 0.00762	45/12/12	0.00166/ 0.00166	37/21/19	0.00840/ 0.00849	35/20/19	0.00587/ 0.00591	36/18/10	0.00514/ 0.00518	42/44/27	0.01489/ 0.01519
2	48	47/16/12	0.00356/ 0.00358	48/10/9	0.00126/ 0.00127	46/15/12	0.00392/ 0.00394	47/14/12	0.00366/ 0.00368	47/4/5	0.00101/ 0.00101	47/30/22	0.01230/ 0.01250
3	44	45/8/7	0.00428/ 0.00431	43/5/5	0.00038/ 0.00038	43/16/12	0.00737/ 0.00744	37/13/9	0.00727/ 0.00734	44/6/6	0.00153/ 0.00153	41/22/12	0.01047/ 0.01062

Table 3.5. Estimates of pairwise F_{ST} among putative *Xanthoparmelia* species (below diagonal) and the significance level (above diagonal); ns, not significant (two nonsignificant P-values are show). Numbers on top row correspond to numbered taxa in the first column.

	1	**2**	**3**	**4**	**5**	**6**	**7**
1 ***X. chlorochroa***	-	ns	0.0000	ns	0.0811 n.s.	ns	ns
2. ***X. coloradoënsis***	0.00125	-	0.0721 n.s	ns	ns	ns	ns
3 ***X. cumberlandia***	0.11097	0.03794	-	ns	ns	ns	ns
4 ***X. neochlorochroa***	-0.10319	-0.09908	-0.03473	-	0.02703	ns	ns
5 ***X. neowyomingica***	0.05264	0.01516	0.01692	0.50591	-	0.01802.	0.0000
6 ***X. vagans***	-0.11701	-0.12695	-0.07955	0.17329	0.52033	-	ns
7 ***X. wyomingica***	0.12696	0.06507	0.00664	-0.03751	0.08599	-0.10867	-

Table 3.6. Estimates of pairwise F_{ST} between population clusters inferred in STRUCTURE analyses and major chemotypes

	Comparison	F_{ST}	**Significance**
Structure	K1-K2	0.42285	0.0000
Structure	K1-K3	0.35209	0.0000
Structure	K2-K3	0.43664	0.0000
Chemotypes	Stictic - Salzinic	0.14303	0.0000

Table 3.7. Results of the paired Shimodaira-Hasegawa topological constraint tests of our best ML topology compared to three alternative hypotheses of relationships in the Intermountain *Xanthoparmelia* group proposed in this study.

Tree	ln L	Difference ln L	Significantly Worse	Topology compared
this article (Fig. 2)	-11165.19	(best)	-	This article
	-12048.58	883.39	yes	Species
	-11645.43	480.24	yes	Chemotypes
	-11175.35	10.16	no	STRUCTURE

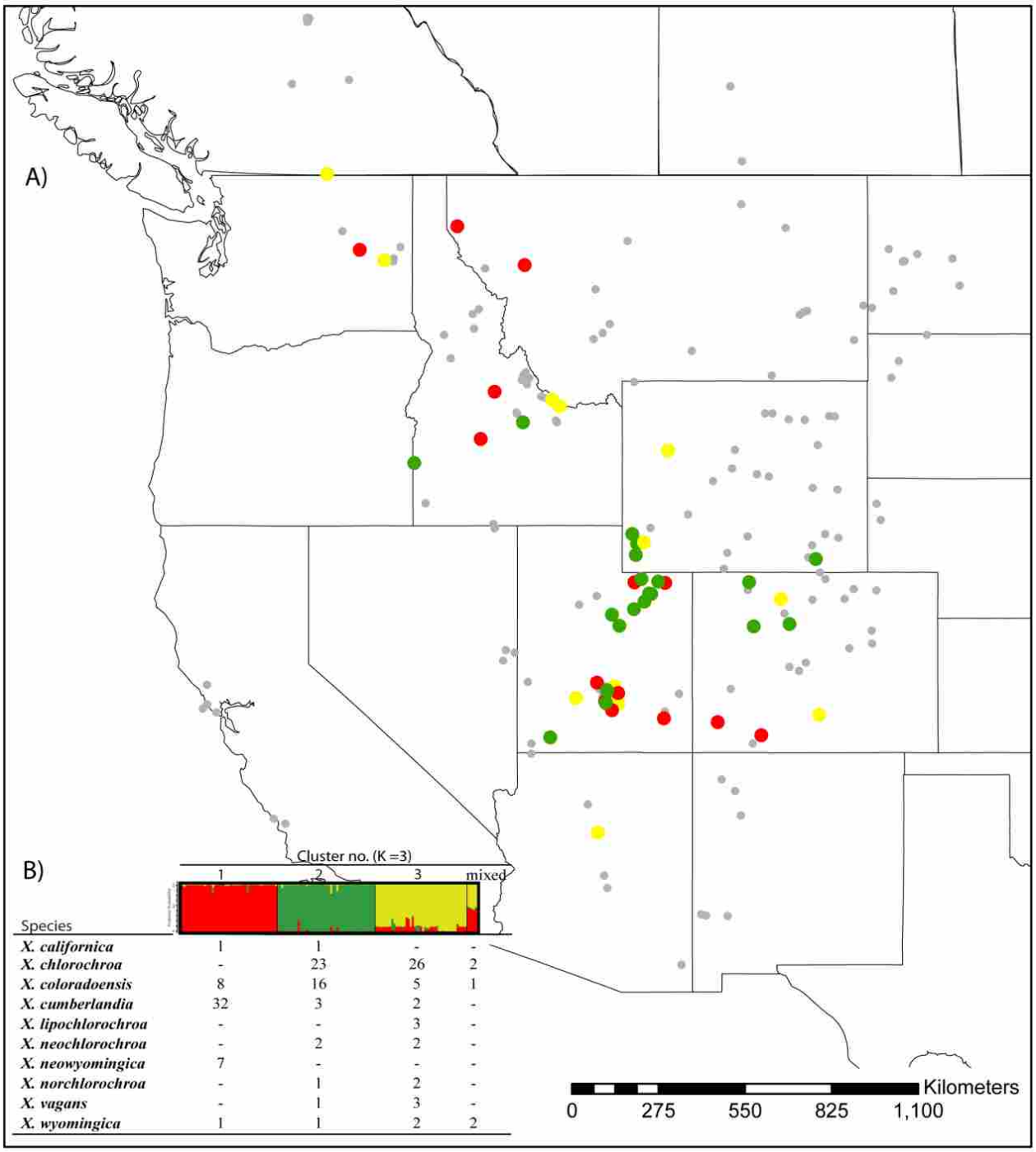

Figure 3.1. (A) Geographic distributions of sampled *Xanthoparmelia* specimens and inferred population clusters in the western United States. (B) Population subdivision and the occurrence of putative lineages in each inferred population cluster inferred from the STRUCTURE analysis; each accession is shown by a thin vertical line that is partitioned into three colored segments. The accessions in which members' probability is < 70 % are classified into a mixed category.

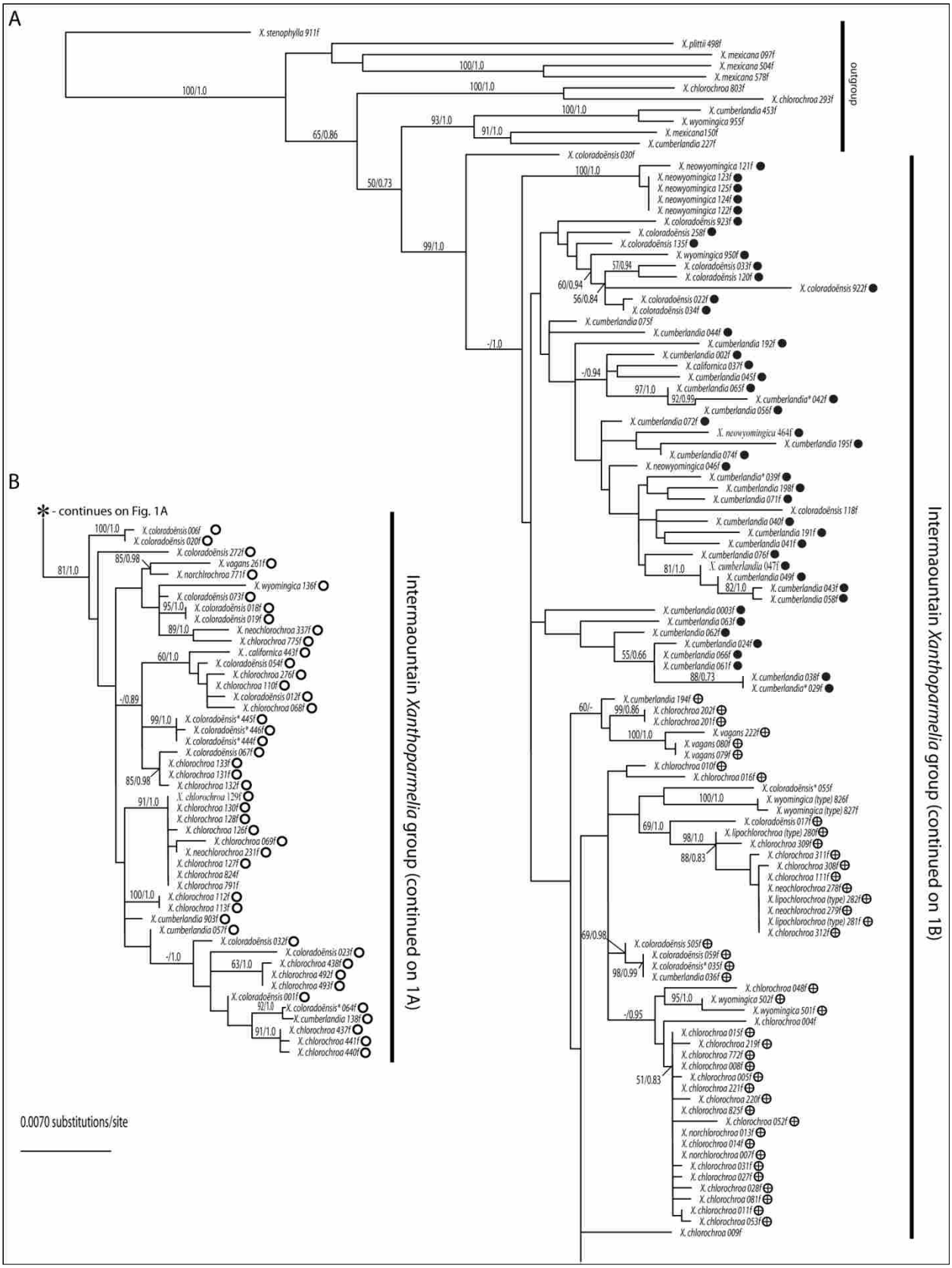
A
X. stenophylla 911f
X. plittii 498f
X. mexicana 097f
X. mexicana 504f
X. mexicana 578f
X. chlorochroa 803f
X. chlorochroa 293f
X. cumberlandia 453f
X. wyomingica 955f
X. mexicana150f
X. cumberlandia 227f
outgroup
100/1.0
100/1.0
100/1.0
93/1.0
91/1.0
65/0.86
50/0.73
99/1.0
-/1.0
X. coloradoënsis 030f
X. neowyomingica 121f
X. neowyomingica 123f
X. neowyomingica 125f
X. neowyomingica 124f
X. neowyomingica 122f
X. coloradoënsis 923f
X. coloradoënsis 258f
X. coloradoënsis 135f
X. wyomingica 950f
X. coloradoënsis 033f
X. coloradoënsis 120f
X. coloradoënsis 922f
X. coloradoënsis 022f
X. coloradoënsis 034f
X. cumberlandia 075f
X. cumberlandia 044f
X. cumberlandia 192f
X. cumberlandia 002f
X. californica 037f
X. cumberlandia 045f
X. cumberlandia 065f
X. cumberlandia* 042f
X. cumberlandia 056f
X. cumberlandia 072f
X. neowyomingica 464f
X. cumberlandia 195f
X. cumberlandia 074f
X. neowyomingica 046f
X. cumberlandia* 039f
X. cumberlandia 198f
X. cumberlandia 071f
X. coloradoënsis 118f
X. cumberlandia 040f
X. cumberlandia 191f
X. cumberlandia 041f
X. cumberlandia 076f
X. cumberlandia 047f
X. cumberlandia 049f
X. cumberlandia 043f
X. cumberlandia 058f
X. cumberlandia 0003f
X. cumberlandia 063f
X. cumberlandia 062f
X. cumberlandia 024f
X. cumberlandia 066f
X. cumberlandia 061f
X. cumberlandia 038f
X. cumberlandia* 029f
X. cumberlandia 194f
X. chlorochroa 202f
X. chlorochroa 201f
X. vagans 222f
X. vagans 080f
X. vagans 079f
X. chlorochroa 010f
X. chlorochroa 016f
X. coloradoënsis* 055f
X. wyomingica (type) 826f
X. wyomingica (type) 827f
X. coloradoënsis 017f
X. lipochlorochroa (type) 280f
X. chlorochroa 309f
X. chlorochroa 311f
X. chlorochroa 308f
X. chlorochroa 111f
X. neochlorochroa 278f
X. lipochlorochroa (type) 282f
X. neochlorochroa 279f
X. lipochlorochroa (type) 281f
X. chlorochroa 312f
X. coloradoënsis 505f
X. coloradoënsis 059f
X. coloradoënsis* 035f
X. cumberlandia 036f
X. chlorochroa 048f
X. wyomingica 502f
X. wyomingica 501f
X. chlorochroa 004f
X. chlorochroa 015f
X. chlorochroa 219f
X. chlorochroa 772f
X. chlorochroa 008f
X. chlorochroa 005f
X. chlorochroa 221f
X. chlorochroa 220f
X. chlorochroa 825f
X. chlorochroa 052f
X. norchlorochroa 013f
X. chlorochroa 014f
X. norchlorochroa 007f
X. chlorochroa 031f
X. chlorochroa 027f
X. chlorochroa 028f
X. chlorochroa 081f
X. chlorochroa 011f
X. chlorochroa 053f
X. chlorochroa 009f
Intermaountain Xanthoparmelia group (continued on 1B)
B
* - continues on Fig. 1A
X. coloradoënsis 006f
X. coloradoënsis 020f
X. coloradoënsis 272f
X. vagans 261f
X. norchlrochroa 771f
X. wyomingica 136f
X. coloradoënsis 073f
X. coloradoënsis 018f
X. coloradoënsis 019f
X. neochlorochroa 337f
X. chlorochroa 775f
X. . californica 443f
X. coloradoënsis 054f
X. chlorochroa 276f
X. chlorochroa 110f
X. coloradoënsis 012f
X. chlorochroa 068f
X. coloradoënsis* 445f
X. coloradoënsis* 446f
X. coloradoënsis* 444f
X. coloradoënsis 067f
X. chlorochroa 133f
X. chlorochroa 131f
X. chlorochroa 132f
X. chlorochroa 129f
X. chlorochroa 130f
X. chlorochroa 128f
X. chlorochroa 126f
X. chlorochroa 069f
X. neochlorochroa 231f
X. chlorochroa 127f
X. chlorochroa 824f
X. chlorochroa 791f
X. chlorochroa 112f
X. chlorochroa 113f
X. cumberlandia 903f
X. cumberlandia 057f
X. coloradoënsis 032f
X. coloradoënsis 023f
X. chlorochroa 438f
X. chlorochroa 492f
X. chlorochroa 493f
X. coloradoënsis 001f
X. coloradoënsis* 064f
X. cumberlandia 138f
X. chlorochroa 437f
X. chlorochroa 441f
X. chlorochroa 440f
100/1.0
85/0.98
81/1.0
95/1.0
89/1.0
60/1.0
-/0.89
99/1.0
85/0.98
91/1.0
100/1.0
-/1.0
63/1.0
92/1.0
91/1.0
Intermaountain Xanthoparmelia group (continued on 1A)
0.0070 substitutions/site

Figure 3.2 (on previous page). ML phylogenetic relationships of *Xanthoparmelia* taxa inferred from a combined analysis of nuclear ribosomal markers ITS, IGS, LSU, and intron and protein-coding fragments from β-tubulin and *MCM7* genes. Values at each node indicate non-parametric bootstrap support (BS)/ posterior probability (PP), only values ≥ BS 50/PP 0.5 are listed. The focal group "Intermountain *Xanthoparmelia* group is indicated in Fig. 1A and Fig 1B. Filled circles at the end of taxon labels indicate individuals assigned membership in population cluster one inferred from the STRUCTURE analysis, open circles indicate population cluster two, and circles with cross indicate population cluster three.

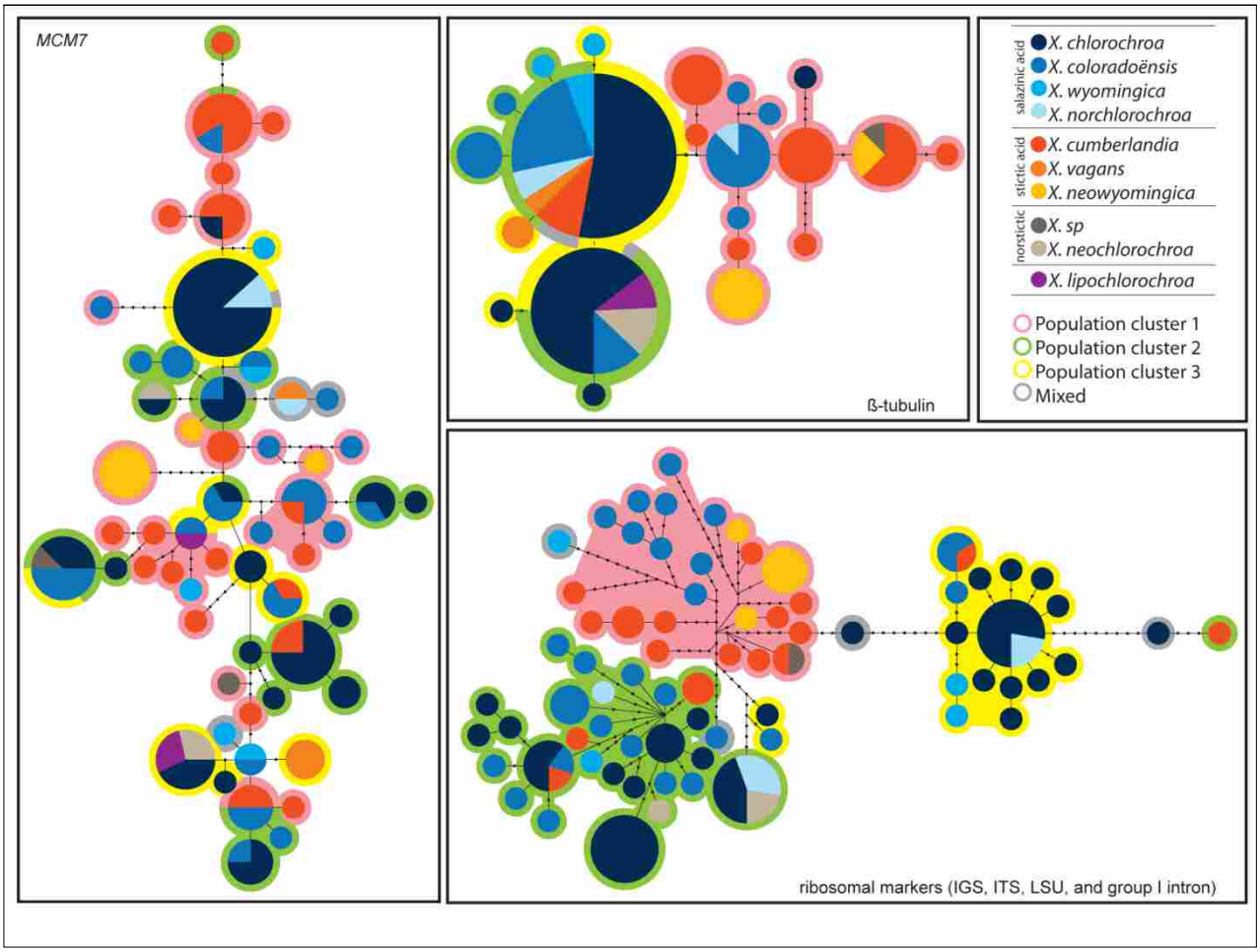

Figure 3.3. Unrooted statistical parsimony haplotype networks at 95% probability for (A) ribosomal (ITS, IGS, LSU, and intron), (B) β-tubulin, and (C) *MCM7* loci within the Intermountain *Xanthoparmelia* group. The sizes of the circles in each haplotype networks are proportional to the number of individuals in each given haplotype, and small circles are inferred from haplotypes not sampled. Putative species are color coded in all networks; and outline color signifies membership in population clusters inferred from the STRUCTURE analysis.

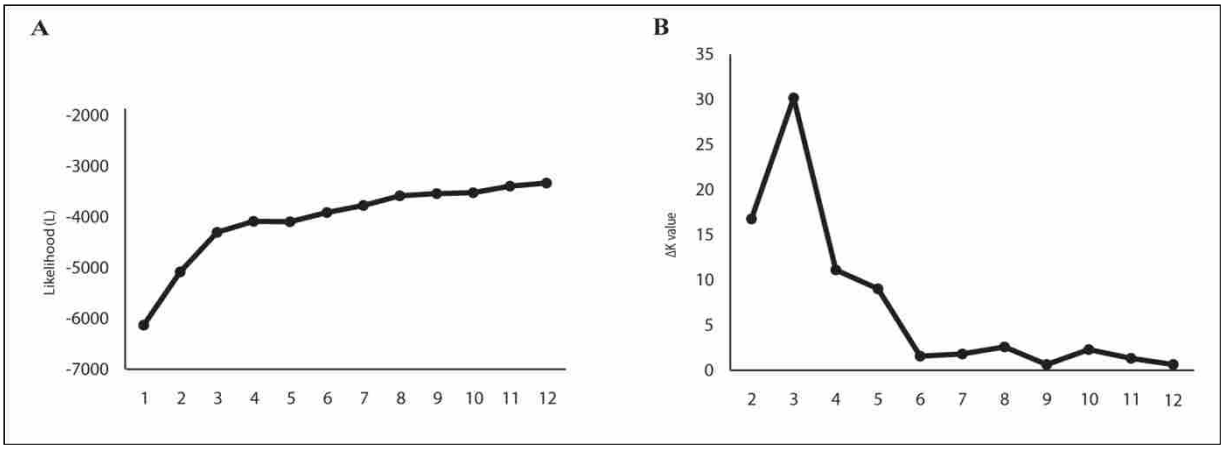

Figure 3.4. A) The median likelihoods for 12 runs for each *K* estimate are shown on the likelihood plot for STRUCTURE analysis of sampled *Xanthoparmelia* species. B) Δ*K* calculated as $\Delta K = m|L''(K)|/s[L(K)]$. The modal value of this distribution is the uppermost level of structure (*K*).

Supplementary data 3.1. Collection information for specimens included in the present study.

ID	Species	Herbarium Acc. No.	Major Acid	Form	Reproductive mode	Structure	Location	Lat.	Lon.	Ele.	Collector (s)
037f	*X. californica*	BRY-55185	norstictic	erratic	not observed	1	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
443f	*X. californica*	BRY-55387	norstictic	saxicolous	not observed	2	USA, UT, Duchesne Co.	40.526	-110.3529	2088 m	Leavitt et al.
004f	*X. chlorochroa*	BRY-55154	salazinic	vagrant	fragmentation	mixed	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
005f	*X. chlorochroa*	BRY-55155	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
008f	*X. chlorochroa*	BRY-55158	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
009f	*X. chlorochroa*	BRY-55159	salazinic	vagrant	fragmentation	mixed	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
010f	*X. chlorochroa*	BRY-55160	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
011f	*X. chlorochroa*	BRY-55161	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
014f	*X. chlorochroa*	BRY-55164	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
015f	*X. chlorochroa*	BRY-55165	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
016f	*X. chlorochroa*	BRY-55166	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
027f	*X. chlorochroa*	BRY-55175	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
028f	*X. chlorochroa*	BRY-55176	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
031f	*X. chlorochroa*	BRY-55179	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
048f	*X. chlorochroa*	BRY-55196	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
052f	*X. chlorochroa*	BRY-55198	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
053f	*X. chlorochroa*	BRY-55199	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
068f	*X. chlorochroa*	BRY-55213	salazinic	vagrant	fragmentation	2	USA, WY, Uinta Co.	41.3769	-110.6621	2057 m	Leavitt et al.
069f	*X. chlorochroa*	BRY-55214	salazinic	vagrant	fragmentation	2	USA, UT, Duchesne Co.	40.3697	-110.4128	2005 m	Leavitt et al.
081f	*X. chlorochroa*	BRY-55224	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
110f	*X. chlorochroa*	BRY-55236	salazinic	vagrant	fragmentation	2	USA, WY, Uinta Co.	41.3769	-110.6621	2057 m	Leavitt et al.
111f	*X. chlorochroa*	BRY-55237	salazinic	vagrant	fragmentation	3	USA, WY, Uinta Co.	41.3769	-110.6621	2057 m	Leavitt et al.

112f	*X. chlorochroa*	BRY-55238	salazinic	vagrant	fragmentation	2	USA, ID, Owyhee Co.	43.3202	-116.9795	1271 m	Leavitt et al.
113f	*X. chlorochroa*	BRY-55239	salazinic	vagrant	fragmentation	2	USA, ID, Owyhee Co.	43.3202	-116.9795	1271 m	Leavitt et al.
126f	*X. chlorochroa*	BRY-55247	salazinic	vagrant	fragmentation	2	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
127f	*X. chlorochroa*	BRY-55248	salazinic	vagrant	fragmentation	2	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
128f	*X. chlorochroa*	BRY-55249	salazinic	vagrant	fragmentation	2	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
129f	*X. chlorochroa*	BRY-55250	salazinic	vagrant	fragmentation	2	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
130f	*X. chlorochroa*	BRY-55251	salazinic	vagrant	fragmentation	2	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
131f	*X. chlorochroa*	BRY-55252	salazinic	vagrant	fragmentation	2	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
132f	*X. chlorochroa*	BRY-55253	salazinic	vagrant	fragmentation	2	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
133f	*X. chlorochroa*	BRY-55254	salazinic	vagrant	fragmentation	2	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
201f	*X. chlorochroa*	BRY-55287	salazinic	vagrant	fragmentation	3	USA, MT, Beaverhead Co.	44.6225	-113.0520	2715 m	St. Clair et al.
202f	*X. chlorochroa*	BRY-55288	salazinic	vagrant	fragmentation	3	USA, MT, Beaverhead Co.	44.6225	-113.0520	2715 m	St. Clair et al.
219f	*X. chlorochroa*	BRY-55295	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
220f	*X. chlorochroa*	BRY-55296	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
221f	*X. chlorochroa*	BRY-55297	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
276f	*X. chlorochroa*	BRY-55315	salazinic	vagrant	fragmentation	2	USA, WY, Lincoln Co.	41.6254	-110.6270	2050 m	Leavitt et al.
308f	*X. chlorochroa*	BRY-55341	salazinic	vagrant	fragmentation	3	MT, Beaverhead Co.	44.4876	-112.8269	2120 m	B. McCune 21280
309f	*X. chlorochroa*	BRY-55342	salazinic	vagrant	fragmentation	3	MT, Beaverhead Co.	44.4876	-112.8269	2120 m	B. McCune 21280
311f	*X. chlorochroa*	BRY-55344	salazinic	vagrant	fragmentation	3	USA, WY, Fremont Co.	43.5774	-109.7370	2469 m	Rosentreter 15445
312f	*X. chlorochroa*	BRY-55345	salazinic	vagrant	fragmentation	3	USA, WY, Fremont Co..	43.5774	-109.7370	2469 m	Rosentreter 15445
437f	*X. chlorochroa*	BRY-55381	salazinic	vagrant	fragmentation	2	USA, UT, Duchesne Co.	40.2039	-110.7130	2088 m	Leavitt et al.
438f	*X. chlorochroa*	BRY-55382	salazinic	vagrant	fragmentation	2	USA, UT, Duchesne Co.	40.2039	-110.7130	2088 m	Leavitt et al.
440f	*X. chlorochroa*	BRY-55384	salazinic	vagrant	fragmentation	2	USA, UT, Duchesne Co..	40.5444	-110.2852	2517 m	Leavitt et al.
441f	*X. chlorochroa*	BRY-55685	salazinic	vagrant	fragmentation	2	USA, UT, Duchesne Co..	40.5444	-110.2852	2517 m	Leavitt et al.

492f	*X. chlorochroa*	BRY-55416	salazinic	vagrant	fragmentation	2	USA, UT, Utah Co.	39.8426	-111.1298	2393 m	Leavitt et al.
493f	*X. chlorochroa*	BRY-55417	salazinic	vagrant	fragmentation	2	USA, UT, Utah Co.	39.8426	-111.1298	2393 m	Leavitt et al.
772f	*X. chlorochroa*	BRY-55448	salazinic	vagrant	fragmentation	3	USA, UT, Beaver/Piute Co.	38.2328	-112.3652	3035 m	Greenwood
775f	*X. chlorochroa*	BRY-55451	salazinic	vagrant	fragmentation	2	USA, CO, Summit Co.	39.8790	-106.2782	2447 m	Leavitt
791f	*X. chlorochroa*	BRY-55467	salazinic	vagrant	fragmentation	2	USA, WY, Lincoln Co.	41.8246	-110.7632	2019 m	Leavitt
824f	*X. chlorochroa*	BRY-55499	salazinic	vagrant	fragmentation	2	USA, CO, Moffat Co.	40.6206	-107.4658	1942 m	Leavitt
825f	*X. chlorochroa*	BRY-55500	salazinic	vagrant	fragmentation	3	USA, CO, Jackson Co.	40.4252	-106.5233	2553 m	Leavitt
001f	*X. coloradoënsis*	BRY-55151	salazinic	saxicolous	not observed	2	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
006f	*X. coloradoënsis*	BRY-55156	salazinic	saxicolous	not observed	2	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
012f	*X. coloradoënsis*	BRY-55162	salazinic	saxicolous	fragmentation	2	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
017f	*X. coloradoënsis*	BRY-55167	salazinic	saxicolous	not observed	3	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
018f	*X. coloradoënsis*	BRY-55168	salazinic	saxicolous	not observed	2	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
019f	*X. coloradoënsis*	BRY-55169	salazinic	saxicolous	not observed	2	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
020f	*X. coloradoënsis*	BRY-55170	salazinic	saxicolous	not observed	2	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
022f	*X. coloradoënsis*	BRY-55171	salazinic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
023f	*X. coloradoënsis*	BRY-55172	salazinic	saxicolous	not observed	2	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
030f	*X. coloradoënsis*	BRY-55178	salazinic	saxicolous	not observed	-	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
032f	*X. coloradoënsis*	BRY-55180	salazinic	saxicolous	not observed	2	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
033f	*X. coloradoënsis*	BRY-55181	Salazinic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
034f	*X. coloradoënsis*	BRY-55182	salazinic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
035f	*X. coloradoënsis**	BRY-55183	Salazinic	erratic	not observed	3	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
054f	*X. coloradoënsis*	BRY-55200	Salazinic	saxicolous	apothecia	2	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
055f	*X. coloradoënsis**	BRY-55201	Salazinic	saxicolous	not observed	mixed	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
059f	*X. coloradoënsis*	BRY-55205	salazinic	saxicolous	apothecia	3	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
064f	*X. coloradoënsis*	BRY-55209	salazinic	erratic	not observed	2	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.

067f	*X. coloradoënsis*	BRY-55212	salazinic	saxicolous	not observed	2	USA, UT, Summit Co.	40.8047	-110.0213	3360 m	EA 80-1108
073f	*X. coloradoënsis*	BRY-55218	salazinic	saxicolous	not observed	2	USA, UT, Wayne Co.	38.4097	-111.4757	3360 m	Leavitt et al.
118f	*X. coloradoënsis*	BRY-55240	salazinic	saxicolous	not observed	mixed	USA, ID, Lemhi Co.	44.6812	-113.3623	1820 m	Leavitt et al.
120f	*X. coloradoënsis*	BRY-55241	salazinic	saxicolous	not observed	1	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
135f	*X. coloradoënsis*	BRY-55255	salazinic	saxicolous	not observed	1	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
258f	*X. coloradoënsis*	BRY-55308	salazinic	saxicolous	not observed	1	USA, ID, Custer Co.	44.7833	-114.6875	2479 m	St. Clair et al.
272f	*X. coloradoënsis*	BRY-55312	salazinic	saxicolous	not observed	2	USA, UT, Washington Co.	37.3474	-113.1010	2110 m	Leavitt et al.
444f	*X. coloradoënsis**	BRY-55388	salazinic	erratic	not observed	2	USA, UT, Duchesne Co.	40.5351	-110.2233	2413 m	Leavitt et al.
445f	*X. coloradoënsis*	BRY-55389	salazinic	erratic	not observed	2	USA, UT, Duchesne Co.	40.5351	-110.2233	2413 m	Leavitt et al.
446f	*X. coloradoënsis*	BRY-55390	salazinic	saxicolous	not observed	2	USA, UT, Duchesne Co.	40.5351	-110.2233	2413 m	Leavitt et al.
505f	*X. coloradoënsis*	BRY-55427	salazinic	saxicolous	not observed	3	USA, AZ, Coconino Co.	35.1534	-111.7409	2220 m	J. Hollinger 20080624.27
922f	*X. coloradoënsis*	BRY-55524	salazinic	saxicolous	not observed	1	USA, MT, Carter Co.	48.0413	-115.7517	1630 m	T. Wheeler 1371
923f	*X. coloradoënsis*	BRY-55525	salazinic	saxicolous	not observed	1	USA, MT, Lake Co.	47.2952	-113.8312	2370 m	T. Wheeler 1409
002f	*X. cumberlandia*	BRY-55152	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
003f	*X. cumberlandia*	BRY-55153	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
024f	*X. cumberlandia*	BRY-55173	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
029f	*X. cumberlandia**	BRY-55177	stictic	erratic	not observed	1	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
036f	*X. cumberlandia*	BRY-55184	stictic	saxicolous	not observed	3	USA, UT, Wayne Co.	38.1202	111.5071	3300 m	Leavitt et al.
038f	*X. cumberlandia*	BRY-55186	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1230	111.5086	3300 m	Leavitt et al.
039f	*X. cumberlandia**	BRY-55187	stictic	erratic	not observed	1	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
040f	*X. cumberlandia*	BRY-55188	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
041f	*X. cumberlandia*	BRY-55189	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1325	-111.4710	3300 m	Leavitt et al.
042f	*X. cumberlandia**	BRY-55190	stictic	erratic	not observed	1	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
043f	*X. cumberlandia*	BRY-55191	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
044f	*X. cumberlandia*	BRY-55192	stictic	saxicolous	apothecia	1	USA, UT, Wayne	38.1230	-111.5086	3300 m	Leavitt et al.

							Co.				
045f	*X. cumberlandia*	BRY-55193	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1625	-111.5358	3300 m	Leavitt et al.
047f	*X. cumberlandia*	BRY-55195	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
049f	*X. cumberlandia*	BRY-55197	stictic	saxicolous	apothecia	1	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
056f	*X. cumberlandia*	BRY-55202	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
057f	*X. cumberlandia*	BRY-55203	stictic	saxicolous	not observed	2	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
058f	*X. cumberlandia*	BRY-55204	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1202	-111.5071	3300 m	Leavitt et al.
061f	*X. cumberlandia*	BRY-55206	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
062f	*X. cumberlandia*	BRY-55207	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
063f	*X. cumberlandia*	BRY-55208	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
065f	*X. cumberlandia*	BRY-55210	stictic	saxicolous	not observed	1	USA, UT, Summit Co.	40.7743	-109.8244	3410 m	Leavitt et al.
066f	*X. cumberlandia*	BRY-55211	stictic	saxicolous	not observed	1	USA, UT, Summit Co.	40.7743	-109.8244	3410 m	Leavitt et al.
071f	*X. cumberlandia*	BRY-55216	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.5812	-111.7700	3040 m	Leavitt et al.
072f	*X. cumberlandia*	BRY-55217	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.5812	-111.7700	3040 m	Leavitt et al.
074f	*X. cumberlandia*	BRY-55219	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
075f	*X. cumberlandia*	BRY-55220	stictic	saxicolous	not observed	1	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
076f	*X. cumberlandia*	BRY-55221	stictic	saxicolous	apothecia	1	USA, UT, Wayne Co.	38.4097	-111.4757	3300 m	Leavitt et al.
138f	*X. cumberlandia*	BRY-55257	stictic	saxicolous	not observed	2	USA, UT, Utah Co.	40.0847	-111.3401	1750 m	Leavitt et al.
175f	*X. cumberlandia*	BRY-55275	stictic	saxicolous	not observed	na	USA, ID, Elmore Co.	43.8167	-115.0861	1682 m	Leavitt et al.
179f	*X. cumberlandia*	BRY-55276	stictic	saxicolous	not observed	na	USA, UT, Summit Co.	40.7882	-110.6981	3060 m	St. Clair et al.
191f	*X. cumberlandia*	BRY-55281	stictic	saxicolous	not observed	1	USA, CO, Dolores Co.	37.6939	-108.3234	2622 m	Leavitt et al.
192f	*X. cumberlandia*	BRY-55282	stictic	saxicolous	not observed	1	USA, CO, Dolores Co.	37.6939	-108.3234	2622 m	St. Clair et al.
194f	*X. cumberlandia*	BRY-55283	stictic	saxicolous	apothecia	3	USA, CO, Saguache Co.	37.8564	-105.4317	3030 m	St. Clair et al.
195f	*X. cumberlandia*	BRY-55284	stictic	saxicolous	not observed	1	USA, CO, Mineral Co.	37.3884	-107.0918	2657 m	St. Clair et al.
198f	*X. cumberlandia*	BRY-55286	stictic	saxicolous	not observed	1	USA, CO, San Juan Co.	37.7807	-109.8587	2133 m	St. Clair et al.

903f	*X. cumberlandia*	BRY-55508	stictic	saxicolous	apothecia	2	CAN, British Columbia.	49.032	-119.466	396 m	Bjork 15213
280ff	*X. lipochlorochroa* (type locality)	BRY-55318	fatty acid	vagrant	fragmentation	3	USA, WY, Lincoln Co.	41.6388	-110.5699	2018 m	Leavitt et al.
281f	*X. lipochlorochroa* (type locality)	BRY-55319	fatty acid	vagrant	fragmentation	3	USA, WY, Lincoln Co.	41.6388	-110.5699	2018 m	Leavitt et al.
282f	*X. lipochlorochroa* (type locality)	BRY-55320	fatty acid	vagrant	fragmentation	3	USA, WY, Lincoln Co.	41.6254	-110.6270	2050 m	Leavitt et al.
231f	*X. neochlorochroa*	BRY-55303	norstictic	vagrant	fragmentation	2	USA, UT, Wayne Co.	38.4941	-111.5357	2471 m	Leavitt et al.
278f	*X. neochlorochroa*	BRY-55316	norstictic	vagrant	fragmentation	3	USA, WY, Lincoln Co.	41.6388	-110.5699	2018 m	Leavitt et al.
279f	*X. neochlorochroa*	BRY-55317	norstictic	vagrant	fragmentation	3	USA, WY, Lincoln Co.	41.6254	-110.6270	2050 m	Leavitt et al.
337f	*X. neochlorochroa*	BRY-55366	norstictic	vagrant	fragmentation	2	USA, WY, Laramie Co.	41.2916	-105.5247	2137 m	Rosentreter s.n.
046f	*X. neowyomingica*	BRY-55194	stictic	erratic	not observed	1	USA, UT, Wayne Co.	38.1230	-111.5086	3300 m	Leavitt et al.
121f	*X. neowyomingica*	BRY-55242	stictic	vagrant	not observed	1	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
122f	*X. neowyomingica*	BRY-55243	stictic	vagrant	not observed	1	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
123f	*X. neowyomingica*	BRY-55244	stictic	vagrant	not observed	1	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
124f	*X. neowyomingica*	BRY-55245	stictic	vagrant	not observed	1	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
125f	*X. neowyomingica*	BRY-55246	stictic	vagrant	not observed	1	USA, UT, Summit Co.	40.8581	-110.5012	3600 m	Leavitt et al.
464f	*X. neowyomingica*	BRY-55407	stictic	erratic	not observed	1	USA, UT, Summit Co.	40.8581	-110.5012	3645 m	Leavitt et al.
007f	*X. norchlorochroa*	BRY-55157	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1626	-111.5352	3300 m	Leavitt et al.
013f	*X. norchlorochroa*	BRY-55163	salazinic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.1309	-111.4695	3300 m	Leavitt et al.
771f	*X. norchlorochroa*	BRY-55447	norstictic	vagrant	fragmentation	2	USA, CO, Indian Camp Pass	39.8278	-107.2985	3020 m	Leavitt et al.
079f	*X. vagans*	BRY-55222	stictic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.4097	-111.4757	3300m	Leavitt et al.
080f	*X. vagans*	BRY-55223	stictic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.4097	-111.4757	3300m	Leavitt et al.
222f	*X. vagans*	BRY-55298	stictic	vagrant	fragmentation	3	USA, UT, Wayne Co.	38.4097	-111.4757	3300m	Leavitt et al.
261f	*X. vagans*	BRY-55309	stictic	vagrant	fragmentation	2	USA, ID, Lemhi Co.	44.1578	-113.8794	2069 m	St. Clair et al.
136f	*X. wyomingica*	BRY-55256	salazinic	terricolous	not observed	2	USA, UT, Summit Co.	40.8581	-110.5012	3600m	Leavitt et al.
501f	*X. wyomingica*	BRY-55424	salazinic	terricolous	not observed	3	USA, WA, Lincoln Co.	47.3894	-117.8357	689m	Leavitt et al.

502f	*X. wyomingica*	BRY-55425	salazinic	terricolous	not observed	3	USA, WA, Lincoln Co.	47.3894	-117.8357	689m	Leavitt et al.
826f	*X. wyomingica (type)*	BRY-55501	salazinic	semi-attached	not observed	Mixed	USA, WY, Johnson Co.	44.3394	-106.9768	2462m	Leavitt
827f	*X. wyomingica (type)*	BRY-55502	salazinic	semi-attached	not observed	Mixed	USA, WY, Johnson Co.	44.3394	-106.9768	2462m	Leavitt
950f	*X. wyomingica*	BRY-55552	salazinic	semi-attached	not observed	1	USA, WA, Lincoln Co.	47.5902	-118.5359	670 m	Leavitt et al.

Supplementary data 3.2. Species, taxon and study identification number; Herbarium Acc. No., voucher specimen in the Herbarium of Non-vascular cryptogams (BRY); and GenBank accession numbers for all sequences included in the present study (LSU, ITS, IGS, group I intron, *MCM7*, and β-tubulin).

Species	**Herbarium Acc. No.**	**LSU**	**ITS**	**IGS**	**Intron**	***MCM7***	**β-tubulin**
***X. californica* 037f**	BRY-55185	HM579053	HM578641	HM57738	HM578326	HM579460	HM577550.
***X. californica* 443f**	BRY-55387	HM579294	HM578837	-	HM578482	HM579647	HM577735.
***X. chlorochroa* 004f**	BRY-55154	HM579022	HM578610	HM577908	HM578299	HM579492	HM577519.
***X. chlorochroa* 005f**	BRY-55155	HM579023	HM578611	HM577909	HM578300	HM579430	HM577520.
***X. chlorochroa* 008f**	BRY-55158	HM579026	HM5786164	HM577912	HM578303	HM579433	HM577523.
***X. chlorochroa* 009f**	BRY-55159	HM579027	HM578615	HM577913	HM578304	HM579443	HM577524.
***X. chlorochroa* 010f**	BRY-55160	HM579028	HM578616	HM577914	HM578305	HM579465	HM577525.
***X. chlorochroa* 011f**	BRY-55161	HM579029	HM578617	HM577915	HM578306	HM579436	HM577526.
***X. chlorochroa* 014f**	BRY-55164	HM579032	HM578620	HM577918	HM578309	HM579469	HM577529.
***X. chlorochroa* 015f**	BRY-55165	HM579033	HM578621	HM577919	-	HM579440	HM577530.
***X. chlorochroa* 016f**	BRY-55166	HM579034	HM578622	HM577920	HM578310	HM579441	HM577531.
***X. chlorochroa* 027f**	BRY-55175	HM579043	HM578631	HM577928	HM578317	HM579027	HM577540.
***X. chlorochroa* 028f**	BRY-55176	HM579044	HM578632	HM577929	HM578318	HM579451	HM577541.
***X. chlorochroa* 031f**	BRY-55179	HM579047	HM578635	HM577932	HM578320	HM579454	HM577544.
***X. chlorochroa* 048f**	BRY-55196	HM579064	HM578652	HM577947	HM578333	HM579470	HM577545.
***X. chlorochroa* 052f**	BRY-55198	HM579066	HM578654	HM577949	HM578335	HM579472	HM577562.
***X. chlorochroa* 053f**	BRY-55199	HM579067	HM578655	HM577950	HM578336	HM579473	HM577563.
***X. chlorochroa* 068f**	BRY-55213	HM579078	HM578668	HM577960	HM578347	HM579483	HM577573.
***X. chlorochroa* 069f**	BRY-55214	HM579079	HM578669	HM577961	HM578348	HM579484	HM577574.
***X. chlorochroa* 081f**	BRY-55224	HM579089	HM578679	HM577969	HM578355	HM579494	HM577581.
***X. chlorochroa* 110f**	BRY-55236	HM579101	HM578691	HM577981	HM578365	HM579505	HM577593.
***X. chlorochroa* 111f**	BRY-55237	HM579102	HM578692	HM577982	HM578366	HM579506	HM577594.
***X. chlorochroa* 112f**	BRY-55238	HM579103	HM578693	HM577983	HM578367	HM579107	HM577595.
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***X. chlorochroa* 126f**	BRY-55247	HM579123	HM578702	HM577992	HM578374	HM579516	HM577604.
***X. chlorochroa* 127f**	BRY-55248	HM579113	HM578703	HM577993	HM578375	HM579517	HM577605.
***X. chlorochroa* 128f**	BRY-55249	HM579114	HM578704	HM577994	HM578376	HM579518	HM577606.
***X. chlorochroa* 129f**	BRY-55250	HM579115	HM578705	HM577995	HM578377	HM579519	HM577607.
***X. chlorochroa* 130f**	BRY-55251	HM579996	HM578706	HM577996	HM578378	HM579520	HM577608.
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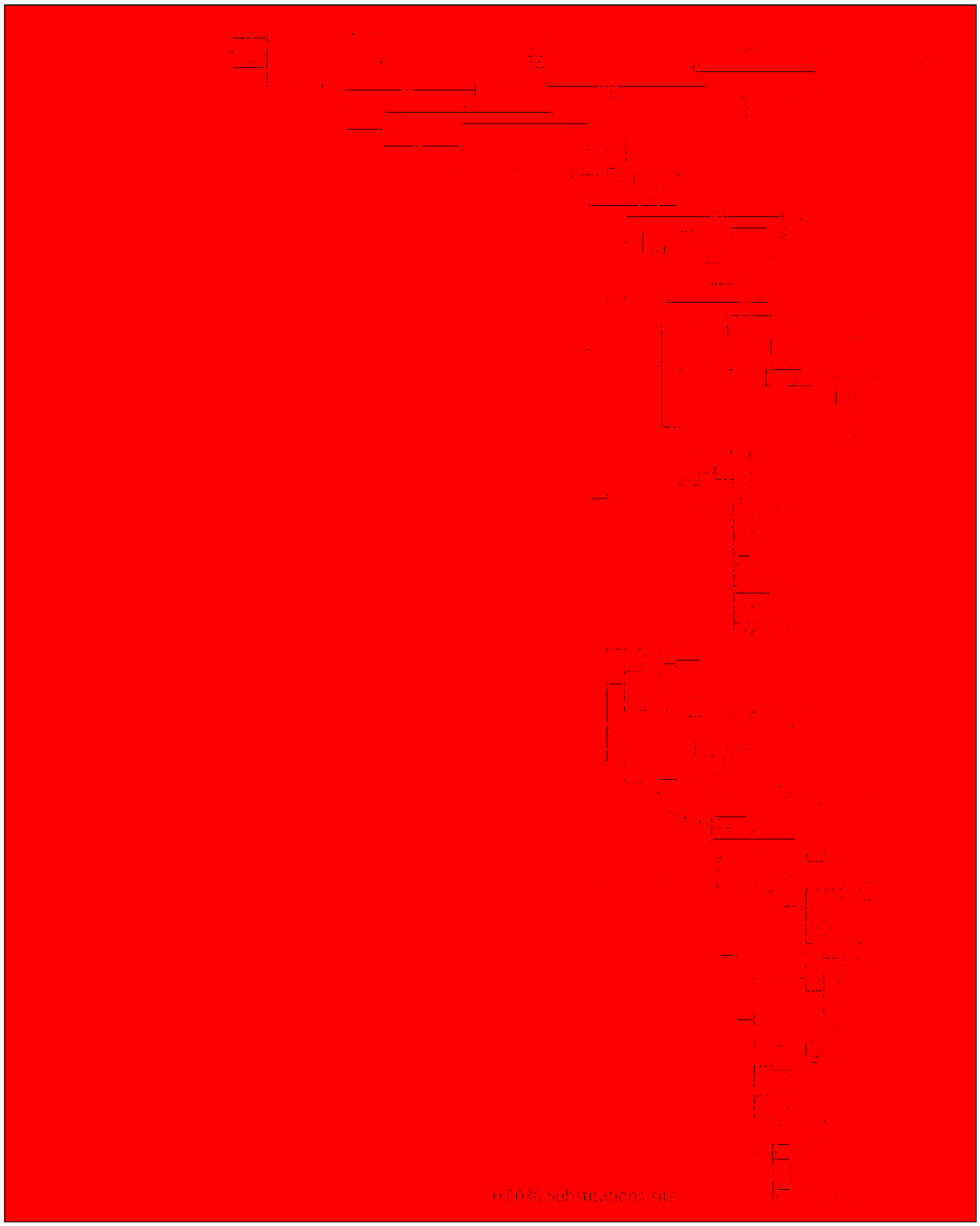
***X. chlorochroa* 132f**	BRY-55253	HM579118	HM578708	HM577998	HM578380	HM579622	HM577610.
***X. chlorochroa* 133f**	BRY-55254	HM579119	HM578709	HM577999	HM578381	HM579523	HM577611.
***X. chlorochroa* 201f**	BRY-55287	HM579152	HM578740	HM578026	-	HM579556	HM577639.
***X. chlorochroa* 202f**	BRY-55288	HM579153	HM578741	HM578027	-	HM579557	HM577640.
***X. chlorochroa* 219f**	BRY-55295	HM579160	HM578748	HM578034	HM578415	HM579564	HM577647.
***X. chlorochroa* 220f**	BRY-55296	HM579161	HM578749	HM578035	HM578416	HM579565	HM577648.
***X. chlorochroa* 221f**	BRY-55297	HM579162	HM578750	HM578036	HM578417	HM579566	HM577649.
***X. chlorochroa* 276f**	BRY-55315	HM579179	HM578767	HM578053	HM578430	HM579583	HM577665.
***X. chlorochroa* 308f**	BRY-55341	-	HM578792	HM578077	HM578454	HM579608	HM577689.
***X. chlorochroa* 309f**	BRY-55342	HM579204	HM578793	HM578078	HM578455	-	HM577690.
***X. chlorochroa* 311f**	BRY-55344	HM579206	HM578795	HM578080	HM578457	HM579610	HM577692.
***X. chlorochroa* 312f**	BRY-55345	HM579207	HM578796	HM578081	HM578458	HM579611	HM577693.
***X. chlorochroa* 437f**	BRY-55381	HM579243	HM578831	HM578115	HM578476	-	HM577729.
***X. chlorochroa* 438f**	BRY-55382	HM579244	HM578832	HM578116	HM578477	HM579644	HM577730.
***X. chlorochroa* 440f**	BRY-55384	HM579246	HM578834	HM578118	HM578479	HM579465	HM577732.
***X. chlorochroa* 441f**	BRY-55685	HM579247	HM578835	HM578119	HM578480	HM579646	HM577733.
***X. chlorochroa* 492f**	BRY-55416	HM579277	HM578866	HM578148	HM578508	HM579661	HM577709.
***X. chlorochroa* 493f**	BRY-55417	HM579278	HM578867	HM578149	HM578509	HM579676	HM577710.
***X. chlorochroa* 772f**	BRY-55448	HM579308	HM578900	HM578179	HM578533	HM579694	HM577789.
***X. chlorochroa* 775f**	BRY-55451	HM579311	HM578903	HM578182	HM578535	HM579697	HM577792.
***X. chlorochroa* 791f**	BRY-55467	HM579327	HM578919	HM578198	HM578548	HM579712	HM577708.
***X. chlorochroa* 824f**	BRY-55499	HM579358	HM578951	HM578230	HM578569	HM579744	HM577839.
***X. chlorochroa* 825f**	BRY-55500	HM579359	HM578952	HM578231	HM578570	HM579745	HM577840.
***X. coloradoënsis* 001**	BRY-55151	HM579019	HM578607	HM577905	HM578296	HM579426	HM577516.
***X. coloradoënsis* 006f**	BRY-55156	HM579024	HM578612	HM577910	HM578301	HM579431	HM577521.
***X. coloradoënsis* 012f**	BRY-55162	HM579030	HM578618	HM577916	HM578307	HM579437	HM577527.
***X. coloradoënsis* 017f**	BRY-55167	HM579035	HM578623	HM577921	HM578311	HM579442	HM577532.
***X. coloradoënsis* 018f**	BRY-55168	HM579036	HM578624	HM577922	HM578312	HM579443	HM577533.
***X. coloradoënsis* 019f**	BRY-55169	HM579037	HM5786265	-	HM578313	HM579444	HM577534.
***X. coloradoënsis* 020f**	BRY-55170	HM579038	HM578626	HM577923	HM578314	HM579445	HM577535.

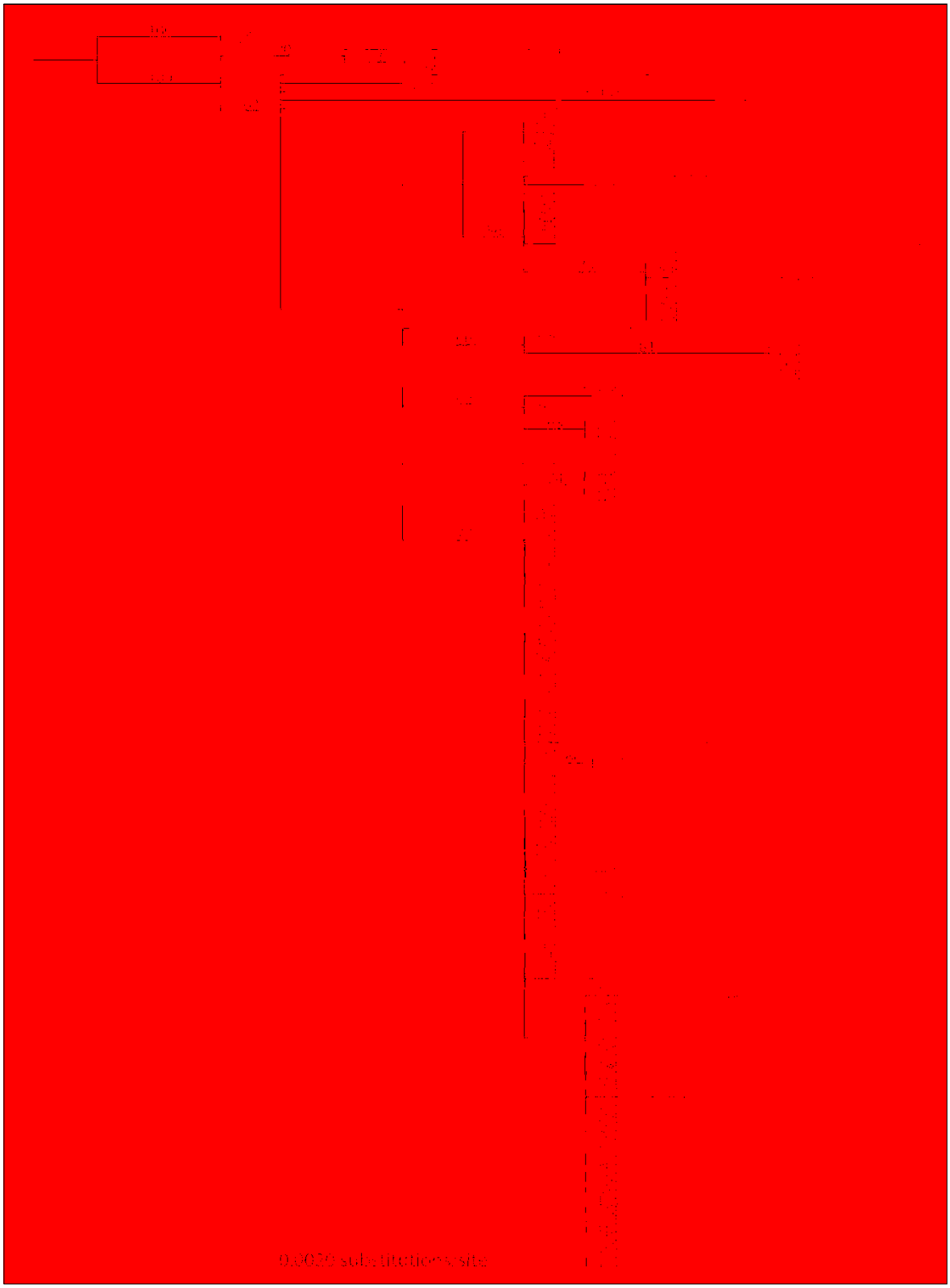
***X. coloradoënsis* 022f**	BRY-55171	HM579039	HM578627	HM577924	HM578315	HM579446	HM577536.
***X. coloradoënsis* 023f**	BRY-55172	HM579040	HM578628	HM577925	HM578316	HM579447	HM577537.
***X. coloradoënsis* 030f**	BRY-55178	HM579046	HM578634	HM577931	HM578319	HM579453	HM577543.
***X. coloradoënsis* 032f**	BRY-55180	HM579048	HM578636	HM577933	HM578321	HM579455	HM577545.
***X. coloradoënsis* 033f**	BRY-55181	HM579049	HM578637	HM577934	HM578322	HM579456	HM577546.
***X. coloradoënsis* 034f**	BRY-55182	HM579050	HM578638	HM577935	HM578323	HM579457	HM577547.
***X. coloradoënsis* 035f**	BRY-55183	HM579051	HM578639	HM577936	HM578324	HM579458	HM577548.
***X. coloradoënsis* 054f**	BRY-55200	HM579068	HM578656	HM577951	HM578337	HM579474	HM577564.
***X. coloradoënsis* 055f**	BRY-55201	HM579069	HM578657	HM577952	HM578338	HM579475	HM577565.
***X. coloradoënsis* 059f**	BRY-55205	HM579073	HM578661	HM577956	HM578342	HM579479	HM577568.
***X. coloradoënsis* 064f**	BRY-55209	HM579075	HM578664	HM577958	HM578344	HM579481	-.
***X. coloradoënsis* 067f**	BRY-55212	HM579077	HM578667	HM577959	HM578346	HM579482	HM577572.
***X. coloradoënsis* 073f**	BRY-55218	HM579083	HM578673	HM577964	HM578351	HM579488	HM577576.
***X. coloradoënsis* 118f**	BRY-55240	HM579105	HM578695	HM577985	-	HM579509	HM577597.
***X. coloradoënsis* 120f**	BRY-55241	HM579106	HM578696	HM577986	HM578369	HM579510	HM577598.
***X. coloradoënsis* 135f**	BRY-55255	HM579120	HM578710	HM578000	HM578382	HM579524	HM577612.
***X. coloradoënsis* 258f**	BRY-55308	HM579172	HM578760	HM578046	HM578426	HM579576	HM577658.
***X. coloradoënsis* 272f**	BRY-55312	HM579176	HM578764	HM578050	HM578428	HM579580	HM577662.
***X. coloradoënsis* 444f**	BRY-55388	HM579250	HM578838	HM578121	HM578483	HM579648	HM577736.
***X. coloradoënsis* 445f**	BRY-55389	HM579251	HM578839	HM578122	HM578484	HM579649	HM577737.
***X. coloradoënsis* 446f**	BRY-55390	HM579252	HM578840	HM578123	HM578485	HM579650	HM577738.
***X. coloradoënsis* 505f**	BRY-55427	HM579288	HM578877	HM578159	HM578515	-	HM577773.
***X. coloradoënsis* 922f**	BRY-55524	HM579383	HM578977	HM578253	HM578585	HM579761	HM577862.
***X. coloradoënsis* 923f**	BRY-55525	HM579384	HM578978	HM578254	HM578586	HM579762	HM577863.
***X. cumberlandia* 002f**	BRY-55152	HM579020	HM578608	HM577906	HM578297	HM579427	HM577517.
***X. cumberlandia* 003f**	BRY-55153	HM579021	HM578609	HM577907	HM578298	HM579428	HM577518.
***X. cumberlandia* 024f**	BRY-55173	HM579041	HM578629	HM577926	-	HM579448	HM577538.
***X. cumberlandia* 029f**	BRY-55177	HM579045	HM578633	HM577930	-	HM579452	HM577542.
***X. cumberlandia* 036f**	BRY-55184	HM579052	HM578640	HM577937	HM578325	HM579459	HM577549.
***X. cumberlandia* 038f**	BRY-55186	HM579054	HM578642	HM577939	-	HM579461	HM577551.
***X. cumberlandia* 039f**	BRY-55187	HM579055	HM578643	HM577940	HM578327	HM579462	HM577552.
***X. cumberlandia* 040f**	BRY-55188	HM579056	HM578644	HM577941	-	HM579463	HM577553.
***X. cumberlandia* 041f**	BRY-55189	HM579057	HM578645	HM577942	HM578328	HM579464	HM577554.
***X. cumberlandia* 042f**	BRY-55190	HM579058	HM578646	HM577943	-	-	HM577555.
***X. cumberlandia* 044f**	BRY-55192	HM579060	HM578648	-	HM578330	HM579466	HM577557.
***X. cumberlandia* 045f**	BRY-55193	HM579061	HM578649	-	-	HM579467	-.
***X. cumberlandia* 047f**	BRY-55195	HM579063	HM578651	HM577946	HM578332	HM579469	HM577559.
***X. cumberlandia* 049f**	BRY-55197	HM579065	HM578653	HM577948	HM578334	HM579471	HM577561.
***X. cumberlandia* 056f**	BRY-55202	HM579070	HM578658	HM577953	HM578339	HM579476	-.
***X. cumberlandia* 057f**	BRY-55203	HM579071	HM578659	HM577954	HM578340	HM579477	HM577566.
***X. cumberlandia* 058f**	BRY-55204	HM579072	HM578660	HM577955	HM578341	HM579478	HM577567.
***X. cumberlandia* 059f**	BRY-55205	HM579073	HM578661	HM577956	HM578342	HM579479	HM577568.
***X. cumberlandia* 061f**	BRY-55206	-	HM578662	-	-	-	-.
***X. cumberlandia* 062f**	BRY-55207	-	-	-	-	-	-.
***X. cumberlandia* 063f**	BRY-55208	HM579074	HM578663	HM577957	HM578343	HM579480	HM577569.
***X. cumberlandia* 064f**	BRY-55209	HM579075	HM578664	HM577958	HM578344	HM579481	-.
***X. cumberlandia* 065f**	BRY-55210	HM579076	HM578665	-	HM578345	-	HM577570.
***X. cumberlandia* 066f**	BRY-55211	-	HM578666	-	-	-	HM577571.
***X. cumberlandia* 071f**	BRY-55216	HM579081	HM578671	-	HM578349	HM579486	-.
***X. cumberlandia* 072f**	BRY-55217	HM579082	HM578672	HM577963	HM578350	HM579487	-.
***X. cumberlandia* 074f**	BRY-55219	HM579084	HM578674	-	HM578352	HM579489	-.
***X. cumberlandia* 075f**	BRY-55220	HM579085	HM578675	HM577965	HM578353	HM579490	HM577577
***X. cumberlandia* 076f**	BRY-55221	HM579086	HM578676	HM577966	HM578354	HM579491	HM577578
***X. cumberlandia* 138f**	BRY-55257	HM579122	HM578712	HM578002	HM578384	HM579526	HM577614
***X. cumberlandia* 175f**	BRY-55275	HM579140	HM578728	HM578020	HM578400	HM579544	HM577631
***X. cumberlandia* 179f**	BRY-55276	HM579141	HM578729	HM578021	HM578401	HM579545	HM577632
***X. cumberlandia* 191f**	BRY-55281	HM579146	HM578734	-	HM578406	HM579550	-
***X. cumberlandia* 192f**	BRY-55282	HM579147	HM578735	-	HM578407	HM579551	-
***X. cumberlandia* 194f**	BRY-55283	HM579148	HM578736	-	-	HM579552	HM577635
***X. cumberlandia* 195f**	BRY-55284	HM579149	HM578737	-	HM578408	HM579553	HM577636
***X. cumberlandia* 198f**	BRY-55286	HM579151	HM578739	HM578025	HM578410	HM579555	HM577638
***X. cumberlandia* 903f**	BRY-55508	HM579367	HM578960	HM578237	HM578575	HM579753	HM577848
***X. lipochlorochroa* 280f**	BRY-55318	HM579182	HM578770	HM578056	HM578433	HM579586	HM577668
***X. lipochlorochroa* 281f**	BRY-55319	HM579183	HM578771	HM578057	HM578434	HM579587	HM577669
***X. lipochlorochroa* 282f**	BRY-55320	HM579184	HM578772	HM578058	HM578435	HM579588	HM577670
***X. neochlorochroa* 231f**	BRY-55303	HM579168	HM578756	HM578042	HM578422	HM579572	HM577655
***X. neochlorochroa* 278f**	BRY-55316	HM579180	HM578768	HM578054	HM578431	HM579584	HM577666
***X. neochlorochroa* 279f**	BRY-55317	HM579181	HM578769	HM578055	HM578432	HM579585	HM577667

***X. neochlorochroa* 337f**	BRY-55366	HM579228	HM578816	HM578102	HM578463	HM579630	HM577714
***X. neowyomingica* 046f**	BRY-55194	HM579062	HM578650	HM577945	HM578331	HM579468	HM577558
***X. neowyomingica* 121f**	BRY-55242	HM579107	HM578697	HM577987	-	HM579511	HM577599
***X. neowyomingica* 122f**	BRY-55243	HM579108	HM578698	HM577988	HM578370	HM579512	HM577600
***X. neowyomingica* 123f**	BRY-55244	HM579109	HM578699	HM577989	HM578371	HM579513	HM577601
***X. neowyomingica* 124f**	BRY-55245	HM579110	HM578700	HM577990	HM578372	HM579514	HM577602
***X. neowyomingica* 125f**	BRY-55246	HM579111	HM578701	HM577991	HM578373	HM579515	HM577603
***X. neowyomingica* 464f**	BRY-55407	HM579269	HM578857	HM578139	HM578502	HM579666	HM577755
***X. norchlorochroa* 007f**	BRY-55157	HM579025	HM578613	HM577911	HM578302	HM579432	HM577522
***X. norchlorochroa* 013f**	BRY-55163	HM579031	HM578619	HM577917	HM578308	HM579438	HM577528
***X. norchlorochroa* 771f**	BRY-55447	HM579307	HM578899	HM578178	HM578532	HM579693	HM577788
***X. vagans* 079f**	BRY-55222	HM579087	HM578677	HM577967	-	HM579492	HM577579
***X. vagans* 080f**	BRY-55223	HM579088	HM578678	HM577968	-	HM579493	HM577580
***X. vagans* 222f**	BRY-55298	HM579163	HM578751	HM578037	-	HM579567	HM577650
***X. vagans* 261f**	BRY-55309	HM579173	HM578761	HM578047	-	HM579577	HM577659
***X. wyomingica* 136f**	BRY-55256	HM579121	HM578711	HM578001	HM578383	HM579525	HM577613
***X. wyomingica* 501f**	BRY-55424	HM579285	HM578874	HM578156	HM578512	HM579681	HM577770
***X. wyomingica* 502f**	BRY-55425	HM579286	HM578875	HM578157	-	-	HM577771
***X. wyomingica* 826f**	BRY-55501	HM579360	HM578953	HM578232	HM578571	HM579746	HM577841
***X. wyomingica* 827f**	BRY-55502	HM579316	HM578964	-	HM578572	HM579747	HM577842
***X. wyomingica* 950f**	BRY-55552	HM579411	HM579005	HM578281	-	-	HM577890

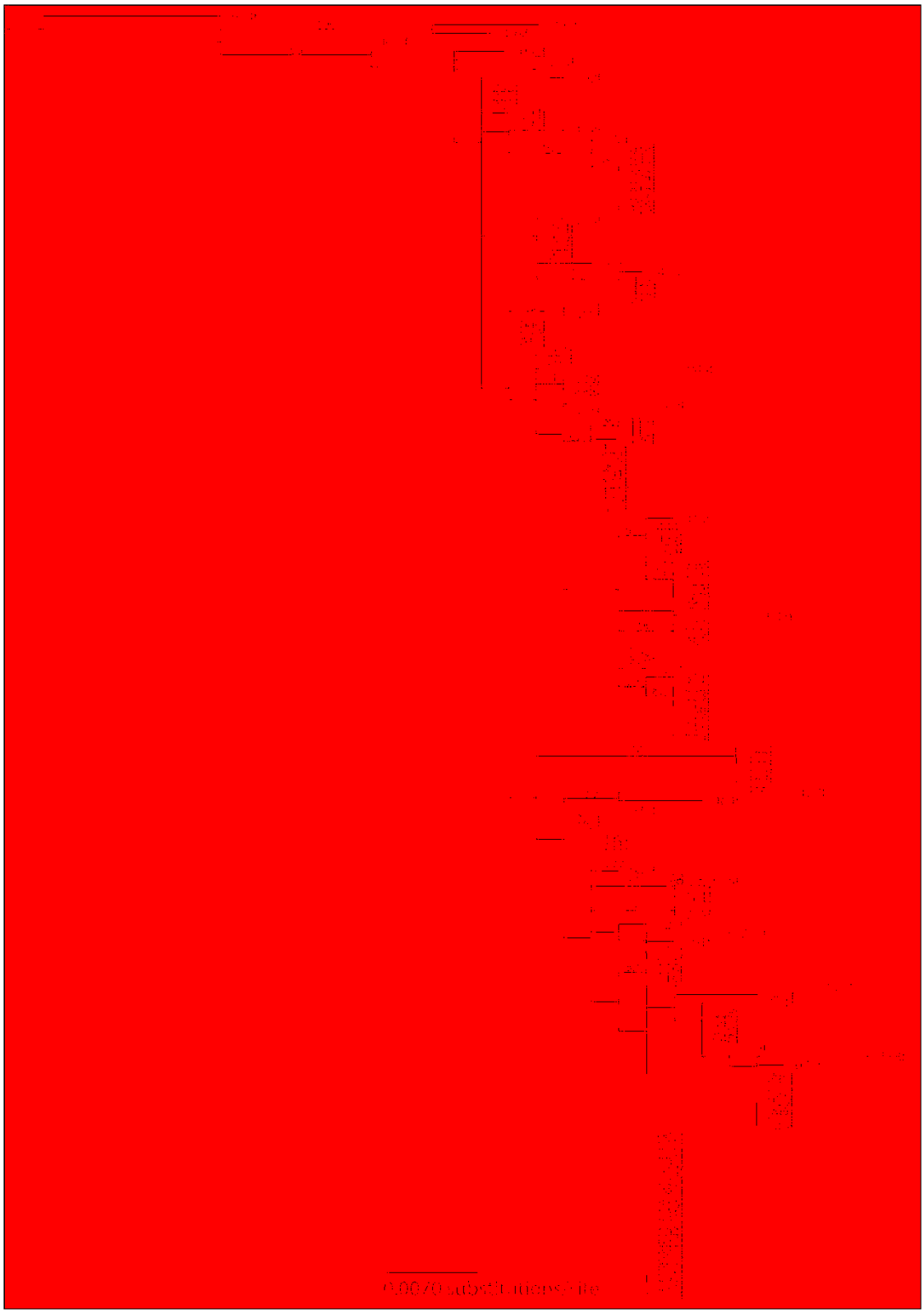
Supplementary data 3.3 (three subsequent pages). Concatenated ribosomal (LSU, ITS, IGS, group I intron), β-tubulin, and *MCM7* gene trees.



Supplementary data 3.3a. ML topology estimated from concatenated ribosomal markers (LSU, ITS, IGS, and group I intron), with bootstrap values > 50 indicated at nodes.



Supplementary data 3.3b. ML topology estimated from β-tubulin fragment, with bootstrap values > 50 indicated at nodes.



Supplementary data 3.3c. ML topology estimated from *MCM7* fragment, with bootstrap values > 50 shown at nodes.