Hidden diversity before our eyes: delimiting and describing cryptic lichen-forming fungal species in camouflage lichens (Parmeliaceae, Ascomycota)

Steven D. Leavitt^{a,*}, Theodore L. Esslinger^b, Pradeep K. Divakar^c, Ana Crespo^c and H. Thorsten Lumbsch^a

^a*Science & Education, The Field Museum, 1400 South Lake Shore Drive, Chicago, Illinois, 60605 USA; sleavitt@fieldmuseum.org*

^b*Department of Biological Sciences #2715, North Dakota State University, PO Box 6050, Stevens Hall, Fargo, ND, 58108-6050, USA*

^c*Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, Madrid E-28040, Spain*

**Corresponding author's phone: 1-312-665-7046*

Abstract: DNA sequence data now play a major role in systematics and taxonomy of lichen-forming fungi. Although molecular data provide unprecedented insight into diversity of lichen-forming fungi, morphologically cryptic species-level lineages circumscribed from sequence data often remain undescribed. The ensuing disconnects between molecular species circumscription and formal taxonomy have resulted in a “taxonomic gap”, even in well-studied groups. Here, we provide a robust, multi-locus phylogeny for the *Melanohalea* clade, comprised of *Emodomelanelia* Divakar & A. Crespo, *Melanelixia* O. Blanco et al., *Melanohalea* O. Blanco et al., *Montanelia* Divakar et al., and *Pleurosticta* Petr. Using diagnostic characters from DNA sequence data and support from the multispecies coalescent model, we formally describe a total of eleven new species and resurrect two others in the hyperdiverse lichen-forming fungal family Parmeliaceae. In addition, the morphological, ecological and geographical features were revised in order to corroborate species descriptions. These species include: four in the genus *Melanelixia* – *M. ahtii* sp. nov., *M. epilosa* comb. nov., *M. hawksworthii* sp. nov., and *M. robertsoniorum* sp. nov.; six in *Melanohalea* – *M. austroamericana* sp. nov., *M. beringiana* sp. nov., *M. clairii* sp. nov., *M. columbiana* sp. nov., *M. davidii* sp. nov., and *M. tahltan* sp. nov.; and three species in *Montanelia* – *M. occultipanniformis* sp. nov., *M. saximontana* comb. nov., and *M. secwepemc* sp. nov. These species can consistently be distinguished by differences in nucleotide position characters in the fungal barcoding marker, the internal transcribed spacer region (ITS), and high speciation probabilities within a multispecies coalescent framework. This study helps close the “taxonomic gap” between molecular species delimitation studies and formal taxonomy by incorporating statistical evaluation of lineage independence, diagnostic differences in molecular sequence data, and additional consideration of differences in morphology and species distributions within a taxonomic framework.

Keywords: coalescent theory; DNA barcoding; integrative taxonomy; molecular taxonomy; Parmeliaceae; species delimitation

1. Introduction

Species serve as a working hypothesis of boundaries among evolutionarily independent metapopulation lineages (de Queiroz, 2007; Pante et al., 2015). Species diagnoses are dependent on consistent and identifiable distinguishing characteristics that separate distinct taxonomic groups (Camargo and Sites, 2013). In practice, most modern species descriptions include phenotype-level, character-based diagnoses, although any suitable feature may be used (Fujita et al., 2012; Sites and Marshall, 2004). Failure to recognize boundaries among species-level lineages can provide biased inferences in a number of biological subdisciplines (e.g., Costello et al., 2013; Manel and Holderegger, 2013; Pante et al., 2015), and the rapid pace of species discovery using DNA sequence data has outpaced the rate of formal species descriptions for many organismal groups.

Following the general lineage species concept (GLC; de Queiroz, 1998; de Queiroz, 1999), researchers can use different empirical properties to test the hypothesis of lineage separation. While phenotypic characters often serve as proxies for assessing lineage independent, genetic data, including DNA sequence data, can provide compelling evidence for lineage independence when analyzed within a rigorous statistical framework (Rannala, 2015). The incorporation of the multispecies coalescent model into species delimitation research has provided a pivotal perspective into molecular systematics and species delimitation (Edwards, 2009; Fujita et al., 2012).

Based on practical and conceptual justifications, Félix et al. (2014) proposed that taxonomy should prioritize evolutionary independence over traditional phenotypic characters as the key feature in delimiting and diagnosing species-level lineages. Hibbett (2016) advanced formal species description based purely on sequence based species delimitation and appealed for changes in the International code of nomenclature for algae, fungi, and plants. According to the *International Code of Nomenclature for algae, fungi, and plants* (ICN), a diagnosis of a taxon is “a statement of that which in the opinion of its author distinguishes the taxon from other taxa.”

(Article 38.2). DNA sequence characters provide suitable character-based taxonomic diagnoses in cases where species-level lineages are morphologically cryptic or otherwise difficult to distinguish (Félix et al., 2014; Jorger and Schrod, 2013; McCune and Altermann, 2009). Recently, it has been argued that renewed attention to sharp diagnoses, rather than species descriptions, may speed up formal taxonomic recognition of species and that DNA-based diagnoses for new species become a recommendation in all Codes (Renner 2016). Furthermore, coalescent-based species delimitation probabilities have also been proposed to serve the same purpose as a standard phenotype-based diagnosis when the species in question is not diagnosable on the basis of morphology alone (Fujita et al., 2012; Leavitt et al., 2013b).

DNA sequence data and molecular-based specimen identification tools play a central role in species delimitation and taxonomic studies of lichen-forming fungi (Divakar and Crespo, 2015; Leavitt et al., 2015b; Lumbsch and Leavitt, 2011; Printzen, 2010). This is largely due to the fact that traditional phenotype-based approaches to species delimitation have been shown to fail to accurately characterize species-level diversity in a number of cases (Crespo and Lumbsch, 2010; Crespo and Pérez-Ortega, 2009). Parmeliaceae is among the most diverse lineages of lichenized fungi (Kraichak et al., 2015; Thell et al., 2012) and has been shown to include a number of morphologically cryptic species (e.g., Divakar et al., 2015b; Leavitt et al., 2011; Singh et al., 2015; Truong et al., 2013).

The brown parmelioids (Parmeliaceae) have received considerable attention over the past half-century (Ahti, 1966; Arup and Sandler Berlin, 2011; Blanco et al., 2004; Esslinger, 1977, 1978; Otte et al., 2005). The majority of brown parmelioid species belong to the *Melanohalea* clade as circumscribed by Crespo et al. (2010) – *Emodomelanelia* Divakar & A. Crespo, *Melanelixia* O. Blanco et al., *Melanohalea* O. Blanco et al., *Montanelia* Divakar et al., and *Pleurosticta* Petr. Genus-wide molecular phylogenetic analyses have been performed previously for *Melanohalea* (Leavitt et al., 2012a; Leavitt et al., 2013a) and *Montanelia* (Divakar et al., 2012; Leavitt et al., 2015a).

In addition, Leavitt et al. (2012b) provided a robust phylogenetic hypothesis for a major clade in the genus *Melanelixia* comprised of *M. fuliginosa*, *M. glabratula*, *M. subaurifera*, and two additional undescribed species-level lineages. While cryptic, species-level lineages have been circumscribed in *Melanelixia*, *Melanohalea*, and *Montanelia*, formal taxonomic recognition of these lineages has been limited to a single species in *Melanelixia* (Divakar et al., 2010).

In this study we propose the formal recognition of eleven previously undescribed species-level lineages in the hyperdiverse family Parmeliaceae, in addition to the resurrection of two additional species. These species had previously been masked within a number of traditionally circumscribed nominal brown parmelioid species (Fig. 1). The specific aims of this study were to: (i) provide a robust, phylogenetic hypothesis for the *Melanohalea* clade; (ii) ascertain additional evidence supporting previously unnamed species-level lineages; and (iii) formally describe morphologically cryptic species-level lineages.

2. Materials and Methods

2.1 Taxonomic sampling

For this study, we examined relationships within and among the five brown parmelioid genera in the *Melanohalea* clade. We included sequence data from a total of 826 specimens collected world-wide representing 55 species/species-level lineages: the monotypic species *Emodomelanelia masonii* (Essl. & Poelt) Divakar & A. Crespo, 17 species in *Melanelixia*, 26 in *Melanohalea*, 10 in *Montanelia*, and one of the two species in *Pleurosticta* (supplementary Table S1). Our sampling included the majority of described species and all previously circumscribed, undescribed species-level lineages (Divakar et al., 2012; Divakar et al., 2010; Leavitt et al., 2015a; Leavitt et al., 2012b; Leavitt et al., 2013a). However, we were unable to obtain sequence data for the following species: *Melanelixia subvillosella* H.Y. Wang & J.C. Wei, *M. huei* (Asahina) O. Blanco et al.; *Melanohalea inactiva* (P.M. Jørg.) O. Blanco et al., *M. nilgirica* Divakar &

Upreti, *M. peruviana* Essl., *M. subverruculifera* (J.C. Wei & Y.M. Jiang) O. Blanco et al. *M. zopheroa* (Essl.) O. Blanco et al.; and *Pleurosticta koflerae* (Clauzade & Poelt) Elix & Lumbsch. Two species representing the genus *Xanthoparmelia* were selected as outgroups for phylogenetic analyses (Divakar et al., 2015a).

2.2 Molecular sequence data

For this study we compiled data from a total of seven loci, including: three from the nuclear ribosomal cistron – the internal transcribed spacer region (ITS), a portion of large subunit (nuLSU), and a portion of the intergenic spacer (IGS); fragments from three nuclear protein-coding loci – RNA polymerase II largest subunit (RPB1), RNA polymerase II second largest subunit (RPB2), and the minichromosome maintenance complex component 7 (MCM7); and a portion of the mitochondrial small subunit (mtSSU). All sampled specimens were represented by at least the ITS marker in phylogenetic analyses, and we attempted to have multiple representatives of all species-level lineages represented by each of the seven selected loci.

In addition to previously published sequences (Divakar et al., 2012; Divakar et al., 2010; Leavitt et al., 2015a; Leavitt et al., 2012b; Leavitt et al., 2013a), new sequence data were generated from 63 *Melanelixia* specimens and five *Melanohalea* specimens. For these specimens, DNA isolation, polymerase chain reactions (PCR), and sequencing followed Leavitt et al. (2012b). We were unable to consistently amplify IGS sequences for specimens in *Melanohalea* and *Montanelia*, and therefore this marker is absent for these genera. Similarly, mtSSU sequences were not generated for specimens representing *Melanelixia* due to difficulties in amplifying this locus with consistency.

Multiple sequence alignments for each marker were performed using the program MAFFT v7 (Katoh et al., 2005; Katoh and Toh, 2008) as described previously (Leavitt et al., 2012a; Leavitt et al., 2012b). For the final ITS alignment, obvious errors in the initial MAFFT alignment were manually corrected. To explore the impact of

ambiguously aligned regions in phylogenetic reconstructions, an additional ITS alignment excluding ambiguously aligned portions of the multiple sequence alignment (MSA) was created. Ambiguous regions were delimited and removed using the GBLOCKS webserver (Talavera and Castresana, 2007); http://molevol.cmima.csic.es/castresana/Gblocks_server.html), implementing the options for a less stringent selection, including the 'Allow smaller final blocks', 'Allow gap positions within the final blocks', and 'Allow less strict flanking positions' options. Multiple sequence alignments for the seven sampled loci – ITS, nuLSU, IGS, MCM7, RPB1, RPB2, and mtSSU – were concatenated into a partitioned supermatrix for subsequent phylogenetic analyses.

2.3 Phylogenetic analyses

A number of recent studies suggest that in many cases concatenation can perform as well as species tree methods that explicitly account for incomplete lineage sorting, particularly in scenarios with low levels of incomplete lineage sorting (ILS) (Lambert et al., 2015; Tonini et al., 2015). Previous studies in *Melanelixia*, *Melanohalea*, and *Montanelia* have recovered highly congruent phylogenies using maximum likelihood (ML), Bayesian, and species tree inference methods. Therefore, given the large size of our data set ($n = 826$, including outgroups) and previous observations of congruence among phylogenetic inference methods, only maximum likelihood (ML) topologies were reconstructed from the seven-locus matrix using the program RAxML v8.2.1 (Stamatakis, 2006; Stamatakis et al., 2008) in the CIPRES Science Gateway server (<http://www.phylo.org/portal2/>). The best-fitting partition scheme was inferred using the program PartitionFinder v1.1.1 (Lanfear et al., 2012). The partitioning scheme was selected from a total of 21 pre-defined partitions as follows: ITS1, 5.8S, and ITS2 (from the ITS); the complete nuLSU; the complete IGS; the complete mtSSU; the first, second and third codon positions of the MCM7; the first, second and third codon positions of two coding regions in the RPB1; two introns in the

RPB1; the first, second and third codon positions of the coding region in the RPB2; and an intron in the RPB2. The partitioned RAxML analysis implemented the 'GTRGAMMA' model and evaluated nodal support using 1000 bootstrap pseudo-replicates. Exploratory analyses of alternative partition strategies provided highly similar topologies and nodal support values. Our ML phylogenetic reconstruction was compared with previous inferences for *Melanelixia* (Leavitt et al., 2012b), *Melanohalea* (Leavitt et al., 2013a), *Montanelia* (Leavitt et al., 2015a), and the entire *Melanohalea* clade (Divakar et al., 2015a).

2.4 Species delimitation and validation

We used the multispecies coalescent model implemented in the program BPP v3.2 (Yang and Rannala, 2010, 2014) to validate previously circumscribed candidate species in the *Melanohalea* clade. BPP has been shown to be among the most accurate empirical species delimitation methods (Camargo et al., 2012; Downton et al., 2014) and has been demonstrated to perform sensibly across a range of scenarios (Yang, 2015; Zhang et al., 2011). This method follows the biological species concept, assuming no admixture following a speciation event (Yang and Rannala, 2010). BPP has been successfully used for other species with strong clonal components of reproduction (Falk et al., 2015), as is likely the case for a number of the brown parmelioid species investigated here. The model incorporates coalescent theory and phylogenetic uncertainty into parameter estimation; and the posterior distribution for species delimitation models is sampled using a reversible-jump Markov Chain Monte Carlo (rjMCMC) chain.

We used the unguided species delimitation analysis ('A11'; Yang, 2015). This algorithm explores different species delimitation models and different species phylogenies, with fixed specimen assignments to populations. This analysis attempts to merge populations into one species, and uses the nearest neighbor interchange (NNI) or subtree pruning and regrafting (SPR) algorithms to change the species tree topology

(Yang and Rannala, 2014). Unguided species delimitation ('A11') analyses in BPP were performed for separately for each genus – *Melanelixia*, *Melanohalea*, and *Montanelia*, with candidate species shown in Fig. 2. The earliest diverging species in the genus *Melanelixia* – *M. calva* (Essl.) A. Crespo, Divakar & Elix, *M. piliferella* (Essl.) A. Crespo, Divakar & Elix, and *M. subglabra* (Räsänen) A. Crespo, Divakar & Elix – were excluded due to small sample sizes and incomplete gene sampling. For similar reasons, *M. austroamericana* Essl. et al. and *M. ushuaiensis* (Zahlbr.) O. Blanco et al. were excluded from the BPP analyses of *Melanohalea*. Equal probabilities for the labeled histories were used to assign probabilities to the models, rates were allowed to vary among loci (locusrate=1), and the analyses were set for automatic fine-tune adjustments. Multiple analyses using different combinations of the theta (θ) and tau (τ) priors spanning a range of possible population sizes and divergence times were performed for each genus. We used the relationships inferred from the ML topology as a starting tree for each genus, and the rjMCMC analysis was run for 200,000 generations, sampling every 2 generations discarding the first 10% as burnin. Each analysis was run twice using a different search algorithm (algorithm 0 or 1) to confirm consistency between runs. Speciation probabilities greater than 0.95 were considered supported species delimitations. In our opinion, speciation probabilities can serve as a diagnostic feature distinguishing taxa (see Fujita et al., 2012).

2.5 Taxonomic conclusions

The internal transcribed spacer region (ITS) of the nuclear ribosomal tandem repeat has been formally proposed as the official barcoding marker for fungi (Schoch et al., 2012) and represented the most comprehensively sampled locus in this study (supplementary Table S1). Therefore, we selected distinct nucleotide position characters from alignments of ITS sequence data to diagnose morphologically cryptic species, in addition to support from BPP analyses (see section 2.4). Diagnostic characters from aligned ITS sequences are reported relative to archived alignments

available in TreeBase (<http://treebase.org/>): (i) the genus *Melanelixia* (TreeBase ID: S18577); (ii) the genus *Melanohalea* (TreeBase ID: S12364); and (iii) the genus *Montanelia* (TreeBase ID: S16237). Only species-level lineages represented by two or more specimens and supported with high speciation probabilities (>0.95) in the BPP analyses are formally described here.

3. Results

3.1 Sequence data

New sequences generated in association with this study have been deposited in GenBank under accession numbers: KX349505– KX349705. The complete brown parmelioid data matrix (n=826 specimens) included 4375 aligned nucleotide position characters. The complete alignment was submitted to TreeBase under study ID S18577. The ITS alignment, with ambiguously aligned portions removed using GBLOCKS, was comprised of 379 aligned bp, as opposed to 554 in the complete ITS alignment.

3.2 Phylogenetic reconstructions

Our phylogenetic analyses recovered a generally well-supported hypothesis of evolutionary relationships for the *Melanohalea* clade (Fig. 2; supplementary Fig. S1). The RAxML analysis of the alternative concatenated data matrix with ambiguously aligned nucleotide position removed from the ITS alignment resulted in highly similar phylogenetic relationships, although nodal support was notably lower for a number of clades (results not shown). Therefore, we report below the results from the complete concatenated alignment.

Melanelixia and *Melanohalea* were recovered with 100% bootstrap support (BS), with *Emodomelanelia* recovered as sister to *Melanelixia*+*Melanohalea* with strong support (BS = 97%). *Pleurosticta* and *Montanelia* were recovered within a well-supported monophyletic clade (BS = 100%), and *Montanelia* was supported as

monophyletic (BS = 79%). Well-supported relationships among species-level lineages within *Melanelixia*, *Melanohalea*, and *Montanelia* were congruent with previous estimates and are not reported in detail here. The vast majority of species were recovered as monophyletic lineages with BS > 80%. Exceptions include: *Melanelixia albertana* (Ahti) O. Blanco et al. (BS=53%), *M. subargentifera* (Nyl.) O. Blanco et al. (BS=66%), and *Melanohalea subolivacea* (Nyl. ex Hasse) O. Blanco et al. (BS<50%). The closely related taxa *Melanohalea mexicana* Essl. & R.-E. Pérez, *M. subolivacea*, and *M. clairii* sp. nov. were recovered within a well-supported monophyletic clade (BS=92%), with *M. clairii* as sister to a weakly supported clade comprised of *M. mexicana*+*M. subolivacea* s. str. (Fig. 2; supplementary Fig. S1). All specimens representing *M. mexicana* were recovered nested within the *M. subolivacea* s. str. clade, with specimens collected from the state of Puebla, Mexico comprising a well-supported (BS=87%), monophyletic clade and a single specimen from the state of Morelos fell outside of the *M. mexicana* clade (supplementary Fig. S1).

Consistent with previous observations, nominal taxa recovered in distinct species-level lineages were not always each other's closest relative (Fig. 2). Clade 'Melanelixia albertana' (Fig. 2), was comprised of specimens identifiable to multiple nominal taxa: a single specimen identified as *M. villosella* (Essl.) O. Blanco et al. (China), specimens identified as *M. glabra* (Schaer.) O. Blanco et al. (China), a specimen identified as *M. glabroides* (Essl.) O. Blanco et al. from northern India, and two specimens from the Russian Far East (Spribille 23677 and Spribille 23949) (supplementary Table S1). The remaining five specimens in this clade were morphologically similar to *M. albertana* (Ahti) O. Blanco et al., including a specimen from the northern Great Plains region of North America (the type specimen for *M. albertana* was collected from the Great Plains region near Edmonton, Canada; Esslinger, 1977).

3.3 Speciation probabilities

All species assessed using the multispecies coalescent validation approach BPP, including those described in this study, were delimited with > 0.95 posterior probability in all analyses performed under different prior combinations (Table 1). In the only case where putative species were recovered as polyphyletic in multilocus phylogenetic reconstructions, *M. mexicana* nested within *M. subolivacea*, both species were supported with high speciation probabilities (PP=1.0) even under the most conservative combination of priors.

TAXONOMY

Based on these results we propose a total of eleven new species within the *Melanohalea* clade: *Melanelixia ahtii*, *M. hawksworthii*, and *M. robertsoniorum*; *Melanohalea austroamericana*, *M. beringiana*, *M. clairii*, *M. columbiana*, *M. davidii*, and *M. tahlitan*; and *Montanelia occulti panniformis*, *M. secwepemc*. Four additional candidate species were supported with high speciation probabilities in the BPP analyses but were represented by only a single individual: *Melanelixia* 'aff. *glabroides* 3953' (USA: Colorado, Henson 53, TLE), *Melanohalea* 'aff. *elegantula* 6042' (Spain: Madrid, Matalpino, Crespo & Divakar 10224, MAF-Lich), *Montanelia* 'aff. *tominii* B' (China: Heilongjiang, Qiang Ren s.n., SDNU), *Montanelia* 'aff. *tominii* E' (China: Heilongjiang, Qiang Ren s.n., SDNU). Therefore, due to the limited current sampling these candidate species are not recognized formally here. We also resurrect two species – *Melanelixia epilosa* and *Montanelia saximontana*.

Previously published provisional names, reference phylogenies, nodal support, and species delimitation probabilities are summarized for each taxon in Table 1.

The species

Melanelixia ahtii S. Leavitt, Essl., Divakar, A. Crespo & Lumbsch, sp. nov.

MYCOBANK #817307

Etymology: This species is named in honor of Dr. Teuvo Ahti for his contributions to understanding diversity in brown parmelioid lichens.

Type: USA: Washington, Klickitat Co., north side of Columbia River Gorge, in mixed oak-ponderosa forest, along Courtney Road, 3.5 km east of Bingen. Epiphyte on *Quercus* sp. Lat. 45.7000° N; Lon. -121.4076° W; alt. ca. 75 m.a.s.l., 03 November 2010, S. Leavitt 4306 (BRY-C - holotype; F – isotype).

Diagnosis: As currently circumscribed, this taxon contains specimens morphologically similar to *M. subargentifera* (Esslinger, 1977) and consists of specimens recovered within clade '*M. ahtii*' (Fig. 2; supplementary Fig. S1). *Melanelixia ahtii* can be distinguished from *M. subargentifera* s. str. by fixed differences in nuclear ribosomal ITS sequence data (Table 2), common occurrence in western North American distribution, and generally sparser hyaline cortical hairs on lobes (missing from some lobes altogether) and finer/shorter cortical hairs than specimens belonging to *M. subargentifera* s. str. (10–25 µm vs. 20–50 µm). *Melanelixia ahtii* is supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of multiple genetic loci (Table 1).

Reference sequences: GenBank Nos. KX349530 (ITS), KX349623 (nuLSU), KX349646 (MCM7), and KX349687 (RPB2).

Ecology and distribution: Based on current sampling, this taxon is currently known from four states – California, Idaho, Oregon, and Washington – east of the Cascade Mountains, USA. This species occurs on bark. The majority of sampled specimens representing this taxon were collected at low elevations in vicinity of the Columbia River and its tributaries (e.g., Snake River, Salmon River, Spokane River), with the exception of two specimens collected in San Luis Obispo Co., California.

Discussion: This species is morphologically similar to *M. subargentifera* s. str., and additional investigations will be required to determine if any phenotypic characters can be used to consistently distinguish *M. ahtii* from *M. subargentifera*. Hyaline cortical hairs on lobes in *M. ahtii* material are generally sparser and finer/shorter (missing from

some lobes altogether) than specimens belong to *M. subargentifera* s. str. This scarcity/size difference in cortical hairs loosely parallels a similar pattern in the morphologically similar species *M. californica* and *M. glabra*. This pattern of differences in cortical hairs also generally coincides with evolutionary relationships. In our phylogenetic reconstruction, taxa with sparser and finer/shorter hyaline cortical hairs – *M. ahtii* and *M. californica* – were recovered as closely related sister species. In contrast, *M. subargentifera* s. str. belongs to a group of closely related species, including *M. epilosa* and *M. glabra*, with more developed hyaline cortical hairs (Fig. 2).

The type specimen representing *M. subargentifera* s. str. was described from the Republic of Karelia in northwestern Russia (Norrlin, 1874: H-NYL 34578), and in this study all European collections representing *M. subargentifera* s. lat. were recovered in the '*M. subargentifera*' clade (supplementary Fig. S1; supplementary Table S1). While we anticipate that *M. ahtii* will be found to be restricted to western North America, more thorough sampling of *M. subargentifera* s. lat. specimens across their entire distribution ranges will be required to better understand the distribution of this new species. We note that *M. ahtii* is more closely related to *M. californica* and *M. glabroides* than to *M. subargentifera* s. str. (Fig. 2). In contrast, *M. subargentifera* s. str. belongs to a well-supported lineage that also comprises two other taxa with old World distributions, *M. epilosa* and *M. glabra* s. str. (Fig. 2). However, our sampling of *M. subargentifera* s. str. also included a number of specimens collected in North America, including populations in the Great Lakes region and western North America (supplementary Table S1).

Additional specimens examined: **USA:** California, San Luis Obispo Co., Dart 440 (TLE) and Dart 444 (TLE); Idaho, Idaho Co., ca. 2 air miles NE of Pittsburgh Landing on the Snake River, Esslinger 18759 (TLE); Idaho, Idaho Co., ca. 10 air miles WSW of Grangeville, near Pine Bar Rapids on the Salmon River, Esslinger 18711 (TLE); Idaho, Idaho Co., 25 km north of Riggins, along the Salmon River near the Slate Creek Recreation Area, ca. 470 m. elevation, on bark and branches of *Cercocarpus ledifolius*,

Esslinger 18784 (TLE; a duplicate in MAF-Lich); Oregon, Klamath Co., Björk 21516 (UBC); Washington, Klickitat Co., ca. 1 km NE of Horsethief Lake State Park along Hwy 14, Esslinger 18637 (TLE); Washington, Klickitat Co., along Hwy 14, in Columbia River Gorge, in Oak forest on basalt outcrop, ca. 9 km east of Murdock, Leavitt 4305 (F); Washington, Klickitat Co., ca. 1.6 km W of "Doug's Beach," Björk 14292 (UBC); Washington, Spokane Co., Riverside State Park, north end, Björk 19199 (UBC).

***Melanelixia epilosa* (J. Steiner)** A. Crespo, Divakar, Gasparyan, V. J. Rico, Essl. S. Leavitt & Lumbsch, comb. nov.

MYCOBANK #817339

Basionym: *Parmelia glabra* var. *epilosa* J. Steiner, Annales Mycologici 17(1): 23 (1919). *Type:* USSR. Georgia: Adzhariya, the gorge Petzkir, Woronow 78 (W-holotype). *Parmelia epilosa* (J. Steiner) Gyeln., Feddes Repert. 30: 221 (1932)

Comments: The type of *M. epilosa* was collected in southern Georgia, and recently collected specimens from neighboring Armenia were recovered within 'clade B' (Divakar et al., 2010). Given the close geographic location and morphological similarities of the Armenian *M. glabra* s. lat. specimens to the type for *Parmelia epilosa* (J. Steiner) Gyeln., we resurrect this name as *Melanelixia epilosa* for specimens recovered within 'clade B' (Divakar et al., 2010). The type specimen of *M. glabra* s. str. was described from Switzerland. The morpho-species is distributed in southern central Europe and common in the Mediterranean region including North Africa (but apparently absent from the British Isles). Almost all samples collected from western Europe and the single collection from Morocco were clustered in the *M. glabra* clade, 'clade A' in Divakar et al., (2010) (supplementary Fig. S1, supplementary Table S1). Therefore, we assume that 'clade A' (Divakar et al., 2010) most likely represents *M. glabra* s. str.

Melanelixia epilosa can be distinguished from *M. glabra* s. str. by fixed differences in nuclear ribosomal ITS sequence data (Table 2) and is supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of multiple

genetic loci (Table 1). This taxon contains specimens morphologically similar to *M. glabra* (Esslinger, 1977) and consists of specimens recovered within clade 'clade B' (Divakar et al., 2010) and '*M. epilosa*' (Fig. 2; supplementary Fig. S1).

Current sampling indicates that *M. epilosa* is distributed in Asia, Turkey, the Caucasus regions and Spain. In Spain it is sympatric with *M. glabra* s. str. in some central zones of the Peninsula but occurs chiefly in eastern regions with continental climate. However, additional studies are necessary to better understand the geographic distribution of the two species. Otherwise, no apparent morphological, chemical or geographical characters have been observed which can consistently be applied to distinguish these two species (Divakar et al., 2010).

Ecology and distribution: It grows as an epiphyte on *Fagus*, *Quercus*, *Salix* and *Ulmus* spp. at elevations between 1200 and 2000 m.

Additional specimens examined: **Armenia:** Ararat, A. Crespo et al. 7099 (DNA5053, MAF-Lich); Ararat, A. Crespo et al. 7147 (DNA5054, MAF-Lich); Gegharkunik, A. Crespo et al. 7220 (DNA5057, MAF-Lich); Tavush, A. Crespo et al. 7260 (DNA5056, MAF-Lich) **India:** Jammu & Kashmir, Srinagar, Dachigam National Park, M. Sheikh DNA 1103 (MAF-Lich); Jammu & Kashmir, Pahlgam, M. Sheikh DNA 1104 (MAF-Lich); Jammu & Kashmir, Srinagar, Dachigam National Park, M. Sheikh DNA 1105 (MAF-Lich). **Spain:** Guadalajara, A. Crespo et al. 10228 (MAF-Lich); Selas, León, Mansilla, N. Hladun 14715 (BCN); Madrid, Navacerrada A. Crespo, & Arnaiz 15653 (BCN). **Turkey:** Bursa, Uldaj, A. Crespo et al. 15558 (MAF-Lich); Zonguldak, Eregli, A. Crespo et al. 15557 (MAF-Lich).

Melanelixia hawksworthii S. Leavitt, Essl. A. Divakar, Crespo & Lumbsch, sp. nov.
MYCOBANK #817308

Etymology: This species is named in honor of Professor David Leslie Hawksworth for his immense contributions to fungal systematics.

Type: United Kingdom, Scotland: West Ross, Loch Carron, Glen Attadale, on the north side of River Attadale, opposite of Home Farm in ash-alder wood by track at edge of pasture. Epiphyte on *Fagus* sp. Lat. 57.3924° N; Lon. -5.4547° W; alt. ca. 5 m.a.s.l., 27 July 2010, Lumbsch 20101a (F - holotype; E - isotype).

Diagnosis: Morphologically similar to *M. fuliginosa* s. lat. [see '*P. glabratula*' in Esslinger (1977)], but consists of specimens recovered within clade '*M. sp. 1*' in Leavitt et al. (2012b). *Melanelixia hawksworthii* can be distinguished from other species in the 'fuliginosa/subaurifera' clade by fixed differences in nuclear ribosomal ITS sequence data (Table 2) and is supported as a lineage distinct from other populations according to coalescent-based genetic analysis of multiple genetic loci (Table 1).

Reference sequences: GenBank Nos. JX126183 (ITS), JX126311 (nuLSU), JX126353 (MCM7), JX126396 (RPB1), and JX126441 (RPB2).

Ecology and distribution: This taxon is known from two corticolous specimens collected in coastal areas of Scotland and a single corticolous specimen collected in a montane region of northwestern Spain, suggesting an atlantic-submediterranean distribution.

Discussion: A number of morphologically similar species are known from the 'fuliginosa/subaurifera' clade, including *M. fuliginosa* (Fr. ex Duby) O. Blanco et al., *M. glabratula* (Lamy) Sandler & Arup, *M. hawksworthii* sp. nov. (this study), and *M. robertsoniorum* sp. nov. (this study). However, the divergence among these species has been estimated to have occurred during the Miocene, ranging from nearly 19 Ma (*mega annum*) for the initial observed divergence event for this group to ca. 10 Ma for the most recent split (Leavitt et al., 2012b). The split between *M. hawksworthii* and its extant sister species, *M. subaurifera*, is estimated to have occurred ca. 15 Ma (Leavitt et al., 2012b). It appears that the "fuliginosa" s. lat. phenotype, including *M. fuliginosa* s. str., *M. glabratula*, *M. hawksworthii*, and *M. robertsoniorum*, is highly conserved within this clade throughout a relatively deep diversification history. However, it is worth noting that the "fuliginosa" phenotype is polyphyletic with *M. subaurifera* (Fig. 2). We here consider the widely distributed specimens within the '*M. subaurifera*' clade as *M.*

subaurifera s. str. This clade includes the samples from several regions of North America, Canada, and Europe – France, Spain, Sweden and UK (Supplementary Table S1). The type of *M. subaurifera* is from Finland, Keski-Suomi, and all European specimens identified as *M. subaurifera* were recovered within this clade.

Additional specimens examined: **United Kingdom:** Scotland, Isle of Skye, Lumbsch 20100b (F). **Spain:** Burgos, south of Alto Campo (Crespo, A. 6030h, MAF-Lich).

Melanelixia robertsoniorum S. Leavitt, Essl. Divakar, A. Crespo & Lumbsch, sp. nov.
MYCOBANK #817309

Etymology: This species is named in honor of the late Judith and Ronald Robertson, experts on Californian lichens, whose kindness and generosity helped promote lichenological research in the state of California.

Type: USA: California, Napa Co., Booth Napa State Park. Oak woodland. Epiphyte on *Alnus rubra* (red alder). Lat. 38.549167° N; Lon. -122.5425° W; alt. unknown. 16 Nov. 2003, Robertson 7139 (MAF-Lich – holotype, TLE - isotype).

Diagnosis: Morphologically similar to *M. glabratula* s. str. [see '*P. glabratula*' in Esslinger (1977)], but consists of specimens recovered within clade '*M. sp. 2*' (Leavitt et al., 2012b). *Melanelixia robertsoniorum* can be distinguished from other species in the 'fuliginosa/subaurifera' clade by fixed differences in nuclear ribosomal ITS sequence data (Table 2) and its restricted distribution to western North America.

Melanelixia robertsoniorum is also supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of multiple genetic loci (Table 1).

Reference sequences: GenBank Nos. JX126185 (ITS), JX126313 (nuLSU), JX126149 (IGS), JX126355 (MCM7), JX126405 (RPB1), and JX126443 (RPB2).

Ecology and distribution: Based on current sampling, this taxon is known exclusively from California, where it grows on bark.

Discussion: As the type specimen representing *M. fuliginosa* is from France and all studied European specimens were recovered in the '*M. fuliginosa*' clade (Fig. 2), we here consider specimens within the '*M. fuliginosa*' clade as *M. fuliginosa* s. str. See also Arup and Sandler Berlin (2011) and the discussion under *Melanelixia hawksworthii*.

Additional specimens examined: **USA:** California; Mendocino Co. (Crespo A. 6605k MAF-Lich); Mendocino Co. (Crespo A. 6605o MAF-Lich); and Napa Co., (Robertson 7140, 10947 MAF-Lich).

Melanohalea austroamericana Essl., Divakar, A. Crespo, S. Leavitt & Lumbsch sp. nov.

MYCOBANK # 817310

Etymology: The new taxon is named '*austroamericana*' based on its currently known distribution at a single South American site in central Chile.

Type: Chile: VII Región, Parque Nacional de Laja. Cerca del control aduanero.

Austrocedrus sp. forest. Epiphyte on *Austrocedrus* sp. Lat. 37° 23' 53" S; Lon. 71° 26' 22" W ; alt. 946m, 02 April 2009, L. Sancho, S. Perez-Ortega & F. Fernández-Mendoza 332 DNA 1888 (MAF-Lich – holotype)

Diagnosis: *Melanohalea austroamericana* can be distinguished from *M. ushuaiensis* by the presence of both soredia and isidia on the upper surface and fixed differences in nuclear ribosomal ITS sequence data (Table 3). Isidia are thin, cylindrical, developing from soralia margins.

Reference sequences: GenBank Nos. JQ813789 (ITS), JQ813112 (nuLSU), JQ813983 (MCM7), JQ814065 (RPB1), and JQ813302 (RPB2).

Ecology and distribution: The two studied specimens are known from a single population in Chile's Parque Nacional de Laja, occurring on bark in a *Austrocedrus* forest.

Discussion: Although superficially similar to *M. ushuaiensis*, *M. austroamericana* is characterized by the production of both soredia and isidia. The isidia are thin, cylindrical and develop from margins of soralia. The sister taxon to *M. austroamericana*, *M. ushuaiensis* (Zahlbr.) O. Blanco et al., is distinctly non-sorediate (Esslinger 1977) but has sparse to numerous isidia. The specimens of *M. austroamericana* were collected from near the northernmost known localities for *M. ushuaiensis*, but the type specimen of the latter species and of its synonym, *Parmelia roivainenii* Räsänen, are both from Tierra del Fuego, more than 1700 km south. Furthermore, the distinction of *M. austroamericana* from *M. ushuaiensis* is strongly supported by molecular phylogenetic analyses, with an estimated split between the two species occurring ca. 15.7 Ma (Leavitt et al., 2012a).

Additional specimens examined: **Chile:** VII Región, Parque Nacional de Laja (337 MAF-Lich).

Melanohalea beringiana S. Leavitt, Essl., Divakar, A. Crespo & Lumbsch sp. nov.

MYCOBANK #817311

Etym.: The new taxon is named '*beringiana*' based on its currently known distribution, which is restricted to Interior Alaska.

Type: USA: Alaska, Interior Alaska, vicinity of the Richardson Highway (Hwy. 4), ca. 24 km north of Paxon, and ca. 0.66 km along entry road to Fielding Lake Recreation Site. Collected in balsam poplar stand – on bole of *Populus balsamifera*. Lat. 63.2024° N; Lon. -145.6228° W; alt. ca. 937 m.a.s.l., 25 June 2011, Esslinger 19564 (F – holotype; BRY-C – isotype).

Diagnosis: Morphologically similar to *M. olivaceoides* s. str. (see Esslinger, 1977), but consists of specimens recovered within clade '*M. olivaceoides* 2' (Leavitt et al., 2013a).

Melanohalea beringiana can be distinguished from *M. olivaceoides* s. str. by fixed differences in nuclear ribosomal ITS sequence data (Table 3) is supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of

multiple genetic loci (Table 1).

Reference sequences: GenBank Nos.: JQ813645 (ITS), JQ813073 (nuLSU), JQ813956 (MCM7), and JQ814030 (RPB1).

Ecology and distribution: Currently, only two specimens of *M. beringiana* are known, both occurring in Interior Alaska, USA. This taxon occurs on bark.

Discussion: Although morphologically similar, *M. beringiana* and *M. olivaceoides* s. str. belong to two distinct clades with an estimated most recent common ancestor (MRCA) of ca. 20 Ma (Leavitt et al., 2012a). Similar to the situation in the morphologically cryptic species in the *Melanelixia fuliginosa-subaurifera* clade, *M. beringiana* and *M. olivaceoides* s. str. provide another striking example of morphological similarities in relatively distantly related species within brown parmelioid genera. *Melanohalea olivaceoides* s. lat. exhibits a boreal-austral American distribution (Esslinger, 1977). However, specimens from populations in southern South America have not been included in molecular phylogenetic studies to date, and their relationship to these taxa remains unknown.

In phylogenetic analyses, *M. beringiana* was recovered within a closely related clade comprised of *M. beringiana*, *M. lobulata*, and *M. subexasperata* (Fig. 2). However, in contrast to *M. beringiana*, both *M. lobulata* and *M. subexasperata* are only known from a limited number of high altitude (2700–4710 m) sites in China, both species commonly produce apothecia and vegetative diaspores (isidia/soredia), and *M. subexasperata* is characterized by cortical hairs. *Melanohalea olivaceoides* s. str. is morphologically indistinguishable from *M. beringiana*, but the former belongs to a closely related clade comprised of *M. gomukhensis*, *M. olivaceoides*, and *M. poeltii* (Fig. 2). Similar to *M. olivaceoides*, *M. gomukhensis* also produces isidioid to granular soredia, but the latter also produces pseudocyphellae (Zhao et al., 2009). The other closely related taxon, *M. poeltii*, is characterized by cylindrical isidia and by the general absence of pseudocyphellae (although sparse pseudocyphellae may occur on some lobes) (Divakar and Upreti, 2005). The close relationships of these phenotypically

distinct *Melanohalea* species – *M. gomukhensis*, *M. olivaceoides* s. str., and *M. poeltii* – highlights the difficulties in inferring evolutionary relationships based on morphological characteristics alone in some groups of lichen-forming fungi.

Based on current observations, *M. beringiana* and *M. olivaceoides* are morphologically indistinguishable. Therefore, we believe that the interests of nomenclatural stability are best served by epitypifying *M. olivaceoides* against a sequenced specimen of known identity that has been collected from near type locality. See discussion under *M. olivaceoides*.

Additional specimens examined: **USA:** Alaska, Interior Alaska, along the Alaska Highway (Hwy. 2) ca. 23 miles east of Tok at rest area, Esslinger 19895 (F).

Melanohalea clairii S. Leavitt, Essl., Divakar, A. Crespo & Lumbsch, sp. nov.

MYCOBANK #817327

Etymology: This species is named in honor of Dr. Larry St. Clair for his for his many contributions to lichenology in western North America.

Type: USA: Colorado, Garfield Co., White River National Forest, at the Hadley Gulch trailhead (USFS Trail No. 1840), along Cline Top Road (USFS Road No. 603, in juniper-oak montane shrubland. Epiphyte on *Quercus gambelii*, Lat. 39.6782° N; Lon. 107.5720° W; alt. ca. 1900 m.a.s.l, 14 July 2010, S. Leavitt 10-317 (BRY-C – holotype; F – isotype).

Diagnosis: Morphologically similar to *M. subolivacea* s. str. (see Esslinger, 1977), but consists of specimens recovered within clade ‘*M. subolivacea* 1’ (Fig. 1; Leavitt et al., 2013a). *Melanohalea clairii* can be distinguished from *M. subolivacea* s. str. by fixed differences in nuclear ribosomal ITS sequence data (Table 3). *Melanohalea clairii* is supported as a lineage distinct from all other species in the ‘subolivacea’ group (*M. mexicana*, *M. subolivacea* s. str., and *M. clairii*) according to coalescent-based genetic analysis of multiple genetic loci (Table 1).

Reference sequences: GenBank Nos. JQ813697 (ITS), JQ813091 (nuLSU), JQ813185

(mtSSU), JN992705 (MCM7), JQ814045 (RPB1), and JQ813280 (RPB2).

Ecology and distribution: Currently this species is known from the Cline Top Mesa in central Colorado and the Wasatch Front in central Utah where it occurs on *Quercus gambelii* and *Acer grandidentatum*. We anticipate that *M. clairii* could have a broader distribution in western North America than currently known. However, it appears that this taxon is much less common than *M. subolivacea* s. str.

Discussion: *Melanohalea clairii* belongs to a closely related group of species that also includes the isidiate taxon *M. mexicana* and fertile species *M. subolivacea* (Fig. 2; supplementary Fig. S1). In our phylogenetic reconstruction, *M. clairii* was recovered as sister to a weakly supported clade comprised of *M. subolivacea* s. str. and *M.*

mexicana, with *M. mexicana* nested within *M. subolivacea* s. str. *Melanohalea mexicana* can easily be distinguished from the *M. subolivacea* and *M. clairii* by its production of isidia and its distribution in southern Mexico (states of Puebla, Mexico, and Morelos). *Melanohalea subolivacea* s. lat. is most commonly found in western North America, including populations in Baja California and northern Mexico. Disjunct populations are known from Great Lakes Region of North America and the Canary Islands, but specimens from populations in the Canary Islands have not yet been included in phylogenetic studies. However, currently it appears that no striking ecological or geographic patterns can be used to distinguish the morphologically similar taxon *M. subolivacea* from *M. clairii*. *Melanohalea subolivacea* was described from Los Angeles Co., California, USA (H-NYL 34657), and all fourteen *M. subolivacea* s. lat. specimens collected from California were recovered in the '*M. subolivacea*' clade (supplementary Table S1; supplementary Fig. S1). Therefore, we conclude that *M. subolivacea* specimens comprising the '*M. subolivacea*' clade represent *M. subolivacea* s. str.

Additional specimens examined: **USA:** Utah County, Wasatch Mountains, vicinity of confluence of Sixth Water and Diamond Fork River, Leavitt 1036.FW2, (F), Leavitt 1036.FW5 (F), Leavitt 1036.FW6 (F), Leavitt 1036.FW7 (F); Colorado, Garfield County,

White River National Forest, along the 'Cline Top' Road, Leavitt 10-318 (F), Leavitt 10-319 (F), and Leavitt, 10-321 (F).

Melanohalea columbiana S. Leavitt, Essl., Divakar, A. Crespo & Lumbsch, sp. nov.

MYCOBANK #817312

Etymology: The new taxon is named based on its occurrence in the Columbia River Drainage and Columbian Plateau.

Type: USA: Washington, Whitman Co.: Rock Creek drainage in the Palouse Tract of the Channeled Scablands, in the Rock Creek/Escure Ranch recreation area.

Specimens collected in riparian habitat in vicinity of Towell Falls on Rock Creek and found growing on *Crataegus* sp. Lat. 46.9826° N; Lon. -117.9292° W; alt. ca. 420 m.a.s.l., 02 August 2010, S. Leavitt 10-003a (BRY-C – holotype; F – isotype).

Diagnosis: Morphologically similar to *M. multispora* s. str. (see Esslinger, 1977), but consists of specimens recovered within clade '*M. multispora* 1' (Leavitt et al., 2013a).

Melanohalea columbiana can consistently be distinguished from other species in the 'multispora' group (*M. columbiana*, *M. multispora* s. str., *M. tahltan*, and *M. trabeculata*) by fixed differences in nuclear ribosomal ITS sequence data (Table 3). *Melanohalea columbiana* is also supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of multiple genetic loci (Table 1).

Reference sequences. GenBank Nos.: JQ813562 (ITS), JQ813045 (nuLSU), JQ813152 (mtSSU), JN992697 (MCM7), JQ814008 (RPB1), JQ813240 (RPB2).

Ecology and distribution: The species occurs on riparian shrubs in the Columbian Plateau. Currently known from seven sites in western North America, including sites along the Salmon River, Idaho; in the Cheney-Palouse Scablands, Washington; along the Columbia River, Central Oregon, and a single site in the Peninsular Ranges in southern California.

Discussion: The 'multispora' group – *M. columbiana*, *M. multispora* s. str., *M. tahltan*, and *M. trabeculata* (Ahti) O. Blanco et al. – represents another relatively old species

group, with the initial split of *M. columbiana* from *M. multispora*+*M. tahlta*+*M. trabeculata* estimated to have occurred during the Miocene (Leavitt et al., 2012a). All species within this group are known exclusively from western North America. Additional studies will be required to evaluate if consistent, diagnostic phenotypic characters can be identified to effectively identify species in this group without using molecular sequence data. A specimen identified as *M. multispora* s. lat. was collected from near the type locality of *M. multispora* in Bonner Co., Idaho, USA. In phylogenetic analyses, this specimen was recovered within clade '*M. multispora* 3' (Leavitt et al., 2013a); and based on this evidence we assume that clade '*M. multispora* 3' (Leavitt et al., 2013a) represents *M. multispora* s. str.

Additional specimens examined: **USA:** California, Riverside Co., Lendemer 11408 (NY); Idaho, Idaho Co., ca. 3.2 km by air NE of Pittsburgh Landing on the Snake River, Esslinger 18757 (TLE); Idaho, Idaho Co., ca. 25 km north of Riggins, along the Salmon River near the Slate Creek Recreation Area, Esslinger 18783 (TLE); Oregon, Wheeler Co., Umatilla Nat. Forest, along Hwy 207 ca. 35.2 km S of Hardman, Esslinger 16555 (TLE); Washington, Grant Co., vicinity of Grand Coulee Dam, at electrical transfer station, in *Artemisia tridentata*-dominated shrub community, ca. 0.5 km E of Grand Coulee, Leavitt 10.site36.01 (F), Leavitt 10.site36.02 (F), Leavitt 10.site36.03 (F); Washington, Douglas Co., along Columbia River, SE of Brewster on Hwy 173, vic. of Wells Wildlife Area, Leavitt 10.site35.01 (F); Washington, Klickitat Co., along Hwy 14, in Columbia River Gorge, in oak woodland, ca. 9 km east of Murdock, Leavitt 10.site12.01 (F), Leavitt 10.site12.02 (F), Leavitt 10.site12.03 (F), Leavitt 10.site12.04 (F); Washington, Whitman Co., Leavitt 10-003b (F).

Melanohalea davidii S. Leavitt, Essl., Divakar, A. Crespo & Lumbsch, sp. nov.

MYCOBANK #817324

Etymology: This new taxon is named in honor of Professor David Leslie Hawksworth.

Type: Spain, Cadiz, Sierra de Grazalema. Epiphyte on *Pinus pinaster*, alt. ca. 100

m.a.s.l., 30 January 2002, A. Crespo 7636 (MAF-Lich - holotype).

Diagnosis: Morphologically similar to *M. exasperata* s. str. (see Esslinger, 1977), but consists of specimens recovered within clade '*M. exasperata* SPAIN' (Leavitt et al., 2013a). *Melanohalea davidii* can be distinguished from *M. exasperata* s. str. by fixed differences in nuclear ribosomal ITS sequence data (Table 3) and is supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of multiple genetic loci (Table 1).

Reference sequences: GenBank Nos. JQ813416 (ITS), JQ813014 (nuLSU), JQ813127 (mtSSU), JQ813910 (MCM7), JQ813994 (RPB1), JQ813213 (RPB2).

Ecology and distribution: This taxon is currently known from only two mid-elevation (1100–1500 m.a.s.l.) localities in Spain: Province of Guadalajara (Selas) and Province of Cádiz (Sierra de Grazalema) where it occurs on *Quercus* and *Pinus*.

Discussion: Morphologically, this taxon is most similar to *M. exasperata*, although based on current phylogenetic reconstructions it appears to be more closely related to *M. elegantula*. However, relationships among *M. davidii* and other closely related *Melanohalea* taxa generally have weak to moderate statistical support, and additional data will be required to confirm the closest relative to this species. Furthermore, *M. exasperata* populations in North America have not yet been sampled for molecular phylogenetic studies, and the relationship of these New World populations in relation to *M. davidii* and *M. exasperata* s. str. remain unknown. *Melanohalea davidii* is known only from two populations in Spain. Pleistocene glacial refugia in the Iberian Peninsula are well documented for a number of organisms (reviewed in Feliner, 2011), and it is possible that *M. davidii* is also a relict species. However, additional sampling of *M. exasperata* s. lat. populations will be required to better understand the actual extent of the distribution of *M. davidii*. In addition, population demographic analyses may also help to elucidate the potential impact of Pleistocene glacial cycles may have had on this taxon. The type of *M. exasperata* is from Italy and since most of the *M. exasperata* samples from Europe (Greece, Norway, The Netherlands, Sweden, UK, Estonia, and

Spain) are clustered in '*M. exasperata*' clade (Leavitt et al., 2013a), and we consider the '*M. exasperata*' clade *sensu* Leavitt et al. (2013a) as *M. exasperata* s. str.

Additional specimens examined: **Spain:** Guadalajara, Selas (10214, MAF-Lich).

Melanohalea mexicana Essl. & R. Pérez

MYCOBANK #548513

Type: Mexico: Puebla, SW side of Pico de Orizaba, along side road from Ciudad Serdán to Tlacotengo, Lat. 18.9942° N; Lon. -97.2972° W; alt. ca. 3940 m.a.s.l., *T.H. Nash 46034* (ASU – holotype; MEXU, DUKE, TLE – isotypes).

Comments: Here we provide reference sequences generated from the holotype of *M. mexicana*.

Reference sequences: GenBank Nos. KX349620 (ITS), KX349666 (MCM7), and KX349705 (RPB2).

Phylogenetic notes: *Melanohalea mexicana* was recovered as polyphyletic and nested within *M. subolivacea* s. str. Specimens collected from the state of Puebla, Mexico, including the type collection, were recovered within a single well-supported lineage within the *M. subolivacea* s. str. clade. However, a single *M. mexicana* specimen collected from the state of Morelos, Mexico (Perez-Perez & Granados 3731 [HEMIM]) was not recovered within the well-supported clade containing the specimens from Puebla (supplementary Fig. S1). However, the specimen from Morelos differed from other *M. mexicana* specimens at only two fixed nucleotide position characters in the ITS alignment, and *M. mexicana* was supported with high speciation probability inferred from multiple loci (BP&P speciation probability = 1.0, this study).

Discussion: see discussion under *Melanohalea clairii*.

Melanohalea olivaceoides (Krog) O. Blanco et al. (2004)

MYCOBANK #488726.

Type: *Parmelia olivaceoides* Krog, Norsk Polarinstitut Skrifter 144: 109 (1968). Alaska. Central Yukon River District: Steese Highway, Circle Hot Springs, Krog 2552 (O, holotype).

Epitype (**designated here**): **USA**: Alaska, Eastern Alaska, ca. 32 km west of Tok along the Alaska Highway (Hwy 2) in the Cathedral Creeks area, near Cathedral Rapids; Lat. 63.3859° N, Lon. -143.7376° W, alt. ca. 466 m.a.s.l., T.L. Esslinger 19440 (F).

Comments: A full, phenotypical description of *M. olivaceoides* s. str. is found in Esslinger (1977). In phylogenetic analyses, this taxon consists of specimens recovered within clade '*M. olivaceoides* 1' (Leavitt et al., 2013a). Currently, *M. olivaceoides* can only be distinguished from *M. beringiana* by fixed differences in nuclear ribosomal ITS sequence data (Table 3) and is supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of multiple genetic loci (Table 1).

Reference sequence: GenBank Nos. JQ813641 (ITS).

Ecology and distribution: boreal-austral American bipolar distribution; on bark or rarely on rock.

Discussion: We believe that the interests of nomenclatural stability are best served by epitypifying *M. olivaceoides* against a sequenced specimen of known identity that has been collected from near the type locality. We take this step in full agreement with Ariyawansa et al. (2014) that epitypification should not be undertaken except in cases where it can be shown that the holotype in question is demonstrably ambiguous (Jørgensen 2014). Careful comparisons between the types of *M. olivaceoides* and *M. beringiana* failed to reveal any consistent characters separating these two taxa. Furthermore, we were unable to extract DNA from the type representing *M. olivaceoides*. Therefore, given that fixed differences in DNA sequence data are the only characters that can consistently diagnose these morphologically similar taxa and the ambiguity of these characters in the type of *M. olivaceoides*, we propose an epitype here.

Melanohalea tahltan S. Leavitt, Essl., Divakar, A. Crespo & Lumbsch, sp. nov.

MYCOBANK #817328

Etymology: This species is named in honor of the indigenous Tahltan people residing in northern portions of the Canadian province British Columbia.

Type: Canada: British Columbia, along the Cassiar Highway (Hwy. 37), ca. 18 km. south of Kinaskan Lake, at rest area in spruce and lodge pole pine forest. Epiphyte on *Salix* sp. Lat. 57.3801° N; Lon. 130.2462° W; alt. ca. 770 m.a.s.l., 23 June 2011, T.L. Esslinger 20166 (F -holotype).

Diagnosis: Morphologically similar to *M. multispora* s. str. (see Esslinger, 1977), but consists of specimens recovered within clade '*M. multispora* 2' (Leavitt et al., 2013a).

Melanohalea tahltan can be distinguished from other species in the 'multispora' group (*M. columbiana*, *M. multispora* s. str., *M. tahltan*, and *M. trabeculata*) by fixed differences in nuclear ribosomal ITS sequence data (Table 3). *Melanohalea tahltan* is also supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of multiple genetic loci (Table 1).

Reference sequences: GenBank Nos. JQ813575 (ITS), JQ813051 (nuLSU), JQ813158 (mtSSU), JQ813937 (MCM7), JQ814012 (RPB1), and JQ813245 (RPB2).

Ecology and distribution: This corticolous taxon is currently only known from two specimens collected in British Columbia, Canada. One specimen originated in the Klappan Region of northwestern British Columbia and the other from the Thompson Plateau in southern British Columbia.

Discussion: The split between *M. tahltan* and *M. multispora* represents the most recent split between species in the 'multispora' group, estimated to have occurred during the early Pliocene, ca. 5 Ma (Leavitt et al., 2012a). However, similar to other morphologically cryptic *M. multispora* s. lat. species (*M. beringiana* and *M. multispora*

s. str.) diagnostic phenotypic characters separating these taxa have not been observed. See discussion under *Melanohalea beringiana* for additional details.

Additional specimens examined: **Canada:** British Columbia, Thompson Plateau, Opax/Mud Lake Sylviculture Research Forest, Bjork 13320 (UBC).

Montanelia occulti panniformis S. Leavitt, Essl., Divakar, A. Crespo & Lumbsch, sp. nov.

MYCOBANK #817329

Etymology: From Latin *occultus*, secret or hidden, and the species epithet

“*panniformis*”, meaning hidden with the nominal taxon *Montanelia panniformis*.

Type: Russia: Khabarovskiy Krai, Bureinskiy Zapovednik, upper reach of the Pravaya Bureya River, Tsarskaya Doroga. Lat. 52.1601° N, Lon. 134.3283° E, alt. ca. 901 m.a.s.l., lignicolous on log in talus, T. Spribille 31832 (GZU - holotype).

Diagnosis: This taxon contains specimens morphologically similar to *M. panniformis* (Esslinger, 1977) and consists of specimens recovered within clade ‘*M. ‘panniformis* A’ (Fig. 2; in Leavitt et al., 2015a). *Melanelixia occulti panniformis* can be distinguished from *M. panniformis* s. str. by fixed differences in nuclear ribosomal ITS sequence data (Table 4). *Montanelia occulti panniformis* is also supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of multiple genetic loci (Table 1).

Reference sequences: GenBank Nos.: KM386100 (ITS), KM386162 (nuLSU), KM386258 (mtSSU), KM386206 (MCM7), KM386301 (RPB1), and KM386141 (RPB2).

Ecology and distribution: This new species is known only from two disjunct populations: Interior Alaska, Denali National Park, Spribille 31832 (GZU); and the Russian Far East, Khabarovskiy Krai, Spribille 27965 (GZU). The specimen from Denali National Park was collected on rock, while the one from Khabarovskiy Krai, Russia occurred on lignum.

Discussion: No apparent morphological, chemical or geographical characters have been observed which can consistently be applied to distinguish *M. panniformis* from *M. occulti panniformis*, although the two sampled specimens are at one end of the range of variation for what is traditionally considered *M. panniformis*, having very narrow, elongate and regular isidioid lobules. *Montanelia panniformis* s. str. was first described from a collection made in Sweden (Esslinger, 1977). In this study, a specimen (Wedin 8285 [S]) with all characteristic features of *M. panniformis* collected in Sweden, the country of origin for the holotype of *M. panniformis*, was included. This specimen was recovered in a clade with the majority of other *M. panniformis* specimens, and we interpret this clade to represent *M. panniformis* s. str. (Fig. 2). A full, phenotypical description of *M. panniformis* s. str. is found in Esslinger (1977). *Montanelia occulti panniformis* is currently known only from two disjunct populations in Interior Alaska, USA and the Russian Far East, respectively. *Montanelia occulti panniformis* is chemically identical to *M. panniformis* s. str., both producing perlatolic and stenosporic acids. Additional sampling of other similar *M. panniformis* s. lat. specimens will be required to assess whether these phenotypic characters can be used to differentiate *M. occulti panniformis* from *M. panniformis* s. str. Populations from the mountains of Venezuela and central Chile have not yet been included in molecular phylogenetic studies, and their relationship to these two taxa is unknown.

Additional specimens examined: **USA:** Alaska, Denali National Park, Mt. Healy Trail, Spribille 27965 (GZU).

Montanelia saximontana (R. Anderson & W. Weber) S. Leavitt, Essl. Divakar, A. Crespo & Lumbsch, comb. nov.

MYCOBANK #817331

Basionym: *Parmelia saximontana* R. Anderson & W. Weber, Bryologist 65: 236. 1962.

Type: USA. Colorado. Boulder Co.: just NW of Boulder, Anderson L-30072 & Weber, Lich. Exs. Colo. 41 (ASU, DUKE, MIN, WIS - isotypes).

Discussion: *Montanelia saximontana* can be distinguished from *M. tominii* s. str. by fixed differences in nuclear ribosomal ITS sequence data (Table 4) and in having restricted distribution to western North America. *Montanelia saximontana* is also supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of multiple genetic loci (Table 1). Otherwise, no apparent phenotypic characters have been observed which can consistently be applied to distinguish *M. saximontana* from Asian specimens representing *M. tominii* s. lat. Based on our limited sampling, it appears that *M. tominii* s. str. does not occur in western North America; and soresiate forms formerly recognized as *M. tominii* in this region belong to *M. saximontana*. All abundantly soresiate forms from North America are morphologically indistinguishable from the original type specimens of *M. saximontana*. *Montanelia tominii* s. lat. populations in Europe have not yet been included in molecular studies, and their placement within the *M. tominii* complex remains uncertain. North American specimens representing typical soresiate *M. tominii* s. lat. (Esslinger, 1977) are an evolutionarily independent lineage, distinct from those occurring in Asia (Leavitt et al., 2015a). *Montanelia saximontana* is represented by specimens recovered within clade 'M. 'tominii A' in Leavitt et al. (2015a).

Specimens examined: **Canada:** British Columbia, along the Cariboo Highway (Hwy. 97) ca. 8 miles east of town of Williams Lake, Esslinger 20241 (TLE), along the Trans-Canada Highway (Hwy. 16) ca. 65 miles west of Fort Fraser, Esslinger 20210 (TLE); **USA:** California, San Diego Co., Cleveland National Forest, south of Wooded Hill, Lendemer 4494 (NY), Tuolumne Co., Yosemite National Park, Mammoth Peak overlook on Tioga Pass, Lendemer 19701 (NY); Yosemite National Park, Tuolumne River watershed, Gaylor Basin area 8, Hutten 14162 (YOSE); Montana, Park Co., along West Boulder River Road, W of McLeod, Esslinger 16627 (TLE); New Mexico, Cantron Co., Gila Wilderness Area, along Indian Creek Trail, St. Clair 32307 (BRY-C), and Utah, Wayne Co., northwest of Boulder Mountain, Leavitt 1022 (BRY-C).

Montanelia secwepemc S. Leavitt, Essl. Divakar, A. Crespo & Lumbsch, sp. nov.

MYCOBANK #817330

Etymology: This species is named after the Secwepemc people of British Columbia, Canada.

Type: Canada: British Columbia, along the Trans-Canada Highway (High. 16), ca. 10 km west of Fort Fraser; Lat. 54.0576° N; Lon. -124.6964° W, elev. ca. 738 m.a.s.l.; south-facing rock boulder beside highway; on rock; 07 July 2011, T.L. Esslinger 20208 (F – holotype; TLE – isotype).

Diagnosis: This taxon contains esorediate specimens that are otherwise morphologically similar to *M. tominii* (Esslinger, 1977) and consists of specimens recovered within clade '*M. 'tominii* C' in Fig. 2 in Leavitt et al. (2015a). *Montanelia secwepemc* can be distinguished from other lineages in the *M. tominii* complex by an esorediate upper surface, North American distribution, and fixed differences in nuclear ribosomal ITS sequence data (Table 4). *Montanelia secwepemc* is also supported as a lineage distinct from all other populations according to coalescent-based genetic analysis of multiple genetic loci (Table 1). Based on the current sampling, it appears that *M. secwepemc* can be distinguished morphologically from the morphologically similar North American species, *M. saximontana*, by the absence of soralia in the former.

Reference sequences: GenBank Nos.: KF257995 (ITS), KM386180 (nuLSU), KM386273 (mtSSU), KM386231 (MCM7), KM386311 (RPB1), KM386125 (RPB2).

Ecology and distribution: This taxon is currently known only from a total of four sites in western North America: a single site in the Canadian province of British Columbia, two sites in the Yukon Territory, and one site in Alaska, USA (supplementary Table S1). All sampled specimens occurred on rock.

Discussion: Molecular data support the potential for up to five species-level lineages within *M. tominii* s. lat. (Leavitt et al., 2015a). Based on our current sampling, three putative species in the *M. tominii* complex appear to be restricted to Asia (*M. aff.*

tominii, *M. aff. tominii* 'B', and *M. aff. tominii* 'E'), while the other two appear to occur exclusively in North America (*M. secwepemc* and *M. saximontana*) (Fig. 2). The initial diversification of the *M. tominii* species complex is estimated to have begun during the late Miocene and continued into the Pleistocene (Leavitt et al., 2015a). In our phylogenetic analyses the two North American species, *M. secwepemc* and *M. saximontana*, were not recovered within a monophyletic clade. Rather, each species was sister to a different Asian lineage (Fig. 2). While striking biogeographic patterns were observed in the *M. tominii* complex, we note that three other *Montanelia* species – *M. disjuncta*, *M. panniformis* s. str., and *M. solediata* – have broad, intercontinental distributions with no evidence of phylogeographic substructure (Leavitt et al., 2015a).

Montanelia tominii s. lat. specimens generally produce soredia (Esslinger, 1992), vegetative reproductive propagules containing both fungal hyphae and algal cells, including one of the two North American species within the *Montanelia tominii* complex, *M. saximontana*. In contrast, all specimens representing *M. secwepemc*, the other North American species within this complex, were found to be esorediate. Based on the current sampling, the production of soredia serves as a diagnostic character for distinguishing the two *M. tominii* s. lat. OTUs occurring in North America. Additional sampling will be required to confirm that this is a consistent diagnostic feature separating *M. secwepemc* from *M. saximontana*.

Of the Asian lineages, only '*M. aff. tominii*' is represented by multiple individuals – two from the state of Uttaranchal, India and two from Inner Mongolia, China. The other candidate species were both represented by single specimens collected from the Heilongjiang Province in northeastern China. The type specimen for *M. tominii* was collected in the southeastern Russian province Chita (Esslinger, 1992). Currently, we are assuming that the most commonly sampled '*M. aff. tominii*' lineage from Asia corresponds to *M. tominii* s. str.; however, additional sampling throughout Asia will be required to adequately assess which Asian *M. tominii* s. lat. clade represents this taxon.

Additional specimens examined: **Canada:** British Columbia, along the Trans-Canada Highway (Hwy. 16) ca. 6.5 miles west of Fort Fraser, Esslinger 20208 (TLE); Yukon Territory, ca. 1-2 miles south of Whitehorse, at Miles Canyon, along the lower trail, Esslinger 20058 (TLE); along Top of the World Highway (Hwy 9) ca. 38 miles east of the Alaska border, Esslinger 19351 (TLE). **USA:** Alaska, near overlook above Liberty Falls, west of the Copper River, McCune 30793 (TLE).

Conclusions

In this study we described a total of eleven new species and resurrected two species, based in part on DNA sequence characters and coalescent-based species delimitation probabilities for taxonomic diagnoses. Although a number of new taxa are described primarily based on evidence from genetic data, it does not preclude the possibility that additional studies investigating morphological and chemical characters may identify additional independent characters, or combinations of characters, supporting the species circumscribed using molecular data. In fact, under the GLC (de Queiroz, 2007), more independent properties providing evidence supporting putative species boundaries are associated with a higher degree of corroboration, and can result in a more integrative approach to species discovery (Dayrat, 2005). However, in the meantime, we consider that these new species represent robust working hypothesis of boundaries among independently evolving metapopulation lineages (Fujita et al., 2012). Recent studies of *Melanelixia glabra* s. lat. and *M. fuliginosa* s. lat. afford insightful examples of integrating different lines of evidence (e.g., ecological, morphology, and genetic) to robustly delimit species boundaries in brown parmelioid lichens (Arup and Sandler Berlin, 2011; Divakar et al., 2010).

The vast majority of species within the *Melanohalea* clade were recovered as well-supported, monophyletic lineages, with a few notable exceptions (Fig. 2). The general pattern of monophyly, genealogical congruence among independent loci, and Miocene- and Pliocene-dominated diversification history supports the conclusion that

most brown parmelioid species, including the new species described here, are well-differentiated within an evolutionary context (Divakar et al., 2012; Leavitt et al., 2012a, 2012b; Leavitt et al., 2013a; Leavitt et al., 2015a).

One notable exception, the *M. subolivacea* group, is comprised of three closely related species, *M. clairii* sp. nov., *M. mexicana*, and *M. subolivacea* s. str. It appears that these species represent a recent diversification event, and incomplete lineage sorting in *M. mexicana* and *M. subolivacea* results in the polyphyly of these taxa (supplementary Fig. S1). Although these species are not recognizable under a phylogenetic species recognition criterion, all three species within the *M. subolivacea* were validated as evolutionarily distinct lineages as modeled under the multispecies coalescent.

In the genus *Montanelia*, the morphologically distinct species *M. predisjuncta* appears to be closely related, or even conspecific, with *M. disjuncta*. However, given the morphological differences between these two taxa (see Esslinger 1977), it is conceivable that *M. predisjuncta* represents a recent split from *M. disjuncta* that is not identifiable in multilocus phylogenetic reconstructions. However, at this point only a single specimen representing *M. predisjuncta* collected at the type locality in central Japan has been included, and a broader collection size will be required to evaluate the evolutionary independence of these species.

Apart from the brown parmelioid species that have not yet been included in molecular phylogenies (see section 2.1), a number of groups still require taxonomic revision, including the '*Melanelixia albertana*' clade and the *Montanelia tominii* s. lat. group.

In contrast to most *Montanelia* species, previous data reveals the potential for striking biogeographic patterns in the *Montanelia tominii* group (Leavitt et al., 2015a). However, *M. tominii* s. lat. populations throughout this nominal taxon's distribution remain sparsely sampled. Additional sampling of Asian populations will be required in

order to ascertain which species-level clade represents *M. tominii* s. str. and robustly elucidate species' distributions and biogeography in this complex.

The species-level clade '*M. albertana*' was comprised of specimens morphologically similar to *M. albertana* (Hai-ying Wang 20084069, SDNU [China]; Urbanavithus 15554, MAF-Lich [Russia, Southern Siberia]; Esslinger 20491A, TLE [USA]); Esslinger 20476, TLE [USA]; Esslinger 20476, TLE [USA]), *M. glabra* (Spribille 23949, GZU [Russian Far East]; Spribille 23677, GZU [Russian Far East]; Hai-ying Wang 20084060, SDNU [China]), *M. aff. glabroides* (Divakar, BCN-Lich 15661 [India]), and *M. aff. villosella* (Yuan-da Du 20071596-1, SDNU [China]). Based on current sampling and the species boundaries delimited in this study, specimens representing this polymorphic species-level clade are known from Asia (China, India, and Russia) and upper Great Plains regions of North America. *Montanelia albertana* specimens from Asia were generally collected at high elevation sites, between 1410 and 3100 m.a.s.l., with the exception of the two collections from lower elevation sites in Far East, Russia. In contrast, all North American specimens were collected from lower elevation sites (<500 m.a.s.l.). We await additional sequence data from a broader sampling of species within this clade before proposing any formal taxonomic changes for this group.

As molecular sequence data become more readily available across previously understudied organismal groups, the effective incorporation of robust results from empirical species delimitation based on genetic data into formal taxonomic descriptions will most likely continue to play an increasing role. We anticipate that species descriptions based on genetic data will become increasingly important and routinely applied. Other disciplines such as ecology, conservation and physiology will benefit from formal recognition of species-level lineages inferred from molecular data (Pante et al., 2015; Hibbet 2016, Renner 2016). In conclusion, we hope that our formal taxonomic treatment of morphologically cryptic lineages in brown parmelioids (Parmeliaceae) will inspire additional research of these taxa.

Acknowledgements

This study was motivated, in part, by David Hawksworth and his tremendous contributions to fungal systematics. We express appreciation to anonymous reviewers, whose comments greatly improved this manuscript. We sincerely thank generous colleagues for providing brown parmelioid specimens collected from all over the world. Daniel Le (Field Museum) is gratefully acknowledge for his help photographing specimens. This work was financially supported by the National Science Foundation ('Hidden diversity in parmelioid lichens'; DEB-0949147) and the Spanish Ministerio de Ciencia e Innovación (CGL2013-42498-P) projects.

References

- Ahti, T., 1966. *Parmelia olivacea* and the allied non-isidiate and non-sorediate corticolous lichens in the northern hemisphere. *Acta Botanica Fennica* 70, 1-68.
- Ariyawansa, H. A., D. L. Hawksworth, K. D. Hyde, E. B. G. Jones, S. S. N. Maharachchikumbura, D. S. Manamgoda, K. M. Thambugala, D. Udayanga, E. Camporesi, A. Daranagama, R. Jayawardena *et al.*, 2014. Epitypification and neotypification: guidelines with appropriate and inappropriate examples. *Fungal Diversity* 69, 57-91.
- Arup, U., Sandler Berlin, E., 2011. A taxonomic study of *Melanelixia fuliginosa* in Europe. *The Lichenologist* 43, 89-97.
- Blanco, O., Crespo, A., Divakar, P.K., Esslinger, T.L., Hawksworth, D.L., Thorsten Lumbsch, H., 2004. *Melanelixia* and *Melanohalea*, two new genera segregated from *Melanelia* (Parmeliaceae) based on molecular and morphological data. *Mycological Research* 108, 873-884.
- Camargo, A., Morando, M., Avila, L.J., Sites, J.W., 2012. Species delimitation with ABC and other coalescent-based methods: A test of accuracy with simulations and an empirical example with lizards of the *Liolaemus darwini* complex (Squamata: Liolaemidae). *Evolution* 66, 2834-2849.
- Camargo, A., Sites, J.W., 2013. Species Delimitation: A Decade After the Renaissance, in: Pavlinov, I. (Ed.), *The Species Problem - Ongoing Issues*. InTech.
- Costello, M.J., May, R.M., Stork, N.E., 2013. Can we name earth's species before they go extinct? *Science* 339, 413-416.
- Crespo, A., Kauff, F., Divakar, P.K., del Prado, R., Perez-Ortega, S., Amo de Paz, G., Ferencova, Z., Blanco, O., Roca-Valiente, B., Nunez-Zapata, J., Cubas, P., Arguello, A., Elix, J.A., Esslinger, T.L., Hawksworth, D.L., Millanes, A., Molina, M.C., Wedin, M., Ahti, T., Aptroot, A., et al., 2010. Phylogenetic generic classification of parmelioid lichens (Parmeliaceae, Ascomycota) based on molecular, morphological and chemical evidence. *Taxon* 59, 1735-1753.
- Crespo, A., Lumbsch, H.T., 2010. Cryptic species in lichen-forming fungi. *IMA Fungus* 1, 167-170.
- Crespo, A., Pérez-Ortega, S., 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(S1), 71-81.
- 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. Oxford University Press, Oxford.
- de Queiroz, K., 1999. The general lineage concept of species and the defining properties of the species category., in: Wilson, R.A. (Ed.), *Species: New interdisciplinary essays*. MIT Press, Cambridge, Massachusetts, pp. 49-89.
- de Queiroz, K., 2007. Species Concepts and Species Delimitation. *Systematic Biology* 56, 879-886.
- Divakar, P.K., Crespo, A., 2015. Molecular phylogenetic and phylogenomic approaches in studies of lichen systematics and evolution, in: Upreti, D.K., Divakar, P.K., Shukla, V., Bajpai, R. (Eds.), *Recent Advances in Lichenology*. Springer India, India, pp. 45-60.
- Divakar, P.K., Crespo, A., Wedin, M., Leavitt, S.D., Hawksworth, D.L., Myllys, L., McCune, B., Randle, T., Bjerke, J.W., Ohmura, Y., Schmitt, I., Boluda, C.G., Alors, D., Roca-Valiente, B., Del-Prado, R., Ruibal, C., Buaruang, K., Núñez-Zapata, J., Amo de Paz, G., Rico, V.J., Molina, M.C., Elix, J.A., Esslinger, T.L., Tronstad, I.K.K., Lindgren, H., Ertz, D., Gueidan, C., Saag, L., Mark, K., Singh, G., Dal Grande, F., Parnmen, S., Beck, A., Benatti, M.N., Blanchon, D., Candan, M.,

- Clerc, P., Goward, T., Grube, M., Hodkinson, B.P., Hur, J.-S., Kantvilas, G., Kirika, P.M., Lendemer, J., Mattsson, J.-E., Messuti, M.I., Miadlikowska, J., Nelsen, M., Ohlson, J.I., Pérez-Ortega, S., Saag, A., Sipman, H.J.M., Sohrabi, M., Thell, A., Thor, G., Truong, C., Yahr, R., Upreti, D.K., Cubas, P., Lumbsch, H.T., 2015a. Evolution of complex symbiotic relationships in a morphologically derived family of lichen-forming fungi. *New Phytologist*, 208, 1217-1226.
- Divakar, P.K., Del-Prado, R., Lumbsch, H.T., Wedin, M., Esslinger, T.L., Leavitt, S.D., Crespo, A., 2012. Diversification of the newly recognized lichen-forming fungal lineage *Montanelia* (Parmeliaceae, Ascomycota) and its relation to key geological and climatic events. *American Journal of Botany* 99, 2014-2026.
- Divakar, P.K., Figueras, G., Hladun, N., Crespo, A., 2010. Molecular phylogenetic studies reveal an undescribed species within the North American concept of *Melanelixia glabra* (Parmeliaceae). *Fungal Diversity* 42, 47-55.
- Divakar, P.K., Leavitt, S.D., Molina, M.C., Del-Prado, R., Lumbsch, H.T., Crespo, A., 2015b. A DNA barcoding approach for identification of hidden diversity in Parmeliaceae (Ascomycota): *Parmelia* sensu stricto as a case study. *Botanical Journal of the Linnean Society*, n/a-n/a.
- Divakar, P.K., Upreti, D.K., 2005. A new species in *Melanohalea* (Parmeliaceae, Ascomycotina) and new lichen records from India. *The Lichenologist* 37, 511-517.
- Dowton, M., Meiklejohn, K., Cameron, S.L., Wallman, J., 2014. A preliminary framework for DNA barcoding, incorporating the multispecies coalescent. *Systematic Biology*.
- Edwards, S.V., 2009. Is a new and general theory of molecular systematics emerging? *Evolution* 63, 1-19.
- Esslinger, T.L., 1977. A chemosystematic revision of the brown Parmeliae. *Journal of Hattori Botanical Laboratory* 42, 1-211.
- Esslinger, T.L., 1978. A new status for brown Parmeliae. *Mycotaxon* 7, 45-54.
- Esslinger, T.L., 1992. The brown *Parmelia* type specimens of A. N. Oxner. *The Lichenologist* 24, 13-20.
- Falk, B.G., Glor, R.E., Perkins, S.L. 2015. Clonal reproduction shapes evolution in the lizard malaria parasite *Plasmodium floridense*. *Evolution* 69, 1584-1596.
- Feliner, G.N., 2011. Southern European glacial refugia: A tale of tales. *Taxon* 60, 365-372.
- Félix, M.-A., Braendle, C., Cutter, A.D., 2014. A streamlined system for species diagnosis in *Caenorhabditis* (Nematoda: Rhabditidae) with name designations for 15 distinct biological species. *PLoS ONE* 9, e94723.
- Fujita, M.K., Leaché, A.D., Burbrink, F.T., McGuire, J.A., Moritz, C., 2012. Coalescent-based species delimitation in an integrative taxonomy. *Trends in Ecology & Evolution* 9, 480–488.
- Hibbet, D., 2016. The invisible dimension of fungal diversity. *Science* 351, 1150-1151.
- Jorger, K., Schrod, M., 2013. How to describe a cryptic species? Practical challenges of molecular taxonomy. *Frontiers in Zoology* 10, 59.
- Jørgensen, P. M., 2014. Notes on the new Example 9 in Article 9.8 of the International Code of Nomenclature for algae, fungi, and plants. *Taxon* 63,132-133.
- Katoh, K., Kuma, K.-i., Toh, H., Miyata, T., 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. *Nucleic Acids Research* 33, 511-518.
- Katoh, K., Toh, H., 2008. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9, 286-298.
- Kraichak, E., Divakar, P.K., Crespo, A., Leavitt, S.D., Nelsen, M.P., Lücking, R., Lumbsch, H.T., 2015. A tale of two hyper-diversities: Diversification dynamics of the two largest families of lichenized fungi. *Scientific Reports* in press.
- Lambert, S.M., Reeder, T.W., Wiens, J.J., 2015. When do species-tree and concatenated estimates disagree? An empirical analysis with higher-level scincid lizard phylogeny. *Molecular Phylogenetics and Evolution* 82, Part A, 146-155.

- Lanfear, R., Calcott, B., Ho, S.Y.W., Guindon, S., 2012. PartitionFinder: Combined Selection of Partitioning Schemes and Substitution Models for Phylogenetic Analyses. *Molecular Biology and Evolution* 29, 1695-1701.
- Leavitt, S., Esslinger, T., Divakar, P., Lumbsch, H., 2012a. Miocene and Pliocene dominated diversification of the lichen-forming fungal genus *Melanohalea* (Parmeliaceae, Ascomycota) and Pleistocene population expansions. *BMC Evolutionary Biology* 12, 176.
- Leavitt, S.D., Divakar, P.K., Ohmura, Y., Wang, L.-s., Esslinger, T.L., Lumbsch, H.T., 2015a. Who's getting around? Assessing species diversity and phylogeography in the widely distributed lichen-forming fungal genus *Montanelia* (Parmeliaceae, Ascomycota). *Molecular Phylogenetics and Evolution* 90, 85-96.
- Leavitt, S.D., Esslinger, T.L., Divakar, P.K., Lumbsch, H.T., 2012b. Miocene divergence, phenotypically cryptic lineages, and contrasting distribution patterns in common lichen-forming fungi (Ascomycota: Parmeliaceae). *Biological Journal of the Linnean Society* 1007, 920–937.
- Leavitt, S.D., Esslinger, T.L., Spribille, T., Divakar, P.K., Lumbsch, H.T., 2013a. Multilocus phylogeny of the lichen-forming fungal genus *Melanohalea* (Parmeliaceae, Ascomycota): Insights on diversity, distributions, and a comparison of species tree and concatenated topologies. *Molecular Phylogenetics and Evolution* 66, 138–152.
- Leavitt, S.D., Fernández-Mendoza, F., Pérez-Ortega, S., Sohrabi, M., Divakar, P.K., Lumbsch, H.T., St. Clair, L.L., 2013b. DNA barcode identification of lichen-forming fungal species in the *Rhizoplaca melanophthalma* species-complex (Lecanorales, Lecanoraceae), including five new species *MycKeys* 7, 1–22.
- Leavitt, S.D., Johnson, L.A., Goward, T., St. Clair, L.L., 2011. Species delimitation in taxonomically difficult lichen-forming fungi: An example from morphologically and chemically diverse *Xanthoparmelia* (Parmeliaceae) in North America. *Molecular Phylogenetics and Evolution* 60, 317-332.
- Leavitt, S.D., Moreau, C.S., Lumbsch, H.T., 2015b. The Dynamic Discipline of Species Delimitation: Progress Toward Effectively Recognizing Species Boundaries in Natural Populations, in: Upreti, D.K., Divakar, P.K., Shukla, V., Bajpai, R. (Eds.), *Recent Advances in Lichenology*. Springer India, pp. 11-44.
- Lumbsch, H.T., Leavitt, S.D., 2011. Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. *Fungal Diversity* 50, 59-72.
- Manel, S., Holderegger, R., 2013. Ten years of landscape genetics. *Trends in Ecology & Evolution* 28, 614-621.
- McCune, B., Altermann, S., 2009. *Letharia gracilis* (Parmeliaceae), a new species from California and Oregon. *The Bryologist* 112, 375-378.
- Otte, V., Esslinger, T.L., Litterski, B., 2005. Global distribution of the European species of the lichen genus *Melanelia* Essl. *Journal of Biogeography* 32, 1221-1241.
- Pante, E., Puillandre, N., Viricel, A., Arnaud-Haond, S., Aurelle, D., Castelin, M., Chenuil, A., Destombe, C., Forcioli, D., Valero, M., Viard, F., Samadi, S., 2015. Species are hypotheses: avoid connectivity assessments based on pillars of sand. *Molecular Ecology* 24, 525-544.
- Printzen, C., 2010. Lichen Systematics: The Role of Morphological and Molecular Data to Reconstruct Phylogenetic Relationships, pp. 233-275.
- Rannala, B., 2015. The art and science of species delimitation. *Current Zoology* 61, 846-853.
- Renner, S. S., 2016. A return to Linnaeus's focus on diagnosis, not description: The use of DNA characters in the formal naming of species. *Systematic Biology*. doi: 10.1093/sysbio/syw032.
- Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A., Chen, W., Consortium, F.B., 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, doi: 10.1073/pnas.1117018109.

- Singh, G., Dal Grande, F., Divakar, P.K., Otte, J., Leavitt, S.D., Szczepanska, K., Crespo, A., Rico, V.J., Aptroot, A., Cáceres, M.E.d.S., Lumbsch, H.T., Schmitt, I., 2015. Coalescent-Based Species Delimitation Approach Uncovers High Cryptic Diversity in the Cosmopolitan Lichen-Forming Fungal Genus *Protoparmelia* (Lecanorales, Ascomycota). *PLoS ONE* 10, e0124625.
- Sites, J.W., Marshall, J.C., 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., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* 57, 758-771.
- Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Systematic Biology* 56, 564-577.
- Thell, A., Crespo, A., Divakar, P.K., Kärnefelt, I., Leavitt, S.D., Lumbsch, H.T., Seaward, M.R.D., 2012. A review of the lichen family Parmeliaceae – history, phylogeny and current taxonomy. *Nordic Journal of Botany* 30, 641-664.
- Tonini, J., Moore, A., Stern, D., Shcheglovitova, M., Ortí, G., 2015. Concatenation and species tree methods exhibit statistically indistinguishable accuracy under a range of simulated conditions. *PLoS Currents* 7, ecurrents.tol.34260cc27551a34527b34124ec34265f36334b34266be.
- Truong, C., Divakar, P.K., Yahr, R., Crespo, A., Clerc, P., 2013. Testing the use of ITS rDNA and protein-coding genes in the generic and species delimitation of the lichen genus *Usnea* (Parmeliaceae, Ascomycota). *Molecular Phylogenetics and Evolution* 68, 357-372.
- Yang, Z., 2015. The BPP program for species tree estimation and species delimitation. *Current Zoology* 61, 854-865.
- Yang, Z., Rannala, B., 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences* 107, 9264-9269.
- Yang, Z., Rannala, B., 2014. Unguided species delimitation using DNA sequence data from multiple loci. *Molecular Biology and Evolution*.
- Zhang, C., Zhang, D.-X., Zhu, T., Yang, Z., 2011. Evaluation of a Bayesian coalescent method of species delimitation. *Systematic Biology* 60, 747-761.
- Zhao, Z.-T., Meng, F.-G., Li, H.-M., Wang, H.-Y., 2009. A new species of *Melanohalea* (Parmeliaceae) from the Tibetan Plateau. *Mycotaxon* 108, 347-352.

Figure Legends

Figure 1. Morphology in the ‘*Melanohalea* clade’: **(A)**, *Melanelixia ahtii* (Leavitt 4306, BRY-C, type); **(B)**, *Melanelixia hawksworthii* (Lumbsch 20101a, F, type); **(C)**, *Melanelixia robertsoniorum* (Crespo et al. 6005 K, MAF-Lich); **(D)**, *Melanohalea beringiana* (Esslinger 19564, F, type); **(E)**, *Melanohalea columbiana* (Leavitt 10-003a, BRY-C, type); **(F)**, *Melanohalea clairii* (Leavitt 10-317, BRY-C, type); **(G)**, *Montanelia* aff. *panniformis* (habit image: Sweden, Västergötland; photo credit: L. Stridvall); and **(H)**, *Montanelia saximontana* (habit image: USA, New Mexico, vic. of Taos; photo credit - J. Hollinger). Scale bar = 5 mm.

Figure 2. The maximum likelihood (ML) tree estimated from a concatenated data set of ribosomal (ITS, IGS, and nrLSU), nuclear protein-coding (MCM7, RPB1, and RPB2), and mitochondrial (mtSSU) markers generated from a total of 55 species/species-level lineages in the ‘*Melanohalea* clade’. Species described in this study are shown in red text; and clades containing proposed new combinations are shown in blue. Maximum likelihood bootstrap values > 50% are indicated at each node; and thickened branches indicate speciation probabilities >0.96 inferred using the program BPP (see text). Note: *Melanohalea mexicana* was also supported with high speciation probability (=1.0), but was recovered as a monophyletic lineage within the ‘*M. subolivacea*’ clade. The complete ML topology is shown in supplementary Figure S1.

Table 1. Summary of supporting information for the eleven new species and two new combinations proposed in this study. Supporting information includes: a summary of available reference phylogenies and previously published provisional names, nodal support in ITS and concatenated topologies, and speciation probabilities inferred under the multispecies coalescent.

New Taxon	Reference phylogeny	nodal support ITS/concatenated	BPP speciation probability
<i>Melanelixia ahtii</i>	'ME_ahtii', Fig. S1 (this study)	99/96	1.0
<i>Melanelixia epilosa</i>	Clade 'B', Fig. 1 (Divakar et al., 2010)	100/98	1.0
<i>Melanelixia hawksworthii</i>	'M. sp. 1', Figs. 1 & 2 (Leavitt et al., 2012b)	100/100	1.0
<i>Melanelixia robertsoniorum</i>	'M. sp. 2', Figs. 1 & 2 (Leavitt et al., 2012b)	99/100	1.0
<i>Melanohalea austroamericana</i>	'M. ushuaiensis Chile', Figs. 1, 2 & 3 (Leavitt et al., 2013a)	100/100	NA
<i>Melanohalea beringiana</i>	'M. olivaceoides 2', Figs. 1, 2 & 3 (Leavitt et al., 2013a).	100/98	0.96-1.0
<i>Melanohalea clairii</i>	'M. subolivacea 1', Figs. 1 & 3 (Leavitt et al., 2013a)	93/100	1.0
<i>Melanohalea columbiana</i>	'M. multispora 1', Figs. 1, 2 & 3 (Leavitt et al., 2013a)	100/100	1.0
<i>Melanohalea davidii</i>	'M. exasperata SPAIN', Figs. 1, 2 & 3 (Leavitt et al., 2013a)	100/100	1.0
<i>Melanohalea tahltn</i>	'M. multispora 2', Figs. 1, 2 & 3 (Leavitt et al., 2013a)	100/100	1.0
<i>Montanelia occulti panniformis</i>	'M. panniformis A', Fig. 2 (Leavitt et al., 2015a)	100/99	1.0
<i>Montanelia saximontana</i>	'MO_saximontana', Fig. S1 (this study)	100/100	1.0
<i>Montanelia secwepemc</i>	'M. tominii C', Fig. 2 (Leavitt et al., 2015a)	100/100	1.0

Table 2. Diagnostic aligned nucleotide position characters in the fungal barcoding marker, ITS, for morphologically similar *Melanelixia* species, based on the alignment in TreeBase (<http://treebase.org/>; ID: S18577). Comparisons of diagnostic ITS characters separating species are made for the 'subargentifera group' (*M. ahtii* and *M. subargentifera*); the 'glabra group' (*M. epilosa* and *M. glabra*); and the 'fuliginosa/subaurifera group' (*M. fuliginosa* s. str., *M. glabratula*, *M. hawksworthii*, *M. robertsoniorum*, and *M. subaurifera*).

Taxon																			
	76	90	108	120	128	136	137	140	155	173	179	189	205	218	417	511	524	543	544
<i>M. ahtii</i>	C	C	T	T	A	G	A	A	A	C	C	T	-	T	C	A	G	C	A
<i>M. subargentifera</i>	T	T	C	C	G	T	C	G	G	A	T	C	C	C	G	G	C	T	-
	90	118	131	136	146	160	210	218	410	524									
<i>M. epilosa</i>	C	C	C	T	T	T	A	-	C	C									
<i>M. glabra</i>	T	T	T	C	C	C	-	T	T	T									
	8	11	26	33	55	76	77	80	82	87	107	128	131	134	135	137	138	139	154
<i>M. fuliginosa</i>	C	A	C	C	A	T	A	C	A	T	A	A	C	C	T	T	C	G	T
<i>M. glabratula</i>	C	A	C	C	R	T	T	-	R	-	R	G	T	C	T	T	C	G	T
<i>M. hawksworthii</i>	T	-	C	G	G	T	A	C	A	-	A	G	T	C	T	T	C	C	T
<i>M. robertsoniorum</i>	C	G	C	C	G	C	A	C	A	-	G	G	C	C	C	C	T	G	C
<i>M. subaurifera</i>	C	G	G	G	G	C	A	T	G	-	R	G	T	T	T	T	C	G	T
	160	164	172	174	176	177	179	185	186	205	209	210	220	286	398	415	417	423	428
<i>M. fuliginosa</i> (continued)	T	G	C	C	T	T	C	-	-	C	A	A	G	C	G	C	A	-	T
<i>M. glabratula</i> (continued)	T	A	C	C	T	C	T	-	-	C	-	-	G	A	Y	C	C	-	C
<i>M. hawksworthii</i> (continued)	T	A	T	T	A	C	T	-	-	C	-	-	G	C	G	C	C	-	C
<i>M. robertsoniorum</i> (continued)	C	A	T	C	T	T	T	-	-	C	-	-	G	C	G	C	A	-	T
<i>M. subaurifera</i> (continued)	T	A	T	C	T	T	T	A	T	T	-	-	A	C	T	T	C	T	G
	444	445	460	461	466	471	486	488	509	513	518	524	528	529	532	540	543	547	
<i>M. fuliginosa</i> (continued)	G	T	G	A	A	T	C	T	C	C	Y	K	G	G	C	R	T	A	
<i>M. glabratula</i> (continued)	G	T	G	A	A	T	T	C	C	C	C	T	A	G	A	A	T	Y	
<i>M. hawksworthii</i> (continued)	G	T	A	G	A	C	T	C	C	C	C	C	A	A	A	A	T	T	
<i>M. robertsoniorum</i>	G	C	G	A	G	T	A	T	C	C	T	T	A	G	A	G	G	T	

(continued)																			
<i>M. subaurifera</i> (continued)	T	Y	G	R	A	T	R	T	T	T	C	G	A	A	A	G	T	T	

Table 3. Diagnostic aligned nucleotide position characters in the fungal barcoding marker, ITS, for morphologically similar *Melanohalea* species, based on the alignment in TreeBase (<http://treebase.org/>; ID: S12364). Comparisons of diagnostic ITS characters separating species are made for the ‘olivaceoides group’ (*M. beringiana* and *M. olivaceoides*); the ‘multispora’ group’ (*M. columbiana*, *M. multispora* s. str., *M. tahltan*, and *M. trabeculata*); the ‘exasperata group’ (*M. davidii* and *M. exasperata* s. str.); and the ‘subolivacea’ group’ (*M. subolivacea* s. str. and *M. clairii*).

Taxon																		
	110	119	121	124	135	144	150	152	154	155	158	159	161	162	184	185	198	309
<i>M. beringiana</i>	T	G	T	C	T	G	T	T	C	G	C	C	-	A	C	A	A	C
<i>M. olivaceoides</i>	G	C	G	T	C	A	C	C	A	A	T	T	A	T	A	C	G	T
	320	345	392	416	417	454	464	465	466	469	471	473	481	485	493	503	507	
<i>M. beringiana</i> (continued)	T	T	T	G	G	T	T	G	G	G	T	T	T	C	C	T	C	
<i>M. olivaceoides</i> (continued)	C	C	C	A	A	C	C	T	-	A	C	C	C	T	A	C	T	
	8	22	36	59	64	76	95	98	103	104	106	109	113	116	118	135	153	154
<i>M. columbiana</i>	C	C	T	Y	T	C	T	G	C	C	C	C	A	C	C	C	C	C
<i>M. multispora</i>	T	T	T	G	T	T	T	G	T	C	Y	C	G	Y	Y	C	Y	M
<i>M. tahltan</i>	?	?	?	?	?	?	?	?	T	A	C	T	G	C	T	T	C	A
<i>M. trabeculata</i>	T	T	C	T	C	T	C	A	T	C	T	C	G	T	C	C	T	A
	169	179	183	191	203	392	441	448	453	472	502	503	506					
<i>M. columbiana</i> (continued)	C	C	C	T	C	C	A	A	A	T	G	T	A					
<i>M. multispora</i> (continued)	C	C	Y	C	T	Y	A	G	T	T	T	C	T					
<i>M. tahltan</i> (continued)	C	T	K	C	T	T	A	G	T	T	T	T	T					
<i>M. trabeculata</i> (continued)	T	C	T	Y	C	C	C	A	T	C	T	T	T					
	108	110	118	119	127	175	184	191	196									
<i>M. davidii</i>	A	T	C	G	C	T	C	T	A									
<i>M. exasperata</i>	G	C	T	C	G	C	T	C	G									
	143	477																
<i>M. clairii</i>	A	C																
<i>M. subolivacea</i>	G	T																

Table 4. Diagnostic aligned nucleotide position characters in the fungal barcoding marker, ITS, for morphologically similar *Montanelia* species, based on the alignment in TreeBase (<http://treebase.org/>; ID: S16237). Comparisons of diagnostic ITS characters are made among species within the 'tominii' group (*M. saximontana*, *M. secwepemc*, '*M. tominii*', '*M. aff. tominii* B', and '*M. aff. tominii* E') and between *M. occulti panniformis* and *M. panniformis*.

Taxon																	
	10	11	23	30	50	68	69	72	75	98	104	114	116	121	122	143	158
<i>M. saximontana</i>	G	A	T	C	-	T	T	C	T	C	C	G	T	T	T	A	C
<i>M. secwepemc</i>	-	-	T	T	T	T	T	T	G	C	C	G	T	T	T	G	T
<i>M. 'tominii'</i>	G	A	T	T	-	C	T	T	T	C	T	A	T	T	T	A	T
<i>M. 'tominii</i> B'	G	A	T	T	-	T	T	T	T	C	C	G	T	C	T	A	T
<i>M. 'tominii</i> E'	G	A	C	T	-	T	G	T	T	T	C	G	C	T	C	A	T
	159	170	171	184	188	207	313	403	436	456	457	476	496	503	504	512	513
<i>M. saximontana</i> (continued)	T	T	A	T	C	C	T	C	T	T	T	T	C	C	A	Y	M
<i>M. secwepemc</i> (continued)	T	T	A	T	T	C	C	C	T	T	C	G	T	C	T	C	A
<i>M. 'tominii'</i> (continued)	C	T	A	T	T	C	C	C	T	C	T	G	C	C	A	C	A
<i>M. 'tominii</i> B' (continued)	T	T	A	T	C	C	C	T	G	T	T	T	C	C	A	C	A
<i>M. 'tominii</i> E' (continued)	T	C	C	C	T	T	C	C	T	C	T	T	C	T	A	T	C
	106	115	117	129	174	192	199	324	454	473	479	481	513				
<i>M. occulti panniformis</i>	C	C	G	C	A	C	T	T	A	G	T	T	C				
<i>M. panniformis</i>	T	T	A	G	G	A	A	C	G	A	C	C	A				

Figure 1

Figure 2