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Assessing the Role of Hybridization in the Evolution of Two Common Lineages of Lichen-Forming Fungi

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ABSTRACT

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Common Lineages of Lichen-Forming Fungi

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Once thought to be an evolutionary dead-end, hybridization is now being detected in genomes across kingdoms, perhaps even playing an integral role in evolution. In chapter 1, I investigated the potential influences of hybridization on the evolution of a group of vagrant, asexual species in the *Rhizoplaca melanophthalma* species group. I sequenced the mitochondrial and nuclear genomes of 55 specimens and found well-supported nuclear phylogenies of both datasets. There were, however, multiple instances of discordance between the mitochondrial and nuclear trees, which can be caused by hybridization. PhyloNet and ABBA-BABA also detected widespread hybridization among this group. In chapter 2, I shifted to the Holarctic clade of lichen-forming fungi in *Xanthoparmelia* to characterize gene tree conflict and investigate the potential for hybrization. Here, I used three different tests for hybridization that account for incomplete lineage sorting – ABBA-BABA, PhyloNet, and SplitsTree – as well as PhyParts to characterize gene tree conflict. Like the *Rhizoplaca* species group, widespread hybridization was detected in the Holarctic clade despite robust phylogenies. My research underscores the value of investigating hybridization when studying species boundaries and evolutionary history.

Keywords: hybridization, lichen, genome evolution, incomplete lineage sorting, introgression, phylogenomics

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Chapter 1

Genome-scale data reveal the role of hybridization in lichen-forming fungi

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Abstract

Advancements in molecular genetics have revealed that hybridization is common among plants, animals, and fungi, playing a role in evolutionary dynamics and speciation. While hybridization has been well-documented in pathogenic fungi, the effects of these processes on speciation in fungal lineages with different life histories and ecological niches are largely unexplored. Here we investigated the potential influence of hybridization on the emergence of morphologically and reproductively distinct asexual lichens. We focus on vagrant forms (growing obligately unattached to substrates) within a clade of rock-dwelling, sexually reproducing species in the *Rhizoplaca melanophthalma* (Lecanoraceae, Ascomycota)

group. We used phylogenomic data from both mitochondrial and nuclear genomes to infer evolutionary relationships and potential patterns of introgression. We were inferred well-supported phylogenies from both the mitochondrial and nuclear datasets. We observed multiple instances of discordance between the mitochondrial and nuclear trees, including the clade comprising the asexual vagrant species *R. arbuscula*, *R. haydenii*, *R. idahoensis,* and a closely related rock-dwelling lineage. Despite well-supported phylogenies, we recovered strong evidence of reticulation using a network approach that incorporates both incomplete lineage sorting and hybridization. These data suggest that the rock-dwelling western North American subalpine endemic *R. shushanii* is potentially the result of a hybrid speciation event, and introgression may have also played a role in other taxa, including vagrant species *R. arbuscula*, *R. haydenii* and *R. idahoensis*. We discuss the potential roles of hybridization in terms of generating asexuality and novel morphological traits in lichens. Furthermore, our results highlight the need for additional study of reticulate phylogenies when investigating species boundaries and evolutionary history, even in cases with well-supported topologies inferred from genome-scale data.

Introduction

While once considered an evolutionary dead end, hybridization is more pervasive than previously assumed, found among plants, fungi, and animals (Chan and Levin 2005, Hedrick 2013b, Hill 2017, Huang 2016, Mallet 2005, Stukenbrock 2016). Neglecting to consider the potential influence of hybridization on speciation and delimiting species boundaries risks oversimplifying evolutionary inferences or may lead to erroneous or conflicting interpretations. Both the extent of the impact of hybridization on speciation and the ability to detect it are highly debated in the scientific community (Abbott et al. 2013, Chapman and Burke 2007, Feliner et al. 2017, Schumer et al. 2014); nevertheless, there remains value in exploring the influences of interspecific gene flow on species as a whole.

Hybridization can play a role in evolutionary dynamics and the transmittance of adaptive variation (Tigano and Friesen 2016), potentially leading to adaptation to new niches or habitats (Rieseberg et al. 2003) and the introduction of novel phenotypes (Gladieux et al. 2014). Examples include the hybrid exchange of mimicry loci among *Heliconius* butterfly species (Dasmahapatra et al. 2012), transgressive segregation of Darwin's finches (*Geospiza* spp.) (Lamichhaney et al. 2018), and introgressive hybridization enabling the exchange of flower color mutations in monkeyflowers (*Mimulus* spp.) (Stankowski and Streisfeld 2015). Genetic interactions can result in incompatibilities between parental alleles, referred to as Dobzhansky-Muller incompatibilities, which can negatively affect fitness or cause hybrid sterility (Fishman and Sweigart 2018, Mack and Nachman 2017). Additionally, hybridization can manifest in the genome as a discordance between mitochondrial and nuclear markers, which in turn can influence the genome in both adaptive and maladaptive ways (Bonnet et al. 2017, Burton and Barreto 2012, Hill 2017, Lee et al. 2008, Sloan et al. 2017). Adaptive introgression of mitochondrial DNA may play an important role in speciation and phylogeography (Ivanov et al. 2018, Toews and Brelsford 2012).

While hybridization has been well-documented among pathogenic fungi (Anderson et al. 2003, Giordano et al. 2018, Greenspan et al. 2018, Greig et al. 2002, Lee et al. 2008, Silva et al. 2018, Stukenbrock et al. 2012), the role of hybridization on the process of speciation of fungal lineages with

different life histories and ecological functions is not well-understood, including among lichen-forming fungi. Gene flow and the role of hybridization in lichen-forming fungal evolution has been a long-standing question (Anderson and Rudolph 1956, Culberson et al. 1988, Magain et al. 2016, O'Brien et al. 2009, Zoller et al. 1999). Species boundaries in fungi, including symbiotic fungi such as lichen formers, have received substantial attention and become more robust with molecular sequence data (Steenkamp et al. 2018). However, processes involved in speciation in lichen-forming fungi have received far less attention. With poor fossil records (Lucking et al. 2009, Prieto and Wedin 2013) and potentially unreliable phenotype-based classification (Lumbsch and Leavitt 2011), options for elucidating the evolutionary history of lichen-forming fungi was challenging prior to the development of modern molecular tools. Potential evidence of hybridization has been encountered in a number of lichen-forming fungal lineages (Culberson and Hale 1973a, Ekman and Fröberg 1988, Ertz et al. 2009, O'Brien et al. 2009, Widhelm et al. 2019). However, genome-scale datasets have not yet been used to explicitly infer the role of hybridization on the process of speciation lichen-forming fungi.

Characterizing gene flow is further complicated by a diverse range of reproductive strategies used by lichen-forming fungi (Murtagh et al. 2000, Tripp and Lendemer 2017). Species of filamentous fungi with one of two different allelic variants can obligately cross-fertilize (heterothallism), only being able to reproduce with an individual with the opposite allelic variant, in contrast to species which contain both allelic variants and can self-fertilize (homothallism) (Billiard et al. 2012, Taylor et al. 1999, Wilson et al. 2015). Asexual reproduction is also common in many fungi (Taylor et al. 1999),including lichens that commonly reproduce asexually via vegetative fragmentation or propagules (Tripp and Lendemer 2017). Hybridization may occur among interfertile species via sexual reproduction (Giordano et al. 2018), and, while not yet explored in lichen-forming fungi, plant pathogenic fungi have been shown to hybridize asexually via fusion of hyphae (Schardl and Craven 2003, Stukenbrock 2016).

To better understand the potential role of hybridization in the diversification processes in symbiotic fungi, we investigated a morphologically diverse clade of lichen-forming fungi with a Pleistocene-dominated diversification history, the *Rhizoplaca melanophthalma* complex (Fig. 1) (Leavitt

et al. 2013a). While species boundaries within this complex have been well-studied (Leavitt et al. 2011a), processes that drive diversification are poorly understood. Members of this species complex are distributed worldwide, with rock-dwelling (saxicolous) species common in montane regions, hot deserts, and cold deserts, including Antarctica. In many regions, distinct species occur in sympatry with no evidence of ongoing or recent gene flow (Leavitt et al. 2013c). Vagrant forms (Fig. 1a-d) – which grow obligately unattached to substrates, therefore mobile with wind or water movement – occur only in the cold steppe regions of Western North America (Rosentreter 1993). While the rock-dwelling species are abundantly fertile, vagrant forms rarely produce fruiting bodies (apothecia), reproducing instead by vegetative fragmentation. However, in some cases environmentally modified erratic forms may become detached from rocks (facultatively unattached) and continue living on the soil with apothecia. At some sites in the Intermountain Western region of North America, attached, erratic, and vagrant forms of *Rhizoplaca* occur in sympatry (Rosentreter 1993).

These striking differences in growth form (attached, relatively small vs. large, unattached, unusual morphologies) and reproductive strategies (sexual vs. strictly asexual) among closely related species-level lineages (Fig. 1) beg the question of which evolutionary processes drive this disparity. Furthermore, considering the frequently sympatric distributions and recent diversification history (Rosentreter 1993), the origin and establishment of reproductive barriers in the *R. melanophthalma* complex remain unknown. Given the known role of hybridization in developing novel traits and reproductive barriers in fungi (Feurtey and Stukenbrock 2018, Giordano et al. 2018, Lee et al. 2008), here we investigate whether hybridization played a role in the process of diversification and establishment of reproductive barriers among members in the *R. melanophthalma* complex.

To address this question, we targeted vagrant *Rhizoplaca* forms in sage-steppe communities in western North America. While previous phylogenomic studies have provided well-supported hypotheses of evolutionary relationships with the *Rhizoplaca melanophthalma* species complex (Leavitt et al. 2011a, Leavitt et al. 2013b, Leavitt et al. 2016a), the evolutionary origin of vagrancy remains unresolved. Vagrant taxa include *Rhizoplaca arbuscula* Rosentr., St. Clair & Leavitt, *R. haydenii* (Tuck.) W.A.

Weber, *R. idahoensis* Rosentreter & McCune, and two subspecies of *R. melanophthalma*, *R. melanophthalma* subsp. *cerebriforms* Rosentreter & B.D. Ryan and *R. melanophthalma* subsp. *crispa* Rosentreter & B.D. Ryan. *Rhizoplaca arbuscula* was recently elevated from subspecies, *R. haydenii* subsp. *arbuscula*, to species based on phylogenomic data and distinct phenotypic characters (Leavitt et al. 2019a). *Rhizoplaca arbuscula*, *R. haydenii* and *R. idahoensis* are distinct from other species in the complex because they are exclusively asexual, obligately unattached (true vagrants), and morphologically distinct. Previous studies revealed potential discordance at a genomic level (Grewe et al. 2017, Leavitt et al. 2016a), and we hypothesize that hybridization may have played a crucial role in the origin of these deviant lineages. To do this, we used genome-scale data to (1) reconstruct evolutionary relationships, (2) identify potential cases of mtDNA introgression, and (3) infer potential hybridization events to examine patterns of reticulation in the *R. melanophthalma* group.

Methods

Taxon sampling

Metagenomic data was generated from 55 specimens representing the nine formally described species within the *Rhizoplaca melanophthalma* species complex (Supplementary Table S1; short reads generated for this study were deposited in NCBI's Short Read Archive under project PRJNA576709) (Leavitt et al. 2013c), as well as representative specimens of three formally described subspecies (McCune and Rosentreter 2007). Previous studies have provided strong support for some species boundaries and relationships among species-level lineages in this complex (Grewe et al. 2017, Leavitt et al. 2011a, Leavitt et al. 2013b, Leavitt et al. 2016b), with the exception of members of the *R. porteri* group and the vagrant taxa/forms, including: *R. arbuscula*, *R. haydenii*, *R. idahoensis*, *R. melanophthalma* subsp. *cerebriformis*, and *R. melanophthalma* subsp. *crispa* (Fig. 1). Vagrant specimens were sampled from multiple sites in the Lemhi Valley and Big Lost River Valley in central Idaho where distinct vagrant taxa/forms commonly co-occur (McCune and Rosentreter 2007), supplemented by a limited number of specimens collected at multiple sites in Wyoming, USA (Supplementary Table S1). *Rhizoplaca arbuscula*

and *R. melanophthalma* subsp. *cerebriformis* were represented by material collected from the type localities. We also included a saxicolous specimen collected in western Montana that had been recovered as closely related to *R. haydenii* in a previous, multi-locus phylogenetic study (Leavitt et al. 2016b), provisionally called 'saxicolous *haydenii* 715f'. Based on evidence suggesting potential mtDNA introgression in this specimen, multi-locus sequence data was generated for eight additional morphologically similar thalli collected from the same location in western Montana (Supplementary Fig. S1), hereafter called the 'saxicolous *haydenii* population', in hopes of identifying additional specimens of putative hybrid origin. *Rhizoplaca novomexicana* was used as our outgroup (Leavitt et al. 2016a).

DNA extraction and sequencing

For the 25 new specimens collected for metagenomic high-throughput sequencing, total genomic DNA was extracted from a small portion lichen thalli (comprised of the mycobiont, photobiont, and other associated microbes) using the E.Z.N.A. Plant DNA DS Mini Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) following the manufacturers' recommendations. Total genomic DNA was prepared following the standard Illumina whole genome sequencing (WGS) library preparation process using Adaptive Focused Acoustics for shearing (Covaris); followed by an AMPure cleanup step. The DNA was then processed with the NEBNext® Ultra™ II End Repair/dA-Tailing Module end-repair and the NEBNext® Ultra™ II Ligation Module (New England Biolabs) while using standard Illumina index primers. Libraries were pooled and sequenced with the HiSeq 2500 sequencer in high output mode at the DNA Sequencing Center, Brigham Young University, Provo, Utah, USA, using either 250 cycle paired-end reads, 300 cycle paired-end reads, or 50 cycle single-reads (six samples).

For the eight specimens of putative hybrid origin from the 'saxicolous *haydenii* population' (Supplementary Fig. S1), DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). Since the entire genomes of these specimens had not been sequenced with the first 25 specimens, we Sanger sequenced two nuclear markers, the internal transcribed spacer region (ITS) (Schoch et al. 2012b) and a fragment of the HEC/Ndc80p family protein, along with a region of the

mitochondrial genome. The ITS marker was amplified with primers previously published (Leavitt et al. 2013b). For the HEC/Ndc80p marker, we used primers 'rhizoHEC-f' (5'-CTTCGGTTTCTCTCTCGGCA-3') with 'rhizoHEC-r' (5'-ACCTCGCCGACACAAAAAGA-3'). The mitochondrial marker, a variable region of 720 to 965 bp, was amplified with custom-designed primers for this group; i.e. 'rhizoMTsp-f' (5'-GCCCAYGGGTTTGTTTCTTC-3') with 'rhizoMTsp -r' (5'-TGGCCGAGGAGGACTATTGA-3'). Markers were selected based on exploratory analyses of previous genome assemblies (Leavitt et al. 2016a) to identify a single-copy nuclear marker and a mitochondrial marker that consistently recovered *Rhizoplaca* species as monophyletic. We were unable to identify a single genomic region that recovered members of the *R. porteri* group *(R. occulta, R. polymorpha,* and *R. porteri*) as monophyletic, except for the ITS region. PCR cycling conditions for all markers were: initial denaturation at 95°C for 10 min, followed by 38 cycles at 94°C for 1 min, 50°C for 1 min, 72°C for 2 min, and final elongation at 72°C for 10 min. PCR fragments were cleaned using the ExoSAP-IT PCR Product Cleanup Reagent (ThermoFisher Scientific), and complementary strands were sequenced using the same primers used for amplification. Sequencing reactions were performed using Big Dye 3.1 (Applied Biosystems, Foster City, CA), and products were run on an AB 3730xl automated sequencer at the DNA Sequencing Center, Brigham Young University Provo, UT, USA.

Read filtering and phylogenomic data matrices

All reads were filtered using TRIMMOMATIC v0.33 before assembly to remove adapter and primer sequences, low quality reads, and/or included contamination from Illumina adaptors. Reads were trimmed when the average quality of 5-base sliding windows was below 20 and bases at the start and end of reads had a quality below 3 and 10, respectively. Subsequently, all trimmed reads shorter than 36 bp were filtered out.

To infer evolutionary relationships and assess potential incongruences between the nuclear and mitochondrial genomes, we generated genomic-scale datasets for both genomes. For the nuclear genome, we generated (i) a genome-scale alignment, (ii) a SNP dataset derived from variable sites in the

Benchmarking Universal Single-Copy Orthologs (BUSCO) (Simao et al. 2015), and (iii) gene trees from 407 single-copy core eukaryotic genes. Because phylogenomic datasets were generated from short-read, metagenomic data (reads from lichen symbionts and associated organisms), exploratory phylogenetic analyses, BLAST comparisons, and relative gene coverage were assessed to ensure the origin from the mycobiont nuclear and mitochondrial genomes. Datasets and accompanying analyses are summarized in Table 1.

The nuclear phylogenomic alignment was created with REALPHY v1.12 (Bertels et al. 2014), which has previously been shown to construct robust genome-scale datasets (Leavitt et al. 2016a, Zeng et al. 2018). Metagenomic reads were mapped to contigs >5kb from a previously published draft genome assembly for *R. melanophthalma sensu stricto* (Leavitt et al. 2016a) in REALPHY v1.12, implementing Bowtie 2.1.0 (Langmead and Salzberg 2012) for read mapping using the following parameters: -readLength 75 –perBaseCov 5 –gapThreshold 0.2 with the remaining parameters set at default values. With the –gapThreshold parameter set to 0.2, each site had no more than 20% missing data.

For the nuclear SNP dataset, the fungal set of BUSCO regions was extracted from the draft genome assembly for *R. melanophthalma s. str.* (Leavitt et al. 2016a). BUSCO uses reciprocal best hit (RBH), creating a Hidden Markov Model (HMM) profile using the protein sequences of 50 reference genomes for each single-copy gene. Every HMM profile generated was then used as query in tBLASTn searches against each genome to find the putative genomic region. An AUGUSTUS (Stanke et al. 2004) prediction was performed for each of the genomic regions. Predicted genes were then aligned to the HMM profiles of the BUSCO gene, and only the gene with the highest bit-score was kept. The BUSCO analysis was conducted in the Cyverse.org Discovery Environment (Goff et al. 2011, Merchant et al. 2016b). Single-copy BUSCO gene regions predicted in the *R. melanophthalma* reference genome were extracted; and duplicated genes and fragments less than 1 kb were removed. A supermatrix was created concatenating every BUSCO single-copy gene using FASconCAT.pl (Misof et al. 2013). Metagenomic reads were mapped to BUSCO genes larger than 1kb using REALPHY v1.12, implementing the same parameters as above, and variable sites were extracted for a SNP dataset. We also generated gene trees for

a subset of the BUSCO genes, focusing on exon regions of 458 core eukaryotic genes (CEG) and extracted using CEGMA (Parra et al. 2007). We used the 'map_n_extract' pipeline (https://github.com/felixgrewe/map_n_extract/(Leavitt et al. 2016a)) to map metagenomic reads from each specimen to CEG genes predicted in the *R. melanophthalma* reference genome. Resulting consensus sequences from each sample were filtered by removing insertions not present in the reference CEG regions and only including genes in which the reference starts with ATC and ends with TGA, TAA, or TAG. We excluded CEG genes with a stop codon within the coding region or those with coding regions that were not divisible by three. Consensus sequences for each CEG region, including introns and small portions of upstream and downstream regions, were aligned using the program MUSCLE(Edgar 2004). Exons from individual CEG consensus sequences passing filtering parameters were extracted for phylogenetic analysis.

For the mitochondrial genome dataset, metagenomic reads from each library were assembled using the SPAdes v3.12.0 assembler (Bankevich et al. 2012). We selected the longest mitochondrial assembly as a reference for phylogenomic assembly using REALPHY v1.12. The following parameters were implemented in REALPHY, implementing Bowtie v2.1.0 (Langmead and Salzberg 2012) for read mapping: -readLength 100 -perBaseCov 5 -gapThreshold 0.2, with the remaining parameters set at default values. For exploratory comparisons, we generated additional mitochondrial datasets, inferring phylogenies from (i) concatenated CDS (protein coding) regions, (ii) concatenated intronic regions, and (iii) complete, aligned mitochondrial contigs. We used DOGMA (Wyman et al. 2004) to initially demark mitochondrial genomic features. Multiple sequence alignments for individual CDSs and introns were performed using MAFFT (Katoh and Standley 2013), with the resulting CDS and intron alignments concatenated separately and implementing the G-INS-I option. Complete mitochondrial contigs were also aligned using MAFFT, implementing the E-INS-I option.

Species tree inference from phylogenetic data

Evolutionary relationships were inferred using (i) a supermatrix approach(Tonini et al. 2015), (ii) two computationally efficient species tree inference approaches accounting for incomplete lineage sorting, such as ASTRAL-III (Zhang et al. 2018) and SVDQuartets (Chifman and Kubatko 2014), and (iii) a recently developed network approach that incorporates both incomplete lineage sorting and hybridization implemented in PhyloNet (Solis-Lemus and Ane 2016). In some cases, concatenation, or super matrix approaches, have been shown to accurately infer relationships across a wide range of scenarios (Tonini et al. 2015). We inferred relationships from both the nuclear and mitochondrial alignments generated using REALPHY. Phylogenetic trees were reconstructed using maximum likelihood as implemented in IQ-TREE (Version 1.6.7) (Nguyen et al. 2015), with 1,000 ultra-fast bootstrap replicates(Hoang et al. 2018) to assess nodal support, followed by the best-fit substitution model as predicted by ModelFinder (Kalyaanamoorthy et al. 2017). Topologies inferred from the nuclear and mitochondrial datasets were visually assessed to infer cases of mitochondrial introgression with *a posteriori* comparisons of morphological groups with phylogenetic structure.

Because standard concatenation approaches may return incorrect trees with high support in the presence of incomplete lineage sorting (Edwards 2009), we used two "coalescent-based" methods to infer species trees for the *R. melanophthalma* complex: ASTRAL-III, a summary method which accepts gene trees as input to generate a species tree, and SVDQuartets, as implemented in PAUP*, a method that infers relationships among quartets of taxa under the coalescent model. Both ASTRAL-III and SVDQuartets+PAUP* are computationally efficient and are able to accurately infer relationships under a range of scenarios (Chou et al. 2015). Specimens were assigned to species/populations based on previous studies and candidate species-level lineages delimited here. A total of nine species/populations were defined for species tree inference. Based on our analyses, *R. melanophthalma, R. parilis*, and *R. shushanii* are robustly delimited (Leavitt et al. 2016a). Previous studies have highlighted the close relationship between *R. occulata*, *R. polymorpha*, and *R. porteri* (Grewe et al. 2017). Our sampling was not designed to delimit species boundaries or relationships among these three taxa; therefore, we treated them as a

single species/population – the *R. porteri* group. Species boundaries in the vagrant taxa, e.g., *R. arbuscula*, *R. haydenii* and *R. idahoensis sensu lato*, were delimited using an iterative approach comparing morphology with the mitochondrial phylogeny. Four mitochondrial clades were identified that correlated with distinct morphological groups (see Results), and these four clades were treated as distinct species/populations in the species tree reconstructions using nuclear phylogenomic data. The single specimen with clear mitonuclear incongruence, specimen '715f' from western Montana, was treated as a distinct species in all species tree analyses.

We used ASTRAL-III v5.6.3 to generate a summary species tree based on the 407 generated CEG gene trees that passed quality filtering. ASTRAL-III reconstructs species trees using the minimum quartet distance. Branch support values were inferred using local posterior probabilities (Sayyari and Mirarab 2016). SVDquartets (Chifman and Kubatko 2014), as implemented in PAUP* v.4.0a (Swofford 2002), were used to generate a species tree from SNP data obtained from the BUSCO alignment generated by REALPHY. This approach accounts for incomplete lineage sorting (ILS) without the need to assemble gene trees. Support for the final species tree was assessed using 25,000 random quartets and n=1000 bootstrap replicates.

Incongruence among individual gene topologies based on the 407 CEG gene topologies was evaluated using internode certainty (IC) and relative tree certainty (TCA) metrics (Salichos et al. 2014) as implemented by RAxML v8.2.3. The IC value of a given internode reflects its specific degree of incongruence, while the TCA value characterizes the global degree of incongruence between trees.

Tests for reticulated relationships

Mitonuclear incongruence suggested a hybrid origin for members of the 'vagrant clade' clade, comprising *R. arbuscula*, *R. haydenii*, *R. idahoensis*, and the 'saxicolous *haydenii* population' (see Results). We then used maximum pseudolikelihood (MPL) to assess corroborative evidence supporting this conclusion. Patterns of reticulation related to hybrid speciation and introgression do not fit traditional bifurcating models and are often more accurately represented by phylogenetic networks (Burbrink and Gehara 2018). Therefore, we used a phylogenetic network approach that accounts for ILS through the

coalescent model and for horizontal inheritance of genes through reticulation nodes in the network (Solis-Lemus and Ane 2016). This maximum pseudolikelihood approach (Yu and Nakhleh 2015) is implemented in PhyloNet v3.6.8 (Wen et al. 2018b). A total of seven groups were defined in the MPL analyses: *R. melanophthalma*, *R. novomexicana*, *R. parilis, R. shushanii*, members of the 'vagrant clade', and members of the *R. porteri* group, along with the 'saxicolous *haydenii*' specimen, '715f', with a clear mitonuclear conflict. In order to identify potential reticulations among more deeply diverged lineages, specimens recovered in the 'vagrant clade', and the *R. porteri* group were each treated as single groups. Based on the 407 CEG gene trees, the "InferNetwork_MPL" algorithm was run allowing for 1 through 5 reticulations by performing 1000 independent searches to avoid sampling in local optimums. Uncertain nodes were bypassed in the gene tree by applying a bootstrap support threshold of 50 using the -b flag. The ten returned species networks were further optimized for both branch lengths and inheritance probabilities using a full likelihood framework by applying the -o flag. The best-fitting network was selected using the Akaike information criterion (AIC) (Akaike 1998, Sullivan and Joyce 2005) by applying the number of parameters (k) as the number of branch lengths plus the number of reticulations with L as the likelihood value.

Tests of interspecific gene flow

We used the program HyDe (Blischak et al. 2018b) to detect potential interspecific hybridization using the SNP dataset constructed from the BUSCO gene regions. HyDe considers a root, four-taxon network consisting of an outgroup and a triplet of ingroup populations, P1, P2, and P3 (Green et al. 2010b) to detect hybridization from phylogenetic invariants that arise under the coalescent model with hybridization. Introgression between P3 and either P1 or P2 influences the relative frequencies of ABBA and BABA, and the D-statistic measures the imbalance between these frequencies (Green et al. 2010b). We tested all possible triplet comparisons among species, treating *R. arbuscula*, *R. haydenii*, and the single specimen representing the 'saxicolous *haydenii* population' as separate species using the python script run_hyde.py. In all cases, *R. novomexicana* was treated as the outgroup. We only considered hypothesis tests that were significant at an overall $\alpha < 0.05$ level (after incorporating a Bonferonni

correction) with estimates of γ between 0 and 1. Z-scores greater than three are generally interpreted as strong evidence of introgression (Eaton and Ree 2013).

Using individual gene trees to infer mitochondrial introgression

To test if other samples from the 'saxicolous *haydenii* population' in western Montana (Supplementary Fig. S1) also exhibited patterns of mito-nuclear discordance, e.g., nuclear genome in the 'vagrant clade' and mitochondrial genome in the *R. porteri* group, we generated topologies from three short gene regions. Sequences from the three single-marker datasets – the nrDNA, the protein-coding region of HEC/Ndc80p, and the fragment of the mitochondrial genome – were aligned in MAFFT v7 (Katoh and Standley 2013). We implemented the G-INS-i alignment algorithm and '1PAM / K=2' scoring matrix, with an offset value of 0.2, and the remaining parameters were set to default values. The HEC/Ndc80p and mitochondrial datasets were represented by all metagenomic samples and sequences from the eight specimens of putative hybrid origin from the 'saxicolous *haydenii* population' generated using Sanger sequencing. ITS sequences were combined with 443 ITS sequences from Leavitt et al. to put our data within a broader specimen sampling context. ML topologies were reconstructed for each region using the program RAxML v8.2.10 (Stamatakis 2014) in the CIPRES Science Gateway server (http://www.phylo.org/portal2/). Substitution models for each locus were estimated using jModelTest v.2.1.10 (Darriba et al. 2012), and nodal support was evaluated using 1000 bootstrap pseudo-replicates.

Results

Data and phylogenomic datasets

The most comprehensive nuclear dataset assembled using REALPHY included a total of 18,457,947 aligned nucleotide position characters. Metagenomic reads mapped to BUSCO genes larger than 1kb resulted in an alignment of 1,620,052 bp; and from this alignment a total of 142,437 SNPs (polymorphic sites) were extracted. Our final CEG dataset comprised gene trees from 407 of the original 458 CEGs. The mitochondrial dataset generated using REALPHY included a total of 63,877 aligned

nucleotide position characters. ITS, the HEC/Ndc80p protein, and the mitochondrial genome marker for the eight additional specimens of putative hybrid origin from western Montana, USA (Supplementary Fig. S2) were deposited in GenBank under access numbers MN795100-MN795107. All alignments were deposited in FigShare, under (https://doi.org/10.6084/m9.figshare).

Phylogenomic reconstructions

A well-supported phylogeny was inferred from the nuclear REALPHY dataset (Fig. 2). Vagrant forms representing *R. arbuscula*, *R. haydenii*, and *R. idahoensis* were recovered in a well-supported clade, the 'vagrant clade'. Within the 'vagrant clade', two divergent, well-supported clades were recovered: one clade comprising specimens representing *R. arbuscula*, and the other comprised of specimens representing *R. haydenii*, *R. idahoensis*, and 'saxicolous haydenii 715f' specimen from western Montana. Distinct clades within the 'vagrant clade' generally coincided with specimens collected from geographically distinct populations. Vagrant forms representing *R. melanophthalma* subsp. *cerebriformis* and *R. melanophthalma* subsp. *crispa* were recovered nested within the *R. porteri* group. Relationships among other *Rhizoplaca* species were congruent with previous studies based on genome-scale data (Grewe et al. 2017, Leavitt et al. 2016a). Of the targeted vagrant taxa, *R. arbuscula*, *R. haydenii*, and *R. idahoensis*, only *R. arbuscula* was recovered as monophyletic (Fig. 2). We note that as in previous studies (Grewe et al. 2017, Leavitt et al. 2016a), species within the *R. porteri* group, e.g., *R. occulta*, *R. polymorpha*, and *R. porteri*, were not recovered as monophyletic.

The phylogeny inferred from the mitochondrial REALPHY dataset recovered four distinct, major mitochondrial clades, with well-supported differences in phylogenetic relationships in comparison to relationships inferred from the nuclear phylogenomic dataset (Fig. 2). These four distinct clades were also recovered in exploratory phylogenetic analyses of the (i) concatenated CDS regions, (ii) concatenated intronic regions, and (iii) alignments of the complete mitochondrial contig, although relationships among major clades differed in each reconstruction (Supplementary Fig. S3). Of the four major mitochondrial clades, one was comprised of specimens representing *R. melanophthalma* (recovered in two separated

clades in the CDS-derived phylogeny; Supplementary Fig. S3A); a second comprised *R. shushanii* specimens; the third was comprised of members of the *R. porteri* group, including *R. melanophthalma* subsp. *cerebriformis* and *R. melanophthalma* subsp. *crispa* and the 'saxicolous haydenii 715f' specimen from western Montana. The fourth clade included all vagrant forms representing *R. arbuscula*, *R. haydenii*, *R. idahoensis*, and the saxicolous species *R. parilis* (Fig. 2). Within this final clade, four distinct sub-clades were recovered, each corresponding to morphologically distinct specimens: (i) *R. haydenii* and *R. idahoensis* (vagrant forms), (ii) *R. arbuscula* specimens with narrow, finely dissected lobes (see Fig. 1a), (iii) *R. arbuscula* specimens with broader, more robust lobes more similar to *R. haydenii*, and (iv) *R. parilis* (an attached saxicolous species).

Discordance was observed between topologies inferred from the nuclear REALPHY and mt REALPHY datasets (Fig. 2). In the nuclear phylogeny, members of the 'vagrant clade' (*R. arbuscula*, *R. haydenii*, and *R. idahoensis*) were distinct from specimens representing *R. parilis*, in contrast to the mt phylogeny where members of the 'vagrant clade' were recovered within a single clade also comprised highly similar mt genomes from *R. parilis* specimens. Furthermore, the 'saxicolous haydenii 715f' specimen from western Montana, recovered in the 'vagrant clade' in the nuclear topology, showed evidence of mitochondrial introgression. Specifically, the 'saxicolous haydenii 715f' specimen was recovered among specimens representing the *R. porteri* group in the mitochondrial topology and not with other members of the 'vagrant clade' (Fig. 2).

Coalescent-based species tree analyses ASTRAL-III and SVDquartet+PAUP* generated identical branching patterns with bootstrap support values = 100% (Fig. 3). Relationships in species tree inferences were consistent with branching patterns inferred from the nuclear REALPHY datasets (Fig. 2). The TCA value (TCA=0.075) revealed a pattern of genome-scale incongruence among trees, and the degree of incongruence for each internode in a set of gene trees, as determined using internode certainty values, is reported in Fig. 3. IC values indicated that most species level lineages were recovered as monophyletic in the majority of gene regions (Fig. 3), while relationships among these lineages had much lower IC values.

Evidence of reticulated evolution

MPL networks incorporating both incomplete lineage sorting and potential reticulations provided support for reticulate evolution in the *Rhizoplaca melanophthalma* species group (Fig. 4). AIC supported the three-reticulation model as the best-fitting scenario (Table 2).

The one-, two- and three-reticulation MPL networks consistently inferred that *R. shushanii* has a hybrid origin resulting from reticulations between the *R. melanophthalma* and *R. parilis* lineages; and the two-reticulation MPL network supports reticulations involving the 'vagrant' lineage with the *R. parilis* lineage, consistent with the inferred mitonuclear discordance (Fig. 2).

Sanger sequencing of the additional specimens of putative hybrid origin from western Montana (collected from the same population as specimen 'saxicolous haydenii 715f' [BRY-C]) also showed evidence of mitonuclear discordance (Supplementary Fig. S2a, b, & c). Relationships of five of the eight additional samples from the putative hybrid population were consistent with the single specimen included in the phylogenomic portion of the study, while the remaining three specimens showed slightly different patterns of mitonuclear discordance. In the nuclear ITS topology specimens were recovered in two separate clades – the *R. melanophthalma* clade which occurred as sister to the second clade comprised of *R. arbuscula, R. haydenii*, *R. idahoensis*, and *R. parilis.* In the topology inferred from the single-copy nuclear HEC/Ndc80p loci, specimens were recovered in two distinct clades, one group within the *'haydenii*/*idahoensis*' clade and the other clade comprised exclusively of the three putative hybrid species with an unresolved relationship to other clades. In the topology inferred from mitochondrial marker, the putative hybrid specimens from western Montana were all recovered in the mitochondrial *R. porteri* group but in three distinct groups (Supplementary Fig. S2a, b, & c).

The HyDe analysis revealed 24 significant triplet comparisons supporting hybridization, including seven comparisons with Z-scores >3 (Table 3). *Rhizoplaca shushanii* was consistently inferred to be of hybrid origin with the highest Z-scores. *Rhizoplaca* species from the 'vagrant clade' were also consistently inferred to have been involved in hybridization, particularly individuals representing *R. arbuscula* (Table 3).

Discussion

Recently, the roles of hybridization have been highlighted as important mechanisms for facilitating rapid speciation and adaptive radiations (Marques et al. 2019c). In particular, hybridization between early branching lineages can augment standing variation that contributes to later speciation events (Bassham et al. 2018, Bell et al. 2012, Hedrick 2013b, Lewontin 1966, Soltis et al. 2004b, Wallbank et al. 2016). Similarly, our results support the potential role of genomic combinatorial mechanisms in the diversification of symbiotic fungi. Mitonuclear discordance in members of the *R. melanophthalma* complex (Fig. 2), ABBA-BABA tests for introgression (Table 3), and reticulated evolutionary histories inferred from nuclear phylogenomic data (Fig. 4), provide evidence that hybridization played a central role in the diversification of this group. While robust phylogenies were inferred from the nuclear phylogenomic datasets (Figs. 2 & 3), the evolutionary history of the mitochondrial genome appears to be more complex in this group. As has been demonstrated in other studies, mitochondrial genome evolution in fungi is complex (Aguileta et al. 2014, Pogoda et al. 2019). Mitochondrial genome evolution in the *R. melanophthalma* complex also appears to be affected by dynamic evolutionary processes, with different phylogenetic relationships inferred from different portions of the mitochondrial genome (Supplementary Fig. 3). Additional research will be required to appropriately characterize features of mt genome evolution, e.g., intron gains/losses, gene rearrangement, recombination, etc., in lichen-forming fungi.

These data support a complex model of speciation in the *Rhizoplaca melanophthalma* group with multiple reticulation events throughout its diversification history. Despite the high internode certainty values and strongly supported phylogenetic clades corresponding to species in the *R. melanophthalma* complex (considering members of the *R. porteri* group as a single species), our results indicated significant evidence for allele sharing in the diversification history of the group. These results are congruent with an interpretation that hybridization may introduce reproductive barriers, and hybrid lineages become reproductively isolated with limited or no ongoing gene flow once established, e.g.

consistent genome-wide support for species lineages but significant genome-scale conflict for relationships among lineages.

Pure hybrids are rarely encountered due to the genetic incompatibilities that reduce fitness (Stukenbrock 2016), but sometimes parental genomes are compatible enough for the persistence of a new hybrid species, either in a new niche or with high enough fitness to compete with the parent species (Marques et al. 2019c). In the case of our study, our PhyloNet analysis indicated that *R. shushanii* is potentially the result of a hybrid speciation event between the *R. melanophthalma* and *R. parilis* lineages, or at least introgressed with ghost lineages, leading to what is now considered a distinct species. These results are further supported by inferred hybrid origin of *R. shushanii* with high associated Z-scores in the HyDe analysis (Table 3). However, we note that PhyloNet and HyDe tests provided somewhat contrasting inferences for the parental lineages involved in the reticulated evolutionary history in this complex (Fig. 4 & Table 3). While additional work will be required to more fully elucidate the timing, direction, and lineages involved in the reticulated evolutionary history of the *R. melanophthalma* group, our study emphasizes the importance of considering hybridization in an organism's evolutionary history, even when relationships are strongly supported as bifurcating in traditional phylogenomic analyses. Furthermore, by using multiple analytical tools for inferring introgression, operating under different assumptions, e.g., MLP & ABBA/BABA test of introgression, and then viewing congruence between methods can provide greater support for role reticulation in the evolutionary history of the targeted group (Blair and Ané 2019).

To date, *R. shushanii* has only been found at subalpine sites in southern Utah despite worldwide sampling efforts, while *R. melanophthalma* and *R. parilis* can be found worldwide (Leavitt et al. 2013a). Perhaps the hybrid speciation of *R. shushanii* occurred only in places where there was a suitable niche available. Schumer et al. (2014) suggests that in order to have evidence of true hybrid speciation, it is necessary to show that hybridization leads to reproductive isolation (Schumer et al. 2014). This criterion is not readily applicable to fungi, as they can speciate without complete reproductive isolation (Kohn 2005, Steenkamp et al. 2018). Furthermore, hybridization and reproductive isolation are especially difficult to account for with lichen-forming fungi, which cannot be easily cultured in vitro for

experimental testing. Regardless, there is value in accounting for gene flow and utilizing phylogenetic networks to explore evolutionary relationships.

A number of studies show that hybrid sterility develops as a result of incompatibilities in the genome (Janko et al. 2018, Lee et al. 2008, Lodé 2012). As many species of lichen-forming fungi can reproduce both sexually and asexually, it's possible that the evolution of hybrid sterility could trigger a lichen-forming fungus to rely primarily on asexual reproduction. While *R. parilis* is abundantly fertile, members of the 'vagrant clade' with the putative introgressed *R. parilis* mitochondrial genomes are strictly asexual. We speculate that a mitonuclear incompatibility may have contributed to the differences in reproductive strategies. We note that one single member of the nuclear 'vagrant clade', the 'saxicolous haydenii 715f' specimen, along with the five of the eight specimens also collected from the same population in western Montana (Supplementary Fig. S2) appear to have an introgressed mitochondrial genome more similar to those from the *R. porteri* group. These six specimens differ morphologically from other members of the *R. 'haydenii*' clade, in that like other members of the *R. melanophthalma* complex, they are umbilicate and attached to rocks. None of the specimens recovered within the 'vagrant clade' using nuclear phylogenomic datasets and with '*porteri*'-type mitochondrial genomes produce normal, fully developed apothecia, although it appears that stunted or partially developed apothecia occurred on these thalli (Fig. 1c). However, investigating mate type loci on the population level could provide additional insights. Pizarro et al. 2019 recently found a representative from the *Rhizoplaca melanopthalma* complex to be heterothallic, with a loss of primary homothallism in the class Lecanoromycetes. An imbalance of mating types on the population level could paint the picture of why, evolutionarily, these lichen-forming fungi might have shifted to asexuality.

Our study also sheds light on how asexual organisms might fit into the evolutionary picture - how they might come to be and how genetically isolated they truly are. Our results reveal significant phylogeographic structure in the asexual *R. haydenii/idahoensis* clade (Fig. 2), suggesting that populations in this lineage may not rely exclusively on clonal reproduction. Other studies have revealed evidence of recombination in what were thought to be asexual lichens (Buschbom and Mueller 2006,

Kroken and Taylor 2001b). It has been argued that fungal sexuality should be viewed as a spectrum, where obligate sexual reproduction and obligate asexual reproduction in a single species rarely occur (Honegger and Zippler 2007, Tripp 2016, Tripp and Lendemer 2017). It is also possible for fungi to exchange genetic material via fusion of vegetative cells (Clutterbuck 1996, Roper et al. 2011, Stukenbrock 2016). Alternatively, this phylogeographic structure may have arisen from variation acquired before the loss of sexuality, or these distinct lineages may arise from independent hybridization events. It is also possible this lineage is a result of an ancient hybridization event, and local populations have since fixed their own unique genetic properties.

Hybridization can generate novel phenotypes, which can sometimes manifest later in evolutionary history under new selective pressures (Marques et al. 2019c). We originally hypothesized that hybridization had played a role in the evolution of vagrancy within the *R. melanophthalma* species complex, as demonstrated by the distinctly vagrant, asexual *R. haydenii* clade. We found that hybridization doesn't fully explain vagrant forms. Vagrants (erratic vs. obligate forms) in the *R. porteri* group, e.g., *R. melanophthalma* subsp. *crispa*, did not show evidence of mitochondrial introgression, in contrast to the taxa *R. haydenii* with *R. parilis*. However, those with the strongest evidence for hybridization showed the most morphological distinctiveness, e.g. *R. shushanii* and members of the 'vagrant clade'. In future research, it would be worthwhile to investigate the influence of the nonfungal symbionts on the evolution of vagrancy, as symbionts can influence morphology in lichens (Ertz et al. 2018, Spribille 2018).

In conclusion, by analyzing phylogenomic data from both mitochondrial and nuclear genomes of species within the *Rhizoplaca melanophthalma* species complex and by reconstructing a phylogenetic network, we showed evidence of a mitonuclear discordance, as well as a reticulated evolutionary history within the complex. Our results support the need for considering reticulate phylogenies when investigating species boundaries and evolutionary history, even in well-supported topologies inferred from genome-scale data. Similarly, while the role of hybridization and recombination has long been considered in diversification of lichen-forming fungi (Ekman and Fröberg 1988, Ertz et al. 2009, O'Brien

et al. 2009, Widhelm et al. 2019), our results provide important impetus for explicitly considering hybridization/introgression in symbiotic fungi and their potential role in generating novel phenotypes and introducing reproductive barriers.

References

Chan, K. M. A. & Levin, S. A. Leaky prezygotic isolation and porous genomes: Rapid introgression of maternally inherited DNA. *Evolution* **59**, 720-729 (2005).

Mallet, J. Hybridization as an invasion of the genome. *Trends Ecol Evol* **20**, 229-237, doi:10.1016/j.tree.2005.02.010 (2005).

Stukenbrock, E. H. The Role of Hybridization in the Evolution and Emergence of New Fungal Plant Pathogens. *Phytopathology* **106**, 104-112, doi:10.1094/phyto-08-15-0184-rvw (2016).

Hedrick, P. W. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. *Molecular Ecology* **22**, 4606-4618, doi:10.1111/mec.12415 (2013).

Hill, G. E. The mitonuclear compatibility species concept. *The Auk* **134**, 393-409, doi:10.1642/auk-16-201.1 (2017).

Huang, J. P. Parapatric genetic introgression and phenotypic assimilation: testing conditions for introgression between Hercules beetles (*Dynastes*, Dynastinae). *Mol Ecol* **25**, 5513-5526, doi:10.1111/mec.13849 (2016).

Feliner, G. N. *et al.* Is homoploid hybrid speciation that rare? An empiricist's view. *Heredity* **118**, 513-516, doi:10.1038/hdy.2017.7 (2017).

Schumer, M., Rosenthal, G. G. & Andolfatto, P. How common is homoploid hybrid speciation? *Evolution* **68**, 1553-1560, doi:10.1111/evo.12399 (2014).

Abbott, R. *et al.* Hybridization and speciation. *Journal of Evolutionary Biology* **26**, 229-246, doi:10.1111/j.1420-9101.2012.02599.x (2013).

Chapman, M. A. & Burke, J. M. Genetic divergence and hybrid speciation. *Evolution* **61**, 1773-1780, doi:10.1111/j.1558-5646.2007.00134.x (2007).

Tigano, A. & Friesen, V. L. Genomics of local adaptation with gene flow. *Molecular Ecology* **25**, 2144-2164, doi:10.1111/mec.13606 (2016).

Rieseberg, L. H. *et al.* Major ecological transitions in wild sunflowers facilitated by hybridization. *Science* **301**, 1211-1216, doi:10.1126/science.1086949 (2003).

Gladieux, P. *et al.* Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in eukaryotes. *Molecular Ecology* **23**, 753-773, doi:10.1111/mec.12631 (2014).

Dasmahapatra, K. K. *et al.* Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. *Nature* **487**, 94-98, doi:10.1038/nature11041 (2012).

Lamichhaney, S. *et al.* Rapid hybrid speciation in Darwin's finches. *Science* **359**, 224-227, doi:10.1126/science.aao4593 (2018).

Stankowski, S. & Streisfeld, M. A. Introgressive hybridization facilitates adaptive divergence in a recent radiation of monkeyflowers. *Proc Biol Sci* **282**, doi:10.1098/rspb.2015.1666 (2015).

Fishman, L. & Sweigart, A. L. in *Annual Review of Plant Biology, Vol 69* Vol. 69 *Annual Review of Plant Biology* (ed S. S. Merchant) 707-731 (2018).

Mack, K. L. & Nachman, M. W. Gene regulation and speciation. *Trends in Genetics* **33**, 68-80, doi:10.1016/j.tig.2016.11.003 (2017).

Bonnet, T., Leblois, R., Rousset, F. & Crochet, P. A. A reassessment of explanations for discordant introgressions of mitochondrial and nuclear genomes. *Evolution* **71**, 2140-2158, doi:10.1111/evo.13296 (2017).

Burton, R. S. & Barreto, F. S. A disproportionate role for mtDNA in Dobzhansky-Muller incompatibilities? *Mol Ecol* **21**, 4942-4957, doi:10.1111/mec.12006 (2012).

Lee, H. Y. *et al.* Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. *Cell* **135**, 1065-1073, doi:10.1016/j.cell.2008.10.047 (2008).

Sloan, D. B., Havird, J. C. & Sharbrough, J. The on-again, off-again relationship between mitochondrial genomes and species boundaries. *Mol Ecol* **26**, 2212-2236, doi:10.1111/mec.13959 (2017).

Toews, D. P. & Brelsford, A. The biogeography of mitochondrial and nuclear discordance in animals. *Mol Ecol* **21**, 3907-3930, doi:10.1111/j.1365-294X.2012.05664.x (2012).

Ivanov, V., Lee, K. M. & Mutanen, M. Mitonuclear discordance in wolf spiders: Genomic evidence for species integrity and introgression. *Molecular Ecology* **27**, 1681-1695, doi:10.1111/mec.14564 (2018).

Giordano, L., Sillo, F., Garbelotto, M. & Gonthier, P. Mitonuclear interactions may contribute to fitness of fungal hybrids. *Sci Rep* **8**, 1706, doi:10.1038/s41598-018-19922-w (2018).

Greig, D., Louis, E. J., Borts, R. H. & Travisano, M. Hybrid speciation in experimental populations of yeast. *Science* **298**, 1773-1775, doi:10.1126/science.1076374 (2002).

Anderson, J. B. *et al.* Mode of selection and experimental evolution of antifungal drug resistance in *Saccharomyces cerevisiae*. *Genetics* **163**, 1287-1298 (2003).

Stukenbrock, E. H., Christiansen, F. B., Hansen, T. T., Dutheil, J. Y. & Schierup, M. H. Fusion of two divergent fungal individuals led to the recent emergence of a unique widespread pathogen species. *Proc Natl Acad Sci U S A* **109**, 10954-10959, doi:10.1073/pnas.1201403109 (2012).

Greenspan, S. E. *et al.* Hybrids of amphibian chytrid show high virulence in native hosts. *Sci Rep* **8**, 9600, doi:10.1038/s41598-018-27828-w (2018).

Silva, D. N., Varzea, V., Paulo, O. S. & Batista, D. Population genomic footprints of host adaptation, introgression and recombination in coffee leaf rust. *Mol Plant Pathol* **19**, 1742-1753, doi:10.1111/mpp.12657 (2018).

Anderson, E. & Rudolph, E. D. An analysis of variation in a variable population of *Cladonia*. *Evolution* **10**, 147-156 (1956).

Culberson, C. F., Culberson, W. L. & Johnson, A. Gene Flow in Lichens. *American Journal of Botany* **75**, pp. 1135-1139 (1988).

O'Brien, H., Miadlikowska, J. & Lutzoni, F. Assessing Reproductive Isolation in Highly Diverse Communities of the Lichen-Forming Fungal Genus *Peltigera*. *Evolution* **63**, 2076-2086 (2009).

Zoller, S., Lutzoni, F. & Scheidegger, C. Genetic variation within and among populations of the threatened lichen Lobaria pulmonaria in Switzerland and implications for its conservation. *8*, 2049-2059 (1999).

Magain, N., Sérusiaux, E., Zhurbenko, M. P., Lutzoni, F. & Miadlikowska, J. Disentangling the *Peltigera polydactylon* Species Complex by Recognizing Two New Taxa, *P. polydactylon* subsp. *udeghe* and *P. seneca*. *Herzogia* **29**, 514-528, doi:10.13158/heia.29.2.2016.514 (2016).

Steenkamp, E. T., Wingfield, M. J., McTaggart, A. R. & Wingfield, B. D. Fungal species and their boundaries matter - Definitions, mechanisms and practical implications. *Fungal Biology Reviews* **32**, 104-116, doi:10.1016/j.fbr.2017.11.002 (2018).

Prieto, M. & Wedin, M. Dating the diversification of the major lineages of Ascomycota (Fungi). *Plos One* **8**, doi:10.1371/journal.pone.0065576 (2013).

Lucking, R., Huhndorf, S., Pfister, D. H., Plata, E. R. & Lumbsch, H. T. Fungi evolved right on track. *Mycologia* **101**, 810-822, doi:10.3852/09-016 (2009).

Lumbsch, H. T. & Leavitt, S. D. Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. *Fungal Diversity* **50**, 59-72, doi:10.1007/s13225-011-0123-z (2011).

Culberson, C. F. & Hale, M. E. Chemical and morphological evolution in *Parmelia* sect. *Hypotrachyna*: Product of ancient hybridization? *Brittonia* **25**, 162-173, doi:10.2307/2805934 (1973).

Ertz, D. *et al.* Towards a new classification of the *Arthoniales* (*Ascomycota*) based on a three-gene phylogeny focussing on the genus *Opegrapha*. *Mycol Res* **113**, 141-152, doi:10.1016/j.mycres.2008.09.002 (2009).

Ekman, S. & Fröberg, L. Taxonomical problems in *Aspicilia contorta* and *A. hoffmannii* - an effect of hybridization? *International Journal of Mycology and Lichenology* **3**, 215-226 (1988).

Widhelm, T. J. *et al.* Multiple historical processes obscure phylogenetic relationships in a taxonomically difficult group (Lobariaceae, Ascomycota). *Sci Rep* **9**, 8968, doi:10.1038/s41598-019-45455-x (2019).

Tripp, E. A. & Lendemer, J. C. Twenty-seven modes of reproduction in the obligate lichen symbiosis. *Brittonia* **70**, 1-14, doi:10.1007/s12228-017-9500-6 (2017).

Murtagh, G. J., Dyer, P. S. & Crittenden, P. D. Reproductive systems: Sex and the single lichen. *Nature* **404**, 564 (2000).

Billiard, S., Lopez-Villavicencio, M., Hood, M. E. & Giraud, T. Sex, outcrossing and mating types: unsolved questions in fungi and beyond. *J Evol Biol* **25**, 1020-1038, doi:10.1111/j.1420-9101.2012.02495.x (2012).

Taylor, J. W., Jacobson, D. J. & Fisher, M. C. The Evolution of Asexual Fungi: Reproduction, Speciation and Classification. *Annu Rev Phytopathol* **37**, 197-246 (1999).

Wilson, A. M. *et al.* Homothallism: an umbrella term for describing diverse sexual behaviours. *IMA Fungus* **6**, 207-214, doi:10.5598/imafungus.2015.06.01.13 (2015).

Schardl, C. L. & Craven, K. D. Interspecific hybridization in plant-associated fungi and oomycetes: a review. *Molecular Ecology* **12**, 2861-2873, doi:10.1046/j.1365-294X.2003.01965.x (2003).

Leavitt, S. *et al.* DNA barcode identification of lichen-forming fungal species in the *Rhizoplaca melanophthalma* species-complex (Lecanorales, Lecanoraceae), including five new species. *MycoKeys* **7**, 1-22, doi:10.3897/mycokeys.7.4508 (2013).

Leavitt, S. D. *et al.* Complex patterns of speciation in cosmopolitan "rock posy" lichens--discovering and delimiting cryptic fungal species in the lichen-forming *Rhizoplaca melanophthalma* species-complex (Lecanoraceae, Ascomycota). *Mol Phylogenet Evol* **59**, 587-602, doi:10.1016/j.ympev.2011.03.020 (2011).

Leavitt, S. D. *et al.* Local representation of global diversity in a cosmopolitan lichen-forming fungal species complex (*Rhizoplaca*, Ascomycota). *Journal of Biogeography* **40**, 1792-1806, doi:10.1111/jbi.12118 (2013).

Rosentreter, R. Vagrant Lichens in North America. *The Bryologist* **96**, 333-338 (1993).

Feurtey, A. & Stukenbrock, E. H. Interspecific Gene Exchange as a Driver of Adaptive Evolution in Fungi. *Annu Rev Microbiol* **72**, 377-398, doi:10.1146/annurev-micro-090817-062753 (2018).

Leavitt, S. D. *et al.* Resolving evolutionary relationships in lichen-forming fungi using diverse phylogenomic datasets and analytical approaches. *Sci Rep* **6**, 22262, doi:10.1038/srep22262 (2016).

Leavitt, S. D. *et al.* DNA barcode identification of lichen-forming fungal species in the *Rhizoplaca melanophthalma* species-complex (Lecanorales, Lecanoraceae), including five new species *MycoKeys* **7**, 1–22, doi:10.3897/mycokeys.7.4508 (2013).

Leavitt, D. H., Keuler, R., Newberry, C. C., Rosentreter, R. & St. Clair, L. Shotgun sequencing decades-old lichen specimens to resolve phylogenomic placement of type specimens. *Plant and Fungal Systematics* **64**, xx-xx (2019).

Grewe, F., Huang, J. P., Leavitt, S. D. & Lumbsch, H. T. Reference-based RADseq resolves robust relationships among closely related species of lichen-forming fungi using metagenomic DNA. *Sci Rep* **7**, 9884, doi:10.1038/s41598-017-09906-7 (2017).

McCune, B. & Rosentreter, R. *Biotic Soil Crust Lichens of the Columbia Basin*. Vol. 39 (Northwest Lichenologists, 2007).

Leavitt, S. D. *et al.* Cryptic diversity and symbiont interactions in rock-posy lichens. *Mol Phylogenet Evol* **99**, 261-274, doi:10.1016/j.ympev.2016.03.030 (2016).

Schoch, C. L. *et al.* Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proc Natl Acad Sci U S A* **109**, 6241-6246, doi:10.1073/pnas.1117018109 (2012).

Schoch, C. L. *et al.* 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, doi:10.1073/pnas.1117018109 (2012).

Simao, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V. & Zdobnov, E. M. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* **31**, 3210-3212, doi:10.1093/bioinformatics/btv351 (2015).

Bertels, F., Silander, O. K., Pachkov, M., Rainey, P. B. & van Nimwegen, E. Automated reconstruction of whole-genome phylogenies from short-sequence reads. *Mol Biol Evol* **31**, 1077-1088, doi:10.1093/molbev/msu088 (2014).

Zeng, Q. *et al.* Comparative genomics of Spiraeoideae-infecting *Erwinia amylovora* strains provides novel insight to genetic diversity and identifies the genetic basis of a low-virulence strain. *Mol Plant Pathol* **19**, 1652-1666, doi:10.1111/mpp.12647 (2018).

Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. *Nat Methods* **9**, 357-359, doi:10.1038/nmeth.1923 (2012).

Stanke, M., Steinkamp, R., Waack, S. & Morgenstern, B. AUGUSTUS: a web server for gene finding in eukaryotes. *Nucleic Acids Res* **32**, W309-312, doi:10.1093/nar/gkh379 (2004).

Merchant, N. *et al.* The iPlant Collaborative: cyberinfrastructure for enabling data to discovery for the life sciences. *Plos Biology* **14**, doi:10.1371/journal.pbio.1002342 (2016).

Goff, S. A. *et al.* The iPlant Collaborative: Cyberinfrastructure for Plant Biology. *Front Plant Sci* **2**, 34, doi:10.3389/fpls.2011.00034 (2011).

Misof, B. *et al.* Selecting informative subsets of sparse supermatrices increases the chance to find correct trees. *BMC Bioinformatics* **14**, 348, doi:10.1186/1471-2105-14-348 (2013).

Parra, G., Bradnam, K. & Korf, I. CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. *Bioinformatics* **23**, 1061-1067, doi:10.1093/bioinformatics/btm071 (2007).

Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792-1797, doi:10.1093/nar/gkh340 (2004).

Bankevich, A. *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* **19**, 455-477, doi:10.1089/cmb.2012.0021 (2012).

Wyman, S. K., Jansen, R. K. & Boore, J. L. Automatic annotation of organellar genomes with DOGMA. *Bioinformatics* **20**, 3252-3255, doi:10.1093/bioinformatics/bth352 (2004).

Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* **30**, 772-780, doi:10.1093/molbev/mst010 (2013).

Tonini, J., Moore, A., Stern, D., Shcheglovitova, M. & Orti, G. Concatenation and species tree methods exhibit statistically indistinguishable accuracy under a range of simulated conditions. *PLoS Curr* **7**, doi:10.1371/currents.tol.34260cc27551a527b124ec5f6334b6be (2015).

Zhang, C., Rabiee, M., Sayyari, E. & Mirarab, S. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. *BMC Bioinformatics* **19**, 153, doi:10.1186/s12859-018-2129-y (2018).

Chifman, J. & Kubatko, L. Quartet inference from SNP data under the coalescent model. *Bioinformatics* **30**, 3317-3324, doi:10.1093/bioinformatics/btu530 (2014).

Solis-Lemus, C. & Ane, C. Inferring phylogenetic networks with maximum pseudolikelihood under incomplete lineage sorting. *PLoS Genet* **12**, e1005896, doi:10.1371/journal.pgen.1005896 (2016).

Nguyen, L. T., Schmidt, H. A., von Haeseler, A. & Minh, B. Q. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* **32**, 268-274, doi:10.1093/molbev/msu300 (2015).

Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q. & Vinh, L. S. UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Mol Biol Evol* **35**, 518-522, doi:10.1093/molbev/msx281 (2018).

Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A. & Jermiin, L. S. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* **14**, 587-589, doi:10.1038/nmeth.4285 (2017).

Edwards, S. V. Is a new and general theory of molecular systematics emerging? *Evolution* **63**, 1-19, doi:10.1111/j.1558-5646.2008.00549.x (2009).

Chou, J. *et al.* A comparative study of SVDquartets and other coalescent-based species tree estimation methods. *BMC Genomics* **16** (2015).

Sayyari, E. & Mirarab, S. Fast coalescent-based computation of local branch support from quartet frequencies. *Molecular Biology and Evolution* **33**, 1654-1668, doi:10.1093/molbev/msw079 (2016).

Swofford, D. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10*. Vol. Version 4.0 (2002).

Salichos, L., Stamatakis, A. & Rokas, A. Novel information theory-based measures for quantifying incongruence among phylogenetic trees. *Mol Biol Evol* **31**, 1261-1271, doi:10.1093/molbev/msu061 (2014).

Burbrink, F. T. & Gehara, M. The Biogeography of Deep Time Phylogenetic Reticulation. *Syst Biol* **67**, 743-744, doi:10.1093/sysbio/syy019 (2018).

Yu, Y. & Nakhleh, L. A maximum pseudo-likelihood approach for phylogenetic networks. *Bmc Genomics* **16**, doi:10.1186/1471-2164-16-s10-s10 (2015).

Wen, D. Q., Yu, Y., Zhu, J. F. & Nakhleh, L. Inferring phylogenetic networks using PhyloNet. *Systematic Biology* **67**, 735-740, doi:10.1093/sysbio/syy015 (2018).

Sullivan, J. & Joyce, P. Model Selection in Phylogenetics. *Annual Review of Ecology, Evolution, and Systematics* **36**, 445-466, doi:10.1146/annurev.ecolsys.36.102003.152633 (2005).

Akaike, H. in *Selected Papers of Hirotugu Akaike* (eds Emanuel Parzen, Kunio Tanabe, & Genshiro Kitagawa) 199-213 (Springer New York, 1998).

Blischak, P. D., Chifman, J., Wolfe, A. D. & Kubatko, L. S. HyDe: A Python Package for Genome-Scale Hybridization Detection. *Systematic Biology* **67**, 821-829, doi:10.1093/sysbio/syy023 (2018).

Green, R. E. *et al.* A Draft Sequence of the Neandertal Genome. *Science* **328**, 710-722, doi:10.1126/science.1188021 (2010).

Eaton, D. A. R. & Ree, R. H. Inferring Phylogeny and Introgression using RADseq Data: An Example from Flowering Plants (Pedicularis: Orobanchaceae). *Systematic Biology* **62**, 689-706, doi:10.1093/sysbio/syt032 (2013).

Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312-1313, doi:10.1093/bioinformatics/btu033 (2014).

Darriba, D., Taboada, G. L., Doallo, R. & Posada, D. jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* **9**, 772, doi:10.1038/nmeth.2109 (2012).

Marques, D. A., Meier, J. I. & Seehausen, O. A combinatorial view on speciation and adaptive radiation. *Trends Ecol Evol*, doi:10.1016/j.tree.2019.02.008 (2019).

Lewontin, R. C. Hybridization as a new source of variation for adaptation to new environments. *Evolution* **20**, 315-336 (1966).

Soltis, D. E. *et al.* Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. *Biological Journal of the Linnean Society* **82**, 485-501 (2004).

Wallbank, R. W. *et al.* Evolutionary novelty in a butterfly wing pattern through enhancer shuffling. *PLoS Biol* **14**, e1002353, doi:10.1371/journal.pbio.1002353 (2016).

Bell, C. D. *et al.* Rapid diversification of *Tragopogon* and ecological associates in Eurasia. *J Evol Biol* **25**, 2470-2480, doi:10.1111/j.1420-9101.2012.02616.x (2012).

Bassham, S., Catchen, J., Lescak, E., von Hippel, F. A. & Cresko, W. A. Repeated Selection of Alternatively Adapted Haplotypes Creates Sweeping Genomic Remodeling in Stickleback. *Genetics* **209**, 921-939, doi:10.1534/genetics.117.300610 (2018).

Pogoda, C. S. *et al.* Genome streamlining via complete loss of introns has occurred multiple times in lichenized fungal mitochondria. *Ecol Evol* **9**, 4245-4263, doi:10.1002/ece3.5056 (2019).

Aguileta, G. *et al.* High variability of mitochondrial gene order among fungi. *Genome Biol Evol* **6**, 451-465, doi:10.1093/gbe/evu028 (2014).

Blair, C. & Ane, C. Phylogenetic trees and networks can serve as powerful and complementary approaches for analysis of genomic data. *Syst Biol*, doi:10.1093/sysbio/syz056 (2019).

Kohn, L. M. Mechanisms of fungal speciation. *Annu Rev Phytopathol* **43**, 279-308, doi:10.1146/annurev.phyto.43.040204.135958 (2005).

Lodé, T. Adaptive Significance and Long-Term Survival of Asexual Lineages. *Evolutionary Biology* **40**, 450-460, doi:10.1007/s11692-012-9219-y (2012).

Janko, K. *et al.* Hybrid asexuality as a primary postzygotic barrier between nascent species: On the interconnection between asexuality, hybridization and speciation. *Mol Ecol* **27**, 248-263, doi:10.1111/mec.14377 (2018).

Pizarro, D. *et al.* Whole-Genome Sequence Data Uncover Widespread Heterothallism in the Largest Group of Lichen-Forming Fungi. *Genome Biol Evol* **11**, 721-730, doi:10.1093/gbe/evz027 (2019).

Kroken, S. & Taylor, J. W. Outcrossing and recombination in the lichenized fungus *Letharia*. *Fungal Genetics and Biology* **34**, 83-92, doi:10.1006/fgbi.2001.1291 (2001).

Buschbom, J. & Mueller, G. M. Testing "species pair" hypotheses: evolutionary processes in the lichen-forming species complex *Porpidia flavocoerulescens* and *Porpidia melinodes*. *Mol Biol Evol* **23**, 574-586, doi:10.1093/molbev/msj063 (2006).

Honegger, R. & Zippler, U. Mating systems in representatives of Parmeliaceae, Ramalinaceae and Physciaceae (Lecanoromycetes, lichen-forming ascomycetes). *Mycol Res* **111**, 424-432, doi:10.1016/j.mycres.2007.02.005 (2007).

Tripp, E. A. Is asexual reproduction an evolutionary dead end in lichens? *The Lichenologist* **48**, 559-580, doi:10.1017/s0024282916000335 (2016).

Roper, M., Ellison, C., Taylor, J. W. & Glass, N. L. Nuclear and genome dynamics in multinucleate ascomycete fungi. *Curr Biol* **21**, R786-793, doi:10.1016/j.cub.2011.06.042 (2011).

Clutterbuck, A. J. Parasexual recombination in fungi. *Indian Academy of Sciences* **75**, 281-286 (1996).

Ertz, D., Guzow-Krzeminska, B., Thor, G., Lubek, A. & Kukwa, M. Photobiont switching causes changes in the reproduction strategy and phenotypic dimorphism in the Arthoniomycetes. *Sci Rep* **8**, 4952, doi:10.1038/s41598-018-23219-3 (2018).

Spribille, T. Relative symbiont input and the lichen symbiotic outcome. *Curr Opin Plant Biol* **44**, 57-63, doi:10.1016/j.pbi.2018.02.007 (2018).

Figures and Tables

Figures

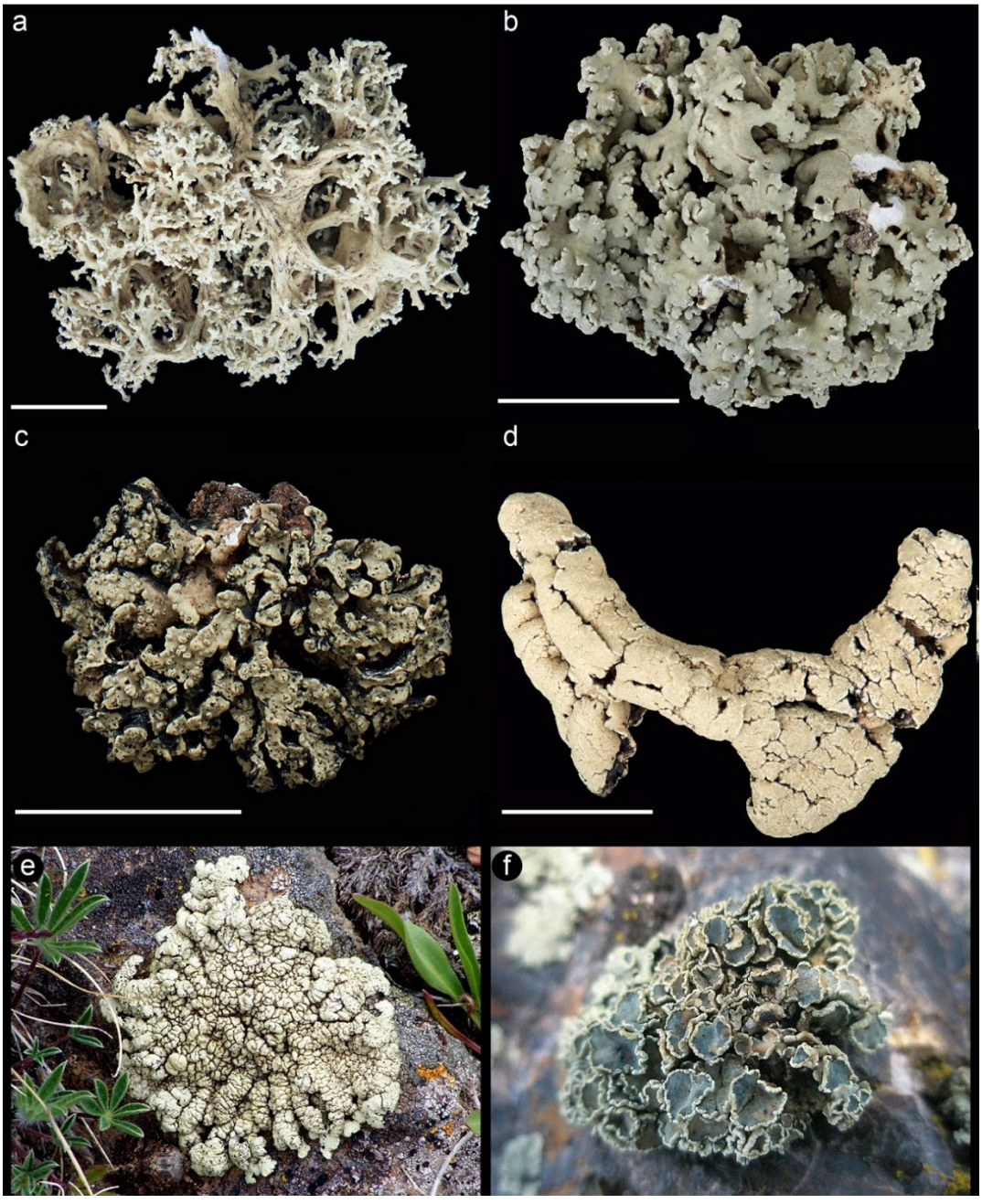

Figure 1. Morphological variation in the *Rhizoplaca melanophthalma* complex. (a) vagrant taxon *Rhizoplaca arbuscula*; (b) vagrant taxon *Rhizoplaca haydenii*; (c) a member of the 'saxicolous *haydenii* population', a putative hybrid population (*Leavitt 715* [BRY-C]); (d) vagrant taxon *Rhizoplaca idahoensis*; (e) saxicolous *Rhizoplaca shushanii*, a taxon inferred here to be of hybrid origin – field

image; and (e) a member of the saxicolous *Rhizoplaca porteri* group with abundant fruiting bodies (apothecia) – field image.

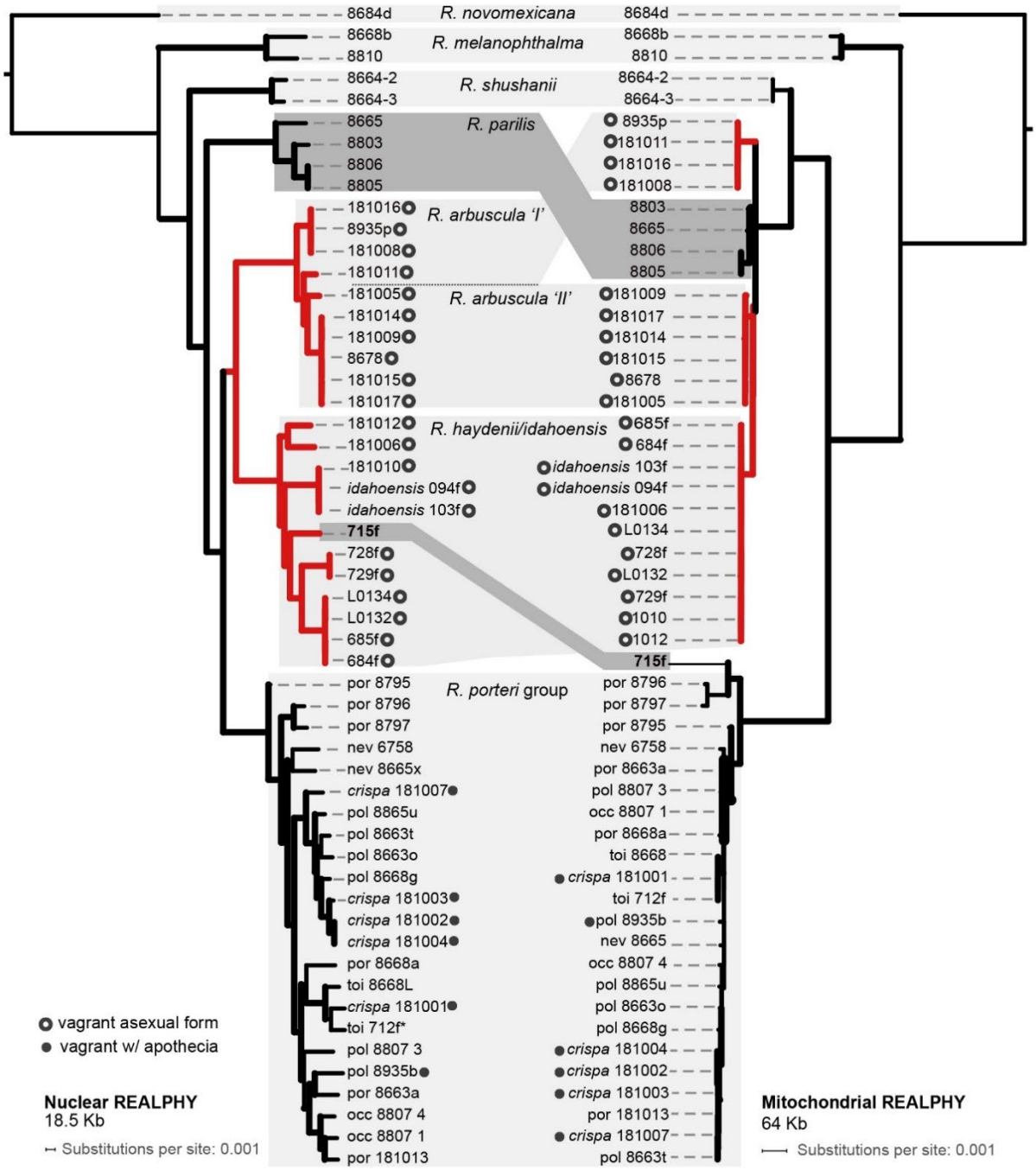

Figure 2. Topologies inferred from nuclear and mitochondrial REALPHY datasets. Thicker lines indicate 95+ bootstrap support. Corresponding clades in the nuclear and mitochondrial phylogenies are highlighted. Red branches indicate 'vagrant clade'.

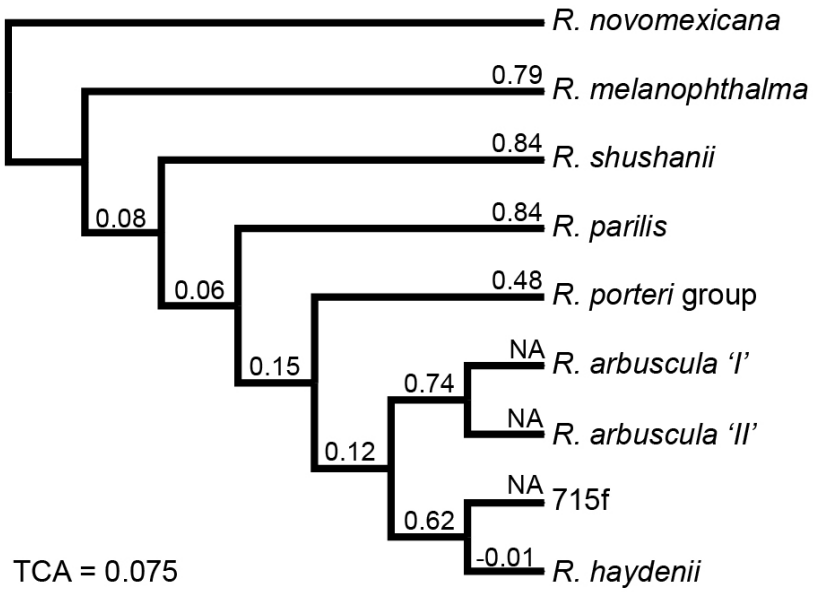

Figure 3. Species tree of the *R. melanophthalma* species complex inferred using ASTRAL-III and SVDquartet+PAUP*. Both species tree approaches inferred identical branching patterns and all nodes were recovered with 100% support. Values above branches correspond to internode certainty (IC) estimated from 407 individual BUSCO gene trees, reflecting the specific degree of incongruence for that internode (scaled between 0 and 1, values closer to 1 indicate no or limited conflict for a given internode, whereas values closer to 0 indicate increasing conflict). The relative tree certainty (TCA) is shown in the bottom left, characterizing the global degree of incongruence among individual gene trees.

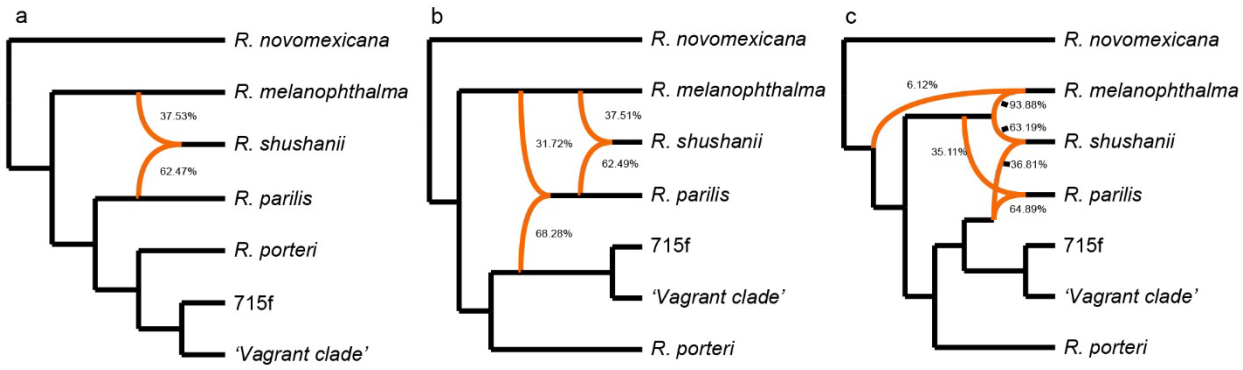

Figure 4. Phylogenetic networks inferred by PhyloNet using Maximum Pseudolikelihood under a one- (a), two- (b), and three-reticulation model (c). AIC supported the three-reticulation model as the best-fitting scenario. Orange branches indicate lineages involved in reticulated histories; and numerical values are the inheritance probabilities for each reticulation.

Tables

Table 1. Summary of phylogenomic datasets and associated analyses. The nuclear REALPHY datasets were generated using contigs >5kb from a draft genome assembly of *R. melanophthalma* as the reference; the mitochondrial REALPHY datasets were generated using the longest assembled mitochondrial contig; the 'BUSCO 1kb+ SNPs' dataset represents all polymorphic sites from concatenated alignments of all BUSCO gene regions >1kb; and the '407 CEG trees' are gene topologies inferred from 407 core eukaryotic gene regions passing quality filters. Alignment lengths and percentage of missing data are given for each alignment.

Dataset	Alignment length	Missing data	Analyses
nuclear REALPHY	18,457,947	6.0%	Concatenated ML (IQ-TREE)
mtDNA REALPHY	63,877	12.4%	Concatenated ML (IQ-TREE)
BUSCO 1kb+ SNPs	142,437	7.0%	Species tree inferred using quartet amalgamation (SVDquartets) & ABBA/BABA test of introgression (HyDe)
407 CEG trees	NA	0%	Summary species tree (ASTRAL-III)/ network approach incorporating both incomplete lineage sorting and hybridization (MPL, PhyloNet)

Table 2. PhyloNet results and AIC calculations to select optimal network, with the optimal network in bold. *L* is the likelihood value, and *k* is number of reticulations plus branch lengths (number of parameters used in the AIC calculation).

# Reticulations	ln*L*	Δln*L*	# Branch lengths	*k*	AIC	ΔAIC
0	-3997622		7	7	7995257.07	8583.05408
1	-3995167	2454.80068	7	8	7990349.47	3675.45272
2	-3994063	1104.14995	7	9	7988143.17	1469.15281
3	**-3993325**	**737.576407**	**9**	**12**	**7986674.02**	**0**
4	-3993355	-29.658988	7	11	7986731.33	57.3179761
5	-3993584	-229.53622	9	14	7987196.41	522.390411

Table 3. HyDe inferences of hybridization in the *Rhizoplaca melanophthalma* species complex. Only putative hybridization events with P-values <0.05 are indicated; results are sorted according the highest Z-scores. 'P1' corresponds to an initial donor species, 'Hybrid' represents the species containing putatively introgressed loci, and 'P2' is a second hybridizing donor. The gamma parameter is an estimate the amount of admixture/introgression from P2 into the hybrid.

P1	Hybrid	P2	Z-score	P-value	Gamma
R. arbuscula	*R. shushanii*	*R. melanophthalma*	**13.49**	0	0.81
R. parilis	*R. shushanii*	*R. melanophthalma*	**13.15**	0	0.81
R. melanophthalma	*R. shushanii*	*R. porteri*	**12.86**	0	0.82
R. haydenii	*R. shushanii*	*R. melanophthalma*	**11.74**	0	0.82
R. '715f'	*R. shushanii*	*melanophthalma*	**11.26**	0	0.19
R. arbuscula	*R. haydenii*	*R. parilis*	**5.14**	1.35E-07	0.90
R. arbuscula	*R. haydenii*	*R. porteri*	**5.01**	2.67E-07	0.77
R. arbuscula	*R. haydenii*	*R.* '715f'	2.82	0.002	0.03
R. shushanii	*R. arbuscula*	*R.* '715f'	2.81	0.002	0.04
R. arbuscula	*R.* '715f'	*R. parilis*	2.79	0.003	0.93
R. arbuscula	*R.* '715f'	*R. porteri*	2.48	0.007	0.85
R. shushanii	*R. arbuscula*	*R. haydenii*	2.45	0.007	0.03
R. arbuscula	*R.* '715f'	*R. melanophthalma*	2.39	0.008	0.98
R. arbuscula	*R. parilis*	*R. parilis*	2.38	0.009	0.91
R. parilis	*R. haydenii*	*R. porteri*	2.27	0.012	0.08
R. arbuscula	*R. haydenii*	*R. melanophthalma*	2.23	0.013	0.98
R. shushanii	*R. parilis*	*R. haydenii*	2.22	0.013	0.10
R. parilis	*R.* '715f'	*R. melanophthalma*	2.22	0.013	0.97
R. shushanii	*R. arbuscula*	*R. porteri*	2.18	0.015	0.05
R. shushanii	*R. parilis*	*R.* '715f'	2.08	0.019	0.10
R. shushanii	*R. parilis*	*R. porteri*	2	0.023	0.10
R. melanophthalma	*R.* '715f'	*R. porteri*	1.9	0.029	0.02
R. '715f'	*R. haydenii*	*R. porteri*	1.79	0.037	0.98
R. parilis	*R. haydenii*	*R.* '715f'	1.72	0.043	0.01

Chapter 2

Interpreting phylogenetic conflict: Hybridization in the most speciose genus of lichen-forming fungi

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Abstract

While advances in sequencing technologies have been invaluable for understanding evolutionary relationships, increasingly large genomic data sets may result in conflicting evolutionary signals which are often caused by biological processes, including hybridization. Hybridization has been detected in a variety of organisms, influencing evolutionary processes, such as generating reproductive barriers and mixing standing genetic variation. Here, we investigate the potential role of hybridization in the diversification of the most speciose genus of lichen-forming fungi, *Xanthoparmelia.* As *Xanthoparmelia*

is projected to have gone through a recent rapid diversification, this genus is particularly suitable for investigating and interpreting the origins of phylogenomic conflict. Focusing on a clade of *Xanthoparmelia* largely restricted to the Holarctic, we used a genome skimming approach to generate 962 single-copy gene regions representing over 2 Mbp of the mycobiont genome. From this genome-scale dataset, we inferred evolutionary relationships using both concatenation and coalescent-based species tree approaches. We also used three independent tests for hybridization. Although different species tree reconstruction methods recovered largely consistent and well-supported trees, there was widespread incongruence among gene trees. Despite challenges in differentiating hybridization from ILS in situations of recent rapid radiations, our genome-wide analyses detected multiple potential hybridization events in the Holarctic clade, suggesting one possible source of trait variability in this hyperdiverse genus. This study highlights the value in using a pluralistic approach for characterizing genome-scale conflict, even in groups with well-resolved phylogenies, while highlighting current challenges in detecting the specific impacts of hybridization.

Introduction

Increasingly large-scale genomic data sets have been instrumental for resolving phylogenetic relationships (Jarvis et al. 2014) and advancing our understanding of evolutionary processes. However, extensive conflict among phylogenetic trees inferred from different regions of the nuclear genome have consistently been revealed in recent studies (Jeffroy et al. 2006, Shen et al. 2017, Smith et al. 2015), in addition to conflict among nuclear, mitochondrial and plastid genomes (Rose et al. 2020, Soltis et al. 2004a, Toews and Brelsford 2012). While many methods of phylogenetic inference aim to reduce the conflict to determine the emergent phylogenetic signal, investigating the causes of the conflict may reveal important evolutionary processes driving diversification (Smith et al. 2015).

Phylogenetic conflict among different loci within a genome are frequently generated by well-known biological causes, such as incomplete lineage sorting (ILS), hybridization, and gene duplication (Jeffroy et al. 2006, Maddison 1997). Hybridization in particular has been increasingly detected across a wide range of organismal groups (Mallet et al. 2016, Runemark et al. 2019), especially in lineages in which highly conflicting gene trees are frequently recovered from different genomic regions (Diaz et al. 2018, Smith et al. 2015, Tang et al. 2018, Zhang et al. 2021). Hybridization can influence evolution in a number of ways, including generating reproductive barriers (Büker et al. 2013), altering the rate of adaptation (Marques et al. 2019a, Samarasinghe et al. 2020), and introducing novel phenotypes (Stankowski and Streisfeld 2015). Here, we use the term “hybridization” to refer to any reticulated evolutionary history, encompassing hybrid speciation, the development of a new species from the reproduction between two intraspecific parents, and introgression, the transfer of genetic material via repeated hybrid backcrossing.

Because hybridization and ILS can occur in similar scenarios—e.g., in recently diverged lineages or rapid radiations that still share ancestral polymorphisms—and can both manifest in phylogenomic datasets as a gene tree incongruence, distinguishing hybridization from ILS remains challenging (Joly et

al. 2009, Reddy et al. 2017). A variety of analytical approaches have been developed to assist distinguishing ILS from hybridization (reviewed in Blair and Ané 2019), including phylogenetic network methods (Solis-Lemus and Ane 2016, Wen et al. 2018a), attempts to detect phylogenetic variants (Blischak et al. 2018a, Durand et al. 2011) and assessing the distribution of internal branch lengths (Edelman et al. 2019). In conjunction with phylogenetic tree reconstruction methods that do not account for hybridization, like supermatrix approaches and many multispecies coalescent approaches, models explicitly accounting for hybridization can be used to infer a more comprehensive perspective of evolutionary history (Blair and Ané 2019).

Hybridization has been shown to be an important evolutionary process among fungi, especially in model species that are amenable to experimental crosses and have relatively fast generation times, like plant pathogens and yeasts (Feurtey and Stukenbrock 2018). Like other organisms, hybridization among fungi can generate reproductive isolation (Anderson et al. 2010), promote adaptive radiation (Feurtey and Stukenbrock 2018), and modify phenotypes (Brion et al. 2020). Genomic conflict can be an indication of hybridization among fungi (Du et al. 2020, Feurtey et al. 2019, Short et al. 2014). In addition to gene tree incongruence, discordance between the mitochondrial and nuclear trees can also be a sign of hybridization (Hill 2017). Mito-nuclear discordance can cause reproductive isolation (Chou et al. 2010), hybrid sterility (Lee et al. 2008), and increased or decreased fitness of the hybrids (Giordano et al. 2018).

Hybridization among lichen-forming fungi, however, has been less studied. A number of studies have detected patterns that are consistent with hybridization (Culberson and Hale 1973b, Ertz et al. 2009, Fontaine et al. 2010, Kroken and Taylor 2001a, Piercey-Normore et al. 2010, Steinová et al. 2013), but few have explicitly tested for it (Keuler et al. 2020, Widhelm et al. 2019). Testing explicitly for hybridization among lichen-forming fungi could inform our understanding of the evolutionary histories that may have led to phylogenetic conflict, ultimately helping resolve relationships and delimit species.

In this study, we investigate the potential for hybridization in a clade of the most diverse genus of lichen-forming fungi *Xanthoparmelia* (Vain.) Hale (Lücking et al. 2017). The monophyletic 'Holarctic clade' in *Xanthoparmelia,* made up of Asian, European, and North American species, originated roughly

7 million years ago (Leavitt et al. 2018). *Xanthoparmelia* exhibits a pattern of diversification that coincides with global aridification, and it has been hypothesized that this rapid radiation was due to expansion into drier habitats (Leavitt et al. 2018, Lumbsch et al. 2008). In the *Xanthoparmelia* Holarctic clade, there is an unusual diversity of secondary metabolites (salazinic, stictic, psoromic acids, etc.) and distinct reproductive strategies (specialized asexual lichen propagules [isidia], vegetative fragmentation, and sexual fungal reproduction [e.g., apothecia]). Additionally, there are a number of ecologically divergent species, like vagrant lichens which grow unattached on the soil, unlike most species of *Xanthoparmelia* which grow attached to rock substrates. The vagrant forms also evolved independently multiple times within the Holarctic clade (Leavitt et al. 2011a) and reproduce primarily asexually by vegetative fragmentation (Rosentreter 1993). The occurrence of potentially adaptive traits occurring in polyphyletic lineages throughout this clade suggests potential for adaptive traits that may have arisen through hybridization (Arnold and Kunte 2017). Because rapid radiations may be driven in part by hybridization (Feurtey and Stukenbrock 2018, Meyer et al. 2017), *Xanthoparmelia* is an ideal group to investigate these processes in. Previous research has revealed the potential for admixed nuclear genomes and ancestry assignment probabilities to multiple genetic clusters for a number of species within the Holarctic *Xanthoparmelia* clade, although the inference was based on limited genetic markers (Leavitt et al. 2011b).

With limited insight into the origins of the high diversity in the ultradiverese genus *Xanthoparmelia* (Leavitt et al. 2018, Lumbsch et al. 2008) and remaining uncertainty in phylogenetic relationships, here we use genomic data to investigate the evolutionary history of the Holarctic *Xanthoparmelia* clade. Specifically, our aims were to (i) reconstruct a robust phylogenetic hypothesis from multiple sequence alignments from hundreds of loci, (ii) characterize conflict in our genome-scale data, and (iii) investigate the potential for hybridization in the evolutionary history of this clade. Using a pluralistic approach, we provide a well-resolved phylogeny for this lineage, while recognizing rampant phylogenomic conflict, including evidence suggesting a role of hybridization in this clade.

Methods

Taxon sampling

Within the Holarctic clade in *Xanthoparmelia* (Leavitt et al. 2018) we selected representatives from species-level lineages recovered from previous studies (Leavitt et al. 2013d), in addition to a number of more recently recognized lineages. Phylogenomic data was generated from 65 specimens spanning the range of phylogenetic diversity and representing ca. 27 candidate species-level lineages circumscribed using multilocus sequence data (Supplementary Table 1). Because traditional taxonomy and genetic-based species circumscriptions are frequently incongruent in this clade of *Xanthoparmelia*, provisional clade and candidate species nomenclature generally follow that proposed in Leavitt et al. (2011b). Specimens were sampled to represent: i) both vagrant vs. rock-dwelling forms, ii) diverse secondary metabolites (salazinic, stictic, psoromic acids, etc.), and (iii) distinct reproductive strategies (isidia, vegetative fragmentation, and sexual fungal reproduction) (Supplementary Table 1). *Xanthoparmelia semiviridis* was selected as the outgroup based on previous reconstructions placing it as sister to the Holarctic clade (Leavitt et al. 2018).

DNA extraction and sequencing

Metagenomic reads were newly generated from a total of 47 *Xanthoparmelia* specimens, in addition to previously published metagenomic data from 18 specimens (Leavitt et al. 2018). For data generated for this study, total genomic DNA was extracted from a small portion of the lichen thallus using the ZR Fungal/Bacterial DNA Miniprep Kit (Zymo Research). Illumina sequencing libraries were constructed following the manufacturer's recommendations, using Illumina's TruSeq DNA PCR-Free Library Preparation Kit or the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA). TruSeq libraries were sequenced on Illumina MiSeq at the Pritzker Laboratory for Molecular Systematics and Evolution at the Field Museum, Chicago, USA. The Nextera libraries were sequenced on the NextSeq platform at the Core Genomics Facility at the University of Illinois at Chicago, USA. For the remaining 18 specimens, total genomic DNA was extracted using the E.Z.N.A Plant DNA DS Mini Kit (Omega

Bio-tek) following manufacturer's recommendations. High-throughput sequencing libraries were prepared from the total genomic DNA, using the standard Illumina whole-genome sequencing (WGS) library preparation process with Adaptive Focused Acoustics for shearing (Covaris). This was followed by AMPure cleanup. DNA was processed using the NEBNext® Ultra™ II End Repair/dA-Tailing Module and the NEBNext® Ultra™ II Ligation Module (New England Biolabs) with standard Illumina index primers. Libraries were run on the Illumina HiSeq with 2×125 paired-end sequencing at the DNA Sequencing Center located on the campus of Brigham Young University, Provo, Utah, USA.

Read filtering and phylogenomic data matrices

Raw reads were trimmed using Trimmomatic v0.39 (Bolger et al. 2014) to remove adapter and primer sequences and low-quality reads. Bases at the start and end of reads were trimmed when they had a quality below 3 and 10, respectively, as well as when the quality of 5-bp sliding windows was < 20. All trimmed reads < 36 bp were filtered out. To identify single-copy nuclear genes for phylogenomic reconstructions, we used Benchmarking Universal Single-Copy Orthologs to extract up to 1438 gene regions (BUSCO; Seppey et al. 2019). Contigs from a SPAdes assembly of paired end reads from specimen *X*. aff. *chlorochroa* (818f) were analyzed in BUSCO in the Cyverse.org Discovery Environment (Goff et al. 2011, Merchant et al. 2016a) using the fungal BUSCO database to extract single-copy gene regions from the draft assembly. The filtered BUSCO genes were used as targets for bait sequence capture using HybPiper (Johnson et al. 2016) to extract these genes regions from each sample.

MAFFT (Katoh and Standley 2013) was used to generate alignments for individual BUSCO genes using the default parameters, and the alignment algorithm for each locus was chosen automatically by MAFFT. For each alignment, any sample which had an average completion (assembly length/target length) of < 0.20 was removed. Genes with average coverage < 75% across all BUSCO genes were removed. Phylogenetic gene trees were reconstructed using maximum likelihood (ML) as implemented by IQ-TREE (Nguyen et al. 2015). The substitution model used for each tree was chosen by ModelFinder (Kalyaanamoorthy et al. 2017). We used PhyParts (Smith et al. 2015) to characterize potential conflict

among the gene trees passing quality filtering. A bipartition analysis was conducted using the default bootstrap filter, ignoring edges with bootstrap values < 50% (see Smith et al. 2015). We note that implementing a higher bootstrap filter value, e.g. 95%, would likely result in an increasing proportion of unsupported relationships (Hoang et al. 2018). The results were visualized on the phylogeny inferred from the concatenated supermatrix using the script phypartspiecharts.py (https://github.com/mossmatters/MJPythonNotebooks).

Because a discordance between nuclear and mitochondrial trees can be evidence of hybridization (Hill 2017), we assembled a mitochondrial dataset. We performed de novo assemblies in SPAdes and identified mitochondrial contigs from each specimen using BLAST and *Xanthoparmelia* mitochondrial sequences downloaded from GenBank. From these, we selected the longest mitochondrial contig—76.5 Kb from *X. stenophylla* (14368)—as the target and used RealPhy v1.12 to align short reads from all samples to generate an alignment. We used the following RealPhy parameters: -readLength 100 -perBaseCov 5 -gapThreshold 0.2, running a rapid bootstrap analysis through RAxML v7.3.0 (Stamatakis 2014). The mitochondrial topology was reconstructed using IQ-TREE (Nguyen et al. 2015), with the substitution model selected using ModelFinder (Kalyaanamoorthy et al. 2017) and nodal support assessed using 1,000 ultra-fast bootstrap replicates (Hoang et al. 2018).

Species tree inference from phylogenetic data

We inferred species trees using both concatenation and coalescent-based summary methods to compare differences in topologies reconstructed under different assumptions, especially in the presence of gene flow and ILS. Because concatenation approaches can provide accurate inferences under a range of conditions (Tonini et al. 2015), we used IQ-TREE v1.6.9 to generate a maximum-likelihood tree from the concatenated BUSCO supermatrix (962 genes, 2.5 MBp alignment), using FASconCAT-G to concatenate each BUSCO gene alignment into a supermatrix. The best-fitting substitution model (TIM2+F+ASC+R6) was selected using ModelFinder, and nodal support was assessed with 1,000 ultra-fast bootstrap replicates (Hoang et al. 2018).

However, concatenation approaches can produce erroneous tree topologies in the presence of ILS (Edwards et al. 2016, Liu et al. 2015), potentially producing high support for the incorrect tree (Bapteste et al. 2008, Warnow 2015). Therefore, we used two coalescent-based species trees methods that accommodate ILS, ASTRAL-III v5.6.3 (Zhang et al. 2018) and SVDQuartets (Chifman and Kubatko 2014) implemented in PAUP* v4.0a167 (Swofford 2002). ASTRAL-III uses individual gene trees to generate a summary species tree, calculating quartet scores on each node. In ASTRAL-III, we generated a summary species tree based on the 962 BUSCO gene trees, with local posterior probabilities calculated at each node (Sayyari and Mirarab 2016). While it is possible to group taxa by species in ASTRAL-III, we elected not to given the uncertainty of species boundaries. We also generated a species tree using SVDQuartets (Chifman and Kubatko 2014) and inferred from the concatenated BUSCO gene alignments as input, and support for the final species tree was assessed using all possible quartets and n=100 bootstrap replicates. We did not group by species in SVDQuartets, keeping each taxon separate due to species boundary uncertainty in this clade.

We used SODA, an ultra-fast species-tree delimitation approach implemented in ASTRAL (Rabiee and Mirarab 2021), to infer candidate species to test for hybridization in a downstream analysis. SODA uses frequencies of quartet topologies to determine if each branch in a guide tree inferred from gene trees is likely to have a positive length, and it uses the results to infer a new species tree that defines species boundaries. We ran SODA with a p-value cut-off of 0.001. SVDQuartets, the second species tree method implemented here, infers relationships among quartets, incorporating sources of variability and estimating the species tree directly from site patterns.

Tests for hybridization

While coalescent-based species tree methods account for ILS, most current methods assume there is no hybridization and can therefore reconstruct the incorrect species tree topologies in the presence of gene flow (Solis-Lemus et al. 2016). Furthermore, hybridization among taxa can be more accurately represented with phylogenetic networks rather than bifurcating models (Blair and Ané 2019, Burbrink and

Gehara 2018). However, many methodological approaches accounting for both ILS and hybridization are computationally intensive, with limitations to the number of terminals that can effectively be analyzed (Rose et al. 2020). Computational limitations with larger datasets (>20 terminals) may require that data can be subdivided, focusing on specific questions. Here we investigated the potential for hybridization among deeper-level lineages rather than individual terminals or putative species-level groups. Leavitt et al. (2011b) described eight major groups within the Holarctic clade, and we used the same groups to divide our taxa into eight groups—A, B3, B4, C, D, E, F (including G), and H—as well as three single-taxon lineages—*X. microspora* (17358), *X*. aff. *dierythra* (098f), and *X.* aff. *dierythra* (14899). We delimited the B clade into two groups, B3 and B4, due to the variable placement of these lineages in species tree reconstructions (see Results). We used the maximum pseudolikelihood (MPL) approach (Yu and Nakhleh 2015) implemented in Phylonet v3.6.8 (Wen et al. 2018a), which takes into consideration both ILS and hybridization. Using on the 962 BUSCO gene trees, we ran the "InferNetwork_MPL" algorithm assigning each terminal to the clade it was recovered in, performing 20 independent searches to avoid sampling in local optimums. We used the Akaike information criterion (AIC) to select the best-fitting network, by applying the number of parameters (*k*) as the number of branch lengths and the number of reticulations with L as the likelihood value. Phylonet is computationally intensive, and we were unable to run more than four reticulations due to computational limitations, exceeding the Office of Research Computing HPC walltime limit of 7 days. Similarly, analyses failed to complete in exploratory runs with the complete 65-terminal dataset and using the 962 BUSCO gene trees as inputs.

To further investigate hybridization, we used the Filtered Super Network method (Whitfield et al. 2008) in SplitsTree4 version 4.17.1 (Huson et al. 2004) to visualize potential areas of hybridization. This approach can help reveal the most common phylogenetic signal when there is a large amount of conflict among individual gene trees. We ran three separate analyses using the Z-closure method (Huson et al. 2004) and filtering to keep splits present in, or fully compatible with, a minimum of 25%, 50%, or 75% of trees, or 240, 481, and 721, respectively, of the BUSCO gene trees.

We used HyDe (Blischak et al. 2018a) to detect introgression using the concatenated BUSCO supermatrix. HyDe uses a rooted, four-taxon network of triplet populations (two parent populations and a hybrid population) and one outgroup to detect phylogenetic invariants, assessing the relative frequencies of ABBA and BABA (Green et al. 2010a, Martin et al. 2015). We partitioned taxa into the same 11 clades as with the Phylonet analysis to detect potential introgression among these major clades within the Holarctic group. In addition to the HyDe analysis on the complete BUSCO supermatrix, we also explored the impact of additional, more stringent gene alignment filtering steps on inferences of hybridization in HyDe. We applied trimAl—a heuristic method that calculates the optimal mode to remove sequence gaps from alignments (Capella-Gutierrez et al. 2009)—to the 400 longest BUSCO loci with the -automated1 flag. The 400 stringently filtered loci were subdivided into four, 100-loci subsets and analyzed individually using HyDe, in addition to the combined stringently filtered 400-loci dataset. Additionally, we ran within-clade HyDe analyses to test for hybridization among species in the same clade by partitioning taxa into SODA-delimited species and analyzing clades with more than 3 putative species. For all HyDe analyses, we incorporated a Bonferonni correction and considered significance at $\alpha < 0.05$, with estimates of γ between 0 and 1. Z-scores > 3 were interpreted as evidence of introgression.

Results

Data and phylogenomic reads

Short reads from 65 *Xanthoparmelia* specimens from the Holarctic clade sequences for this study are available in the NCBI Short Read Archive under BioProject accession # PRJNA848676. Of the 1,438 BUSCO genes searched, 1,345 complete BUSCO genes were recovered (93.5% of all BUSCO groups), with 10 completed and duplicated BUSCO genes which were excluded. From the remaining 1,335 *Xanthoparmelia* BUSCOs, 962 passed additional filtering requirements for generating the phylogenomic dataset using in subsequent analyses (Supplementary Figure 1), The concatenated alignments of 962 nuclear BUSCO markers resulted in a supermatrix of 2,490,354 nucleotide position characters; and

missing data ranged from 1.6% to 78.1%, with an average of 8.4% missing data across all samples. The REALPHY genome skimming approach for generating mitochondrial data resulted in an alignment of 44,251 bp; and missing data ranged from 0.0% to 20.0%, with an average of 4.3% missing data across all samples.

Phylogenomic reconstructions

The concatenation approach, IQ-TREE (Fig. 1), and the two coalescent-based methods which account for ILS, ASTRAL-III (Fig. 2) and SVDQuartets (Supplementary Fig. 2), all consistently recovered the same seven distinct clades: A, B, C, D, E, H, F, and 3 lineages represented by single specimens. ASTRAL-III nested the A clade within the B clade, while SVD-Quartets and IQ-TREE placed the A clade as sister to the entire B clade. ASTRAL-III and IQ-TREE placed H as sister to F, while SVDQuartets placed H as sister to the C, D, and E clades. While IQ-TREE and ASTRAL-III had well-supported backbones, the relationships between clades A-B and the rest of the clade, as well as between H and sister clades C-D-E, are weakly supported. Species-level lineages were consistent between trees, with the exception of SVDQuartets inference that placed *X*. aff. *coloradoensis* (135f) as sister to the rest of E and *X*. aff. *chlorochroa* (L0300) diverging earlier from the rest of its inferred species-level lineage. The SODA species delimitation analysis yielded 42 candidate species (Fig. 2).

PhyParts indicated widespread discordance among gene trees across the Holarctic clade (Fig. 1). 82.8% of the nodes conflicted with the supermatrix topology at their respective node, instead supporting an alternative topology that differed from the most common bipartition.

The mitochondrial tree inferred using IQ-TREE recovered the same 11 clades, including the three single-specimen lineages, as those inferred from the nuclear BUSCO dataset (Fig. 3). There was no evidence of taxa harboring mitochondrial DNA from clades that differed from their nuclear DNA. The backbone of the mitochondrial tree differed slightly from the nuclear IQ-TREE backbone; in the mitochondrial tree, the A clade emerged later than B, and the H clade was placed as sister to C, D, E, and F rather than just F.

Evidence of hybridization

MPL, used to test for hybridization and implemented in Phylonet using individual gene trees, provided support for multiple introgression events at deeper evolutionarily scales in the Holarctic *Xanthoparmelia* clade (Fig. 4). AIC supported four reticulations as the best-fitting model (Table 1); however, due to limitations we were unable to test more than 4 reticulation events, and it is possible that the optimal network is represented by more than 4 reticulations. MPL results showed that most clades had experienced gene flow at some point, with clades A, C, D, and E all inferred to have played a role in the reticulated evolutionary history. The C, D, and A clades each inherited a portion of their genetic material from ancestors that are either extinct or unsampled. Clade A shared loci with the most recent common ancestor (MRCA) of B3, C shared loci with the MRCA of D, and D shared loci with a MRCA of F. Clade E, according to Phylonet, is a hybrid of C and D. The reticulate history depicted with reticulations among clades A, C, and E clades is also reflected in the next two best-scoring networks (Supplementary Fig. 3).

In SplitsTree, we tested hybridization using 25%, 50%, and 75% of gene trees. 25% of the gene trees showed ancestral hybridization within and between A, B, and C clades (Fig. 5). In 50% of the trees hybridization was only inferred within the A clade (Supplementary Fig. 4); and no hybridization was detected when considering the minimum of 75% of the trees.

The HyDe analysis, which used the concatenated BUSCO gene alignments to assess relative frequencies of phylogenetic invariants, revealed hybridization across the Holarctic clade, with 123 out of 496 significant triplet comparisons (all with Z-scores > 3; Supplementary Table 2). Hybridization was inferred among all major clades. Additional HyDe analyses of the more stringently filtered loci were consistent with the initial analysis supporting widespread hybridization (Supplementary Table 3). To test for hybridization within major clades, we also ran HyDe using the candidate species inferred in SODA in clades with more than three putative species (B, C, E, and F). The B, C, E, and F clades had 4, 9, 13, and 122 significant triplet comparisons, respectively (Supplementary Tables 4-7). Overall, the results of the HyDe analyses suggest hybridization at deeper levels, as well as between species within clades.

Discussion

In this study, we generated a genome-scale dataset spanning nearly 2.5 Mbp and 962 single copy gene regions of the fungal genome from 65 specimens in the Holarctic clade of the most speciose genus of lichen-forming fungi, *Xanthoparmelia* (Leavitt et al. 2019b). While relatively consistent phylogenies were inferred from phylogenomic data using both concatenation and coalescent-based species tree methods, we found evidence of widespread hybridization in the phylogenomic data with support from three distinct approaches. We did not detect mito-nuclear discordance/introgression among major lineages in the Holarctic clade, although discordance was not assessed within the major lineages (Fig. 3). Nonetheless, our genome-wide analyses detected multiple hybridization events in the Holarctic clade of *Xanthoparmelia* and sets the stage for future work investigating hybridization among symbiotic fungi. This study highlights the value in investigating sources of phylogenetic conflict that may obscure evolutionary processes, even in groups with well-resolved phylogenies.

It has been hypothesized that *Xanthoparmelia* underwent a radiation during the global aridification of the Oligocene-Miocene boundary (Kraichak et al. 2015, Lumbsch et al. 2008), with at least two migration events into the Holarctic during the late Miocene or Pliocene (Leavitt et al. 2018). Discerning a clear phylogenetic signal is often challenging in clades which have undergone rapid diversification, as there may have not been enough time for polymorphisms to sort (Townsend et al. 2012). Additionally, adaptive radiations can operate in conjunction with hybridization, with the standing variation from hybridization events fueling the evolution of morphological variability among radiated organisms (Hedrick 2013a, Lewontin 1966, Marques et al. 2019b). Because alleles from hybridization are more likely to have been already selectively filtered than new mutations (Hedrick 2013a, Schluter et al. 2004), introgressed alleles can generate novel morphologies or encourage expansion into new niches more quickly than new mutations, thus facilitating rapid speciation (Barrett and Schluter 2008, Feurtey and Stukenbrock 2018, Irisarri et al. 2018, Marques et al. 2019b, Stankowski and Streisfeld 2015). This evolutionary process has been well-studied among a few model groups, such as East African cichlid fish (Irisarri et al. 2018, Marques et al. 2019a, Meier et al. 2017, Svardal et al. 2020), Darwin's finches (Han

et al. 2017, Lamichhaney et al. 2015), and *Heliconius* butterflies (Edelman et al. 2019, Jiggins et al. 2008, Zhang et al. 2016).

Because the study of hybridization, let alone investigating its influence on adaptive radiation, among lichen-forming fungi is still in its infancy, there is ample work to be done to determine the extent of hybridization among these symbiotic fungi and its broader impacts. In the *Xanthoparmelia* Holarctic clade, future research can investigate the timing of hybridization events—specifically, whether they coincide with global aridification. In this study we were unable to verify whether or not trait variability of growth forms and secondary metabolites were caused by hybridization. Future research could focus on identifying genes tied to adaptive traits, as well as constructing annotated reference genomes to further our understanding of the impacts hybridization has on lichen-forming fungal genomes.

While there were areas of congruence between the three methods of detecting hybridization—ABBA-BABA tests in HyDe, MPL in Phylonet, and SplitsTree—the methods we used also inferred different reticulation events. This may be due, in part, to methodological and sampling limitations. Blair and Ané (2019) recommend that researchers should use multiple analyses and investigate areas of congruence between them. In this study, the SplitsTree results suggested the most conservative estimation of hybridization, indicating hybridization between and within A, B, and C clades, represented by only a portion (25%) of the gene trees (Fig. 5). In contrast, both Phylonet (Fig. 4) and HyDe (Supplementary Table 2) analyses indicated widespread hybridization across all major lineages in the Holarctic clade. While the HyDe results consistently inferred widespread hybridization, the inferred reticulation events were not consistently inferred in different HyDe analyses, which is not uncommon with ABBA-BABA tests (Blair and Ané 2019). The HyDe results were also partly in conflict with SplitTree and Phylonet results. Blair and Ané 2019 highlight that violation of model assumptions can influence the number and position of inferred introgression events. Excessive ILS can influence the results of HyDe, though this tends to be less of a problem in larger datasets (Kong and Kutbatko 2020). Additionally, Phylonet relies on gene trees, and results could potentially be affected by gene tree estimation error (DeGiorgio and Degnan, 2014), which the MPL pipeline does not account for. Simulation and empirical studies of factors

that influence network methods for inferring hybridization, such as MPL implemented here, are presently lacking (Blair and Ané. 2019). Alternatively, some Bayesian methods may circumvent gene tree estimation error—i.e. PhyloNetworks (Solís-Lemus et al. 2017) and the SpeciesNetwork package of BEAST2 (Zhang et al., 2018a). Ultimately, we speculate that there is rampant, complex genome-wide admixture across the Holartic *Xanthoparmelia* radiation, which is supported in part by our results. This is not unheard of in other organismal groups, particularly those with a longer history of investigating hybridization (Kozak et al. 2021). Future work will be required to elucidate the extent, timing, and direction of hybridization more fully in this diverse lineage of symbiotic fungi.

Because both concatenation and multispecies coalescent (MSC) methods of species tree reconstruction assume there is no gene flow, neglecting to consider sources of phylogenetic conflict may lead to incorrect topologies (Solis-Lemus et al. 2016) or biased nodal support values (Zhang et al. 2021). In this study, concatenation and MSC methods all recovered the same major subclades in the Holarctic clade in *Xanthoparmelia* but had small differences in the backbone placement of these clades—specifically, the placement of clades A and H. The major subclades inferred from genome-scale data were congruent with inferences based on much smaller multilocus datasets. Our phylogenies, regardless of tree reconstruction method, consistently reconstructed the same seven Holarctic clades: A, B, C, D, E, and F (combined with G in this study), previously delineated in Leavitt et al. (2011b), as well as H, a clade comprised of multiple species-level groups, including *X. hypofusca* (Gyeln.) Hodk. & Lendemer from eastern USA and specimens from western North America resembling the European taxon *X. sublaevis* (Coutinho) Hale. Despite these consistencies, we found evidence for hybridization across the Holarctic clade. Even a small amount of gene flow per generation can influence species tree methods, especially in cases of adaptive radiation (Jiao et al. 2020). Therefore, even with well-supported phylogenies, phylogenetic network methods may reveal vital evolutionary insights.

Phylogenetic conflict can be caused by non-biological causes such as incorrect alignments, incorrect substitution models, and the presence of multi-copy regions (Philippe et al. 2011). Numerous approaches have been proposed for filtering genome-scale datasets, including filtering out genes with low

or conflicting signal (Knowles et al. 2018), removing genes with poor alignment (Tan et al. 2015), or by selecting more slowly evolving genes (Chen et al. 2015, Smith et al. 2018). However, some studies have found that selectively filtering data could bias phylogenetic inferences (Chan et al. 2020, Chen et al. 2015). For this study, we used HybPiper (Johnson et al. 2016) to generate alignments of documented single-copy gene regions in fungi (BUSCO). The BUSCO/HybPiper approach for generating genome-scale datasets has been used to generate robust phylogenetic inferences in other studies of lichen-forming fungi (Grewe et al. 2020, Widhelm et al. 2019). Our results suggest that these loci can also be effectively used to detect signals of hybridization. We detected broad signals of hybridization the initial BUSCO dataset (Supplementary Table 2), as well as more stringently filtered subsets of the BUSCO genes (Supplementary Table 3). However, marker selection for genome-scale datasets for fungi warrants further study to identify appropriate loci and cost-effective strategies for genome sequencing to infer evolutionary processes and relationships.

The analytical approaches we used detect evidence of hybridization also account for ILS. It is important to note that ILS and hybridization can occur simultaneously, and detection of one does not necessarily eliminate the presence of the other (Degnan 2018, Seehausen 2004). While ILS can be present at old nodes and absent among closely related species (Schrempf and Szöllösi 2020), often if species are closely enough related to hybridize, they will have high levels of ILS precisely because they are closely related (Degnan 2018). This is especially true in cases with rapid speciation and large population sizes (Schrempf and Szöllösi 2020). In future research, these analyses could be used to investigate specific questions relating to potential hybridization events, targeting specific taxa and more genes to better understand precise patterns of hybridization in this group with a greater amount of statistical robustness.

References

Abbott, R., D. Albach, S. Ansell, J. W. Arntzen, S. J. E. Baird, N. Bierne, J. W. Boughman, A. Brelsford, C. A. Buerkle, R. Buggs, R. K. Butlin, U. Dieckmann, F. Eroukhmanoff, A. Grill, S. H. Cahan, J. S. Hermansen, G. Hewitt, A. G. Hudson, C. Jiggins, J. Jones, B. Keller, T. Marczewski, J. Mallet, P. Martinez-Rodriguez, M. Most, S. Mullen, R. Nichols, A. W. Nolte, C. Parisod, K. Pfennig, A. M. Rice, M. G. Ritchie, B. Seifert, C. M. Smadja, R. Stelkens, J. M. Szymura, R. Vainola, J. B. W. Wolf, and D. Zinner. 2013. Hybridization and speciation. Journal of Evolutionary Biology 26:229-246.

Aguileta, G., D. M. de Vienne, O. N. Ross, M. E. Hood, T. Giraud, E. Petit, and T. Gabaldon. 2014. High variability of mitochondrial gene order among fungi. Genome Biol Evol 6:451-465.

Akaike, H. 1998. Information Theory and an Extension of the Maximum Likelihood Principle. Pages 199-213 *in* E. Parzen, K. Tanabe, and G. Kitagawa, editors. Selected Papers of Hirotugu Akaike. Springer New York, New York, NY.

Anderson, E., and E. D. Rudolph. 1956. An analysis of variation in a variable population of *Cladonia*. Evolution 10:147-156.

Anderson, J. B., J. Funt, D. A. Thompson, S. Prabhu, A. Socha, C. Sirjusingh, J. R. Dettman, L. Parreiras, D. S. Guttman, A. Regev, and L. M. Kohn. 2010. Determinants of divergent adaptation and Dobzhansky-Muller interaction in experimental yeast populations. Curr Biol 20:1383-1388.

Anderson, J. B., C. Sirjusingh, A. B. Parsons, C. Boone, C. Wickens, L. E. Cowen, and L. M. Kohn. 2003. Mode of selection and experimental evolution of antifungal drug resistance in *Saccharomyces cerevisiae*. Genetics 163:1287-1298.

Arnold, M. L., and K. Kunte. 2017. Adaptive Genetic Exchange: A Tangled History of Admixture and Evolutionary Innovation. Trends Ecol Evol 32:601-611.

Bankevich, A., S. Nurk, D. Antipov, A. A. Gurevich, M. Dvorkin, A. S. Kulikov, V. M. Lesin, S. I. Nikolenko, S. Pham, A. D. Prjibelski, A. V. Pyshkin, A. V. Sirotkin, N. Vyahhi, G. Tesler, M. A.

Alekseyev, and P. A. Pevzner. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455-477.

Bapteste, E., E. Susko, J. Leigh, I. Ruiz-Trillo, J. Bucknam, and W. F. Doolittle. 2008. Alternative methods for concatenation of core genes indicate a lack of resolution in deep nodes of the prokaryotic phylogeny. Mol Biol Evol 25:83-91.

Barrett, R. D., and D. Schluter. 2008. Adaptation from standing genetic variation. Trends Ecol Evol 23:38-44.

Bassham, S., J. Catchen, E. Lescak, F. A. von Hippel, and W. A. Cresko. 2018. Repeated Selection of Alternatively Adapted Haplotypes Creates Sweeping Genomic Remodeling in Stickleback. Genetics 209:921-939.

Bell, C. D., E. V. Mavrodiev, P. S. Soltis, A. K. Calaminus, D. C. Albach, N. Cellinese, N. Garcia-Jacas, and D. E. Soltis. 2012. Rapid diversification of Tragopogon and ecological associates in Eurasia. J Evol Biol 25:2470-2480.

Bertels, F., O. K. Silander, M. Pachkov, P. B. Rainey, and E. van Nimwegen. 2014. Automated reconstruction of whole-genome phylogenies from short-sequence reads. Mol Biol Evol 31:1077-1088.

Billiard, S., M. Lopez-Villavicencio, M. E. Hood, and T. Giraud. 2012. Sex, outcrossing and mating types: unsolved questions in fungi and beyond. J Evol Biol 25:1020-1038.

Blair, C., and C. Ané. 2019. Phylogenetic trees and networks can serve as powerful and complementary approaches for analysis of genomic data. Syst Biol.

Blischak, P. D., J. Chifman, A. D. Wolfe, and L. S. Kubatko. 2018a. HyDe: A Python Package for Genome-Scale Hybridization Detection. Syst Biol 67:821-829.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114-2120.

Bonnet, T., R. Leblois, F. Rousset, and P. A. Crochet. 2017. A reassessment of explanations for discordant introgressions of mitochondrial and nuclear genomes. Evolution 71:2140-2158.

Brion, C., C. Caradec, D. Pflieger, A. Friedrich, and J. Schacherer. 2020. Pervasive phenotypic impact of a large non-recombining introgressed region in yeast. Mol Biol Evol.

Büker, B., E. Petit, D. Begerow, and M. E. Hood. 2013. Experimental hybridization and backcrossing reveal forces of reproductive isolation in *Microbotryum*. BMC Evol Biol 13.

Burbrink, F. T., and M. Gehara. 2018. The Biogeography of Deep Time Phylogenetic Reticulation. Syst Biol 67:743-744.

Burton, R. S., and F. S. Barreto. 2012. A disproportionate role for mtDNA in Dobzhansky-Muller incompatibilities? Mol Ecol 21:4942-4957.

Buschbom, J., and G. M. Mueller. 2006. Testing "species pair" hypotheses: evolutionary processes in the lichen-forming species complex *Porpidia flavocoerulescens* and *Porpidia melinodes*. Mol Biol Evol 23:574-586.

Capella-Gutierrez, S., J. M. Silla-Martinez, and T. Gabaldon. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25:1972-1973.

Chan, K. M. A., and S. A. Levin. 2005. Leaky prezygotic isolation and porous genomes: Rapid introgression of maternally inherited DNA. Evolution 59:720-729.

Chan, K. O., C. R. Hutter, P. L. Wood, Jr., L. L. Grismer, and R. M. Brown. 2020. Larger, unfiltered datasets are more effective at resolving phylogenetic conflict: Introns, exons, and UCEs resolve ambiguities in Golden-backed frogs (Anura: Ranidae; genus *Hylarana*). Mol Phylogenet Evol 151:106899.

Chapman, M. A., and J. M. Burke. 2007. Genetic divergence and hybrid speciation. Evolution 61:1773-1780.

Chen, M. Y., D. Liang, and P. Zhang. 2015. Selecting Question-Specific Genes to Reduce Incongruence in Phylogenomics: A Case Study of Jawed Vertebrate Backbone Phylogeny. Syst Biol 64:1104-1120.

Chifman, J., and L. Kubatko. 2014. Quartet inference from SNP data under the coalescent model. Bioinformatics 30:3317-3324.

Chou, J., A. Gupta, S. Yaduvanshi, R. Davidson, M. Nute, S. Mirarab, and T. Warnow. 2015. A comparative study of SVDquartets and other coalescent-based species tree estimation methods. BMC Genomics 16.

Chou, J. Y., Y. S. Hung, K. H. Lin, H. Y. Lee, and J. Y. Leu. 2010. Multiple molecular mechanisms cause reproductive isolation between three yeast species. PLoS Biol 8:e1000432.

Clutterbuck, A. J. 1996. Parasexual recombination in fungi. Indian Academy of Sciences 75:281-286.

Culberson, C. F., W. L. Culberson, and A. Johnson. 1988. Gene Flow in Lichens. American Journal of Botany 75:pp. 1135-1139.

Culberson, C. F., and M. E. Hale. 1973a. Chemical and morphological evolution in *Parmelia* sect. *Hypotrachyna*: Product of ancient hybridization? Brittonia 25:162-173.

Culberson, C. F., and M. E. Hale. 1973b. Chemical and Morphological Evolution in *Parmelia* Sect. *Hypotrachyna*: Product of Ancient Hybridization? Brittonia 45:162-173.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods 9:772.

Dasmahapatra, K. K., J. R. Walters, A. D. Briscoe, J. W. Davey, A. Whibley, N. J. Nadeau, A. V. Zimin, D. S. T. Hughes, L. C. Ferguson, S. H. Martin, C. Salazar, J. J. Lewis, S. Adler, S. J. Ahn, D. A. Baker, S. W. Baxter, N. L. Chamberlain, R. Chauhan, B. A. Counterman, T. Dalmay, L. E. Gilbert, K. Gordon, D. G. Heckel, H. M. Hines, K. J. Hoff, P. W. H. Holland, E. Jacquin-Joly, F. M. Jiggins, R. T. Jones, D. D. Kapan, P. Kersey, G. Lamas, D. Lawson, D. Mapleson, L. S. Maroja, A. Martin, S. Moxon, W. J. Palmer, R. Papa, A. Papanicolaou, Y. Pauchet, D. A. Ray, N. Rosser, S. L. Salzberg, M. A. Supple, A. Surridge, A. Tenger-Trolander, H. Vogel, P. A. Wilkinson, D. Wilson, J. A. Yorke, F. R. Yuan, A. L. Balmuth, C. Eland, K. Gharbi, M. Thomson, R. A. Gibbs, Y. Han, J. C. Jayaseelan, C. Kovar, T. Mathew, D. M. Muzny, F. Ongeri, L. L. Pu, J. X. Qu, R. L. Thornton, K. C. Worley, Y. Q. Wu, M. Linares, M. L. Blaxter, R. H. Ffrench-Constant, M. Joron, M. R. Kronforst, S. P. Mullen, R. D. Reed, S. E. Scherer, S.

Richards, J. Mallet, W. O. McMillan, C. D. Jiggins, and C. Heliconius Genome. 2012. Butterfly genome reveals promiscuous exchange of mimicry adaptations among species. Nature 487:94-98.

Degnan, J. H. 2018. Modeling Hybridization Under the Network Multispecies Coalescent. Syst Biol 67:786-799.

DeGiorgio, M., and J. H. Degnan. 2014. Robustness to divergence time underestimation when inferring species trees from estimated gene trees. Syst Biol **63**:66-82.

Diaz, F., A. L. A. Luis, A. M. Nakamura, F. Fernandes, I. Sobrinho, Jr., and R. A. de Brito. 2018. Evidence for Introgression Among Three Species of the *Anastrepha fraterculus* Group, a Radiating Species Complex of Fruit Flies. Front Genet 9:359.

Du, X. H., D. Wu, H. Kang, H. Wang, N. Xu, T. Li, and K. Chen. 2020. Heterothallism and potential hybridization events inferred for twenty-two yellow morel species. IMA Fungus 11:4.

Durand, E. Y., N. Patterson, D. Reich, and M. Slatkin. 2011. Testing for ancient admixture between closely related populations. Mol Biol Evol 28:2239-2252.

Eaton, D. A. R., and R. H. Ree. 2013. Inferring Phylogeny and Introgression using RADseq Data: An Example from Flowering Plants (Pedicularis: Orobanchaceae). Systematic Biology 62:689-706.

Edelman, N. B., P. B. Frandsen, M. Miyagi, B. Clavijo, J. Davey, R. B. Dikow, G. Garcia-Accinelli, S. M. Van Belleghem, N. Patterson, D. E. Neafsey, R. Challis, S. Kumar, G. R. P. Moreira, C. Salazar, M. Chouteau, B. A. Counterman, R. Papa, M. Blaxter, R. D. Reed, K. K. Dasmahapatra, M. Kronforst, M. Joron, C. D. Jiggins, W. O. McMillan, F. Di Palma, A. J. Blumberg, J. Wakeley, D. Jaffe, and J. Mallet. 2019. Genomic architecture and introgression shape a butterfly radiation. Science 366:594-599.

Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792-1797.

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

Edwards, S. V., S. Potter, C. J. Schmitt, J. G. Bragg, and C. Moritz. 2016. Reticulation, divergence, and the phylogeography-phylogenetics continuum. Proc Natl Acad Sci U S A 113:8025-8032.

Ekman, S., and L. Fröberg. 1988. Taxonomical problems in *Aspicilia contorta* and *A. hoffmannii* - an effect of hybridization? International Journal of Mycology and Lichenology 3:215-226.

Ertz, D., B. Guzow-Krzeminska, G. Thor, A. Lubek, and M. Kukwa. 2018. Photobiont switching causes changes in the reproduction strategy and phenotypic dimorphism in the *Arthoniomycetes*. Sci Rep 8:4952.

Ertz, D., J. Miadlikowska, F. Lutzoni, S. Dessein, O. Raspe, N. Vigneron, V. Hofstetter, and P. Diederich. 2009. Towards a new classification of the *Arthoniales* (Ascomycota) based on a three-gene phylogeny focussing on the genus *Opegrapha*. Mycol Res 113:141-152.

Feliner, G. N., I. Alvarez, J. Fuertes-Aguilar, M. Heuertz, I. Marques, F. Moharrek, R. Pineiro, R. Riina, J. A. Rossello, P. S. Soltis, and I. Villa-Machio. 2017. Is homoploid hybrid speciation that rare? An empiricist's view. Heredity 118:513-516.

Feurtey, A., D. M. Stevens, W. Stephan, and E. H. Stukenbrock. 2019. Interspecific Gene Exchange Introduces High Genetic Variability in Crop Pathogen. Genome Biol Evol 11:3095-3105.

Feurtey, A., and E. H. Stukenbrock. 2018. Interspecific Gene Exchange as a Driver of Adaptive Evolution in Fungi. Annu Rev Microbiol 72:377-398.

Fishman, L., and A. L. Sweigart. 2018. When Two Rights Make a Wrong: The Evolutionary Genetics of Plant Hybrid Incompatibilities. Pages 707-731 *in* S. S. Merchant, editor. Annual Review of Plant Biology, Vol 69.

Fontaine, K. M., T. Ahti, and M. D. Piercey-Normore. 2010. Convergent evolution in *Cladonia gracilis* and allies. The Lichenologist 42:323-338.

Giordano, L., F. Sillo, M. Garbelotto, and P. Gonthier. 2018. Mitonuclear interactions may contribute to fitness of fungal hybrids. Sci Rep 8:1706.

Gladieux, P., J. Ropars, H. Badouin, A. Branca, G. Aguileta, D. M. De Vienne, R. C. R. De La Vega, S. Branco, and T. Giraud. 2014. Fungal evolutionary genomics provides insight into the mechanisms of adaptive divergence in eukaryotes. Molecular Ecology 23:753-773.

Goff, S. A., M. Vaughn, S. McKay, E. Lyons, A. E. Stapleton, D. Gessler, N. Matasci, L. Wang, M. Hanlon, A. Lenards, A. Muir, N. Merchant, S. Lowry, S. Mock, M. Helmke, A. Kubach, M. Narro, N. Hopkins, D. Micklos, U. Hilgert, M. Gonzales, C. Jordan, E. Skidmore, R. Dooley, J. Cazes, R. McLay, Z. Lu, S. Pasternak, L. Koesterke, W. H. Piel, R. Grene, C. Noutsos, K. Gendler, X. Feng, C. Tang, M. Lent, S. J. Kim, K. Kvilekval, B. S. Manjunath, V. Tannen, A. Stamatakis, M. Sanderson, S. M. Welch, K. A. Cranston, P. Soltis, D. Soltis, B. O'Meara, C. Ane, T. Brutnell, D. J. Kleibenstein, J. W. White, J. Leebens-Mack, M. J. Donoghue, E. P. Spalding, T. J. Vision, C. R. Myers, D. Lowenthal, B. J. Enquist, B. Boyle, A. Akoglu, G. Andrews, S. Ram, D. Ware, L. Stein, and D. Stanzione. 2011. The iPlant Collaborative: Cyberinfrastructure for Plant Biology. Front Plant Sci 2:34.

Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. Zhai, M. H. Fritz, N. F. Hansen, E. Y. Durand, A. S. Malaspinas, J. D. Jensen, T. Marques-Bonet, C. Alkan, K. Prufer, M. Meyer, H. A. Burbano, J. M. Good, R. Schultz, A. Aximu-Petri, A. Butthof, B. Hober, B. Hoffner, M. Siegemund, A. Weihmann, C. Nusbaum, E. S. Lander, C. Russ, N. Novod, J. Affourtit, M. Egholm, C. Verna, P. Rudan, D. Brajkovic, Z. Kucan, I. Gusic, V. B. Doronichev, L. V. Golovanova, C. Lalueza-Fox, M. de la Rasilla, J. Fortea, A. Rosas, R. W. Schmitz, P. L. F. Johnson, E. E. Eichler, D. Falush, E. Birney, J. C. Mullikin, M. Slatkin, R. Nielsen, J. Kelso, M. Lachmann, D. Reich, and S. Paabo. 2010a. A draft sequence of the Neandertal genome. Science 328:710-722.

Green, R. E., J. Krause, A. W. Briggs, T. Maricic, U. Stenzel, M. Kircher, N. Patterson, H. Li, W. W. Zhai, M. H. Y. Fritz, N. F. Hansen, E. Y. Durand, A. S. Malaspinas, J. D. Jensen, T. Marques-Bonet, C. Alkan, K. Prufer, M. Meyer, H. A. Burbano, J. M. Good, R. Schultz, A. Aximu-Petri, A. Butthof, B. Hober, B. Hoffner, M. Siegemund, A. Weihmann, C. Nusbaum, E. S. Lander, C. Russ, N. Novod, J. Affourtit, M. Egholm, C. Verna, P. Rudan, D. Brajkovic, Z. Kucan, I. Gusic, V. B. Doronichev, L. V. Golovanova, C. Lalueza-Fox, M. de la Rasilla, J. Fortea, A. Rosas, R. W. Schmitz, P. L. F. Johnson, E. E. Eichler, D. Falush, E. Birney, J. C. Mullikin, M. Slatkin, R.

Nielsen, J. Kelso, M. Lachmann, D. Reich, and S. Paabo. 2010b. A Draft Sequence of the Neandertal Genome. Science 328:710-722.

Greenspan, S. E., C. Lambertini, T. Carvalho, T. Y. James, L. F. Toledo, C. F. B. Haddad, and C. G. Becker. 2018. Hybrids of amphibian chytrid show high virulence in native hosts. Sci Rep 8:9600.

Greig, D., E. J. Louis, R. H. Borts, and M. Travisano. 2002. Hybrid speciation in experimental populations of yeast. Science 298:1773-1775.

Grewe, F., C. Ametrano, T. J. Widhelm, S. Leavitt, I. Distefano, W. Polyiam, D. Pizarro, M. Wedin, A. Crespo, P. K. Divakar, and H. T. Lumbsch. 2020. Using target enrichment sequencing to study the higher-level phylogeny of the largest lichen-forming fungi family: Parmeliaceae (Ascomycota). IMA Fungus 11:27.

Grewe, F., J. P. Huang, S. D. Leavitt, and H. T. Lumbsch. 2017. Reference-based RADseq resolves robust relationships among closely related species of lichen-forming fungi using metagenomic DNA. Sci Rep 7:9884.

Han, F., S. Lamichhaney, B. R. Grant, P. R. Grant, L. Andersson, and M. T. Webster. 2017. Gene flow, ancient polymorphism, and ecological adaptation shape the genomic landscape of divergence among Darwin's finches. Genome Res 27:1004-1015.

Hedrick, P. W. 2013a. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Mol Ecol 22:4606-4618.

Hedrick, P. W. 2013b. Adaptive introgression in animals: examples and comparison to new mutation and standing variation as sources of adaptive variation. Molecular Ecology 22:4606-4618.

Hill, G. E. 2017. The mitonuclear compatibility species concept. The Auk 134:393-409.

Hoang, D. T., O. Chernomor, A. von Haeseler, B. Q. Minh, and L. S. Vinh. 2018. UFBoot2: Improving the Ultrafast Bootstrap Approximation. Mol Biol Evol 35:518-522.

Honegger, R., and U. Zippler. 2007. Mating systems in representatives of Parmeliaceae, Ramalinaceae and Physciaceae (Lecanoromycetes, lichen-forming ascomycetes). Mycol Res 111:424-432.

Huang, J. P. 2016. Parapatric genetic introgression and phenotypic assimilation: testing conditions for introgression between Hercules beetles (Dynastes, Dynastinae). Mol Ecol 25:5513-5526.

Huson, D. H., T. Dezulian, T. Klopper, and M. A. Steel. 2004. Phylogenetic super-networks from partial trees. IEEE/ACM Trans Comput Biol Bioinform 1:151-158.

Irisarri, I., P. Singh, S. Koblmuller, J. Torres-Dowdall, F. Henning, P. Franchini, C. Fischer, A. R. Lemmon, E. M. Lemmon, G. G. Thallinger, C. Sturmbauer, and A. Meyer. 2018. Phylogenomics uncovers early hybridization and adaptive loci shaping the radiation of Lake Tanganyika cichlid fishes. Nat Commun 9:3159.

Ivanov, V., K. M. Lee, and M. Mutanen. 2018. Mitonuclear discordance in wolf spiders: Genomic evidence for species integrity and introgression. Molecular Ecology 27:1681-1695.

Janko, K., J. Paces, H. Wilkinson-Herbots, R. J. Costa, J. Roslein, P. Drozd, N. Iakovenko, J. Ridl, M. Hroudova, J. Koci, R. Reifova, V. Slechtova, and L. Choleva. 2018. Hybrid asexuality as a primary postzygotic barrier between nascent species: On the interconnection between asexuality, hybridization and speciation. Mol Ecol 27:248-263.

Jarvis, E. D., S. Mirarab, A. J. Aberer, B. Li, P. Houde, C. Li, S. Y. W. Ho, B. C. Faircloth, B. Nabholz, J. T. Howard, A. Suh, C. C. Weber, R. R. da Fonseca, J. Li, F. Zhang, H. Li, L. Zhou, N. Narula, L. Liu, G. Ganapathy, B. Boussau, M. S. Bayzid, V. Zavidovych, S. Subramanian, T. Gabaldón, S. Capella-Gutiérrez, J. Huerta-Cepas, B. Rekepalli, K. Munch, M. Schierup, B. Lindow, W. C. Warren, D. Ray, R. E. Green, M. W. Bruford, X. Zhan, A. Dixon, S. Li, N. Li, Y. Huang, E. P. Derryberry, M. F. Bertelsen, F. H. Sheldon, R. T. Brumfield, C. V. Mello, P. V. Lovell, M. Wirthlin, M. P. C. Schneider, F. Prosdocimi, J. A. Samaniego, A. M. V. Velazquez, A. Alfaro-Núñez, P. F. Campos, B. Petersen, T. Sicheritz-Ponten, A. Pas, T. Bailey, P. Scofield, M. Bunce, D. M. Lambert, Q. Zhou, P. Perelman, A. C. Driskell, B. Shapiro, Z. Xiong, Y. Zeng, S. Liu, Z. Li, B. Liu, K. Wu, J. Xiao, X. Yinqi, Q. Zheng, Y. Zhang, H. Yang, J. Wang, L. Smeds, F. E. Rheindt, M. Braun, J. Fjeldsa, L. Orlando, F. K. Barker, K. A. Jønsson, W. Johnson, K.-P. Koepfli, S. O'Brien, D. Haussler, O. A. Ryder, C. Rahbek, E. Willerslev, G. R. Graves, T. C.

Glenn, J. McCormack, D. Burt, H. Ellegren, P. Alström, S. V. Edwards, A. Stamatakis, D. P. Mindell, J. Cracraft, E. L. Braun, T. Warnow, W. Jun, M. T. P. Gilbert, and G. Zhang. 2014. Whole-genome analyses resolve early branches in the tree of life of modern birds. Science 346:1320-1331.

Jeffroy, O., H. Brinkmann, F. Delsuc, and H. Philippe. 2006. Phylogenomics: the beginning of incongruence? Trends Genet 22:225-231.

Jiao, X., T. Flouri, B. Rannala, and Z. Yang. 2020. The Impact of Cross-Species Gene Flow on Species Tree Estimation. Syst Biol 69:830-847.

Jiggins, C. D., C. Salazar, M. Linares, and J. Mavarez. 2008. Review. Hybrid trait speciation and *Heliconius* butterflies. Philos Trans R Soc Lond B Biol Sci 363:3047-3054.

Johnson, M. G., E. M. Gardner, Y. Liu, R. Medina, B. Goffinet, A. J. Shaw, N. J. Zerega, and N. J. Wickett. 2016. HybPiper: Extracting coding sequence and introns for phylogenetics from high-throughput sequencing reads using target enrichment. Appl Plant Sci 4.

Joly, S., P. A. McLenachan, and P. J. Lockhart. 2009. A statistical approach for distinguishing hybridization and incomplete lineage sorting. Am Nat 174:E54-70.

Kalyaanamoorthy, S., B. Q. Minh, T. K. F. Wong, A. von Haeseler, and L. S. Jermiin. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587-589.

Katoh, K., and D. M. Standley. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772-780.

Keuler, R., A. Garretson, T. Saunders, R. J. Erickson, N. St Andre, F. Grewe, H. Smith, H. T. Lumbsch, J. P. Huang, L. L. St Clair, and S. D. Leavitt. 2020. Genome-scale data reveal the role of hybridization in lichen-forming fungi. Sci Rep 10:1497.

Knowles, L. L., H. Huang, J. Sukumaran, and S. A. Smith. 2018. A matter of phylogenetic scale: Distinguishing incomplete lineage sorting from lateral gene transfer as the cause of gene tree discord in recent versus deep diversification histories. Am J Bot 105:376-384.

Kohn, L. M. 2005. Mechanisms of fungal speciation. Annu Rev Phytopathol 43:279-308.

Kong, S., and L. S. Kubatko. 2020. Comparative Performance of Popular Methods for Hybrid Detection using Genomic Data.

Kraichak, E., P. K. Divakar, A. Crespo, S. D. Leavitt, M. P. Nelsen, R. Lucking, and H. T. Lumbsch. 2015. A Tale of Two Hyper-diversities: Diversification dynamics of the two largest families of lichenized fungi. Sci Rep 5:10028.

Kroken, S., and J. W. Taylor. 2001a. A Gene Genealogical Approach to Recognize Phylogenetic Species Boundaries in the Lichenized Fungus *Letharia*.

Kroken, S., and J. W. Taylor. 2001b. Outcrossing and recombination in the lichenized fungus *Letharia*. Fungal Genetics and Biology 34:83-92.

Lamichhaney, S., J. Berglund, M. S. Almen, K. Maqbool, M. Grabherr, A. Martinez-Barrio, M. Promerova, C. J. Rubin, C. Wang, N. Zamani, B. R. Grant, P. R. Grant, M. T. Webster, and L. Andersson. 2015. Evolution of Darwin's finches and their beaks revealed by genome sequencing. Nature 518:371-375.

Lamichhaney, S., F. Han, M. T. Webster, L. Andersson, B. R. Grant, and P. R. Grant. 2018. Rapid hybrid speciation in Darwin's finches. Science 359:224-227.

Langmead, B., and S. L. Salzberg. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357-359.

Leavitt, D. H., R. Keuler, C. C. Newberry, R. Rosentreter, and L. St. Clair. 2019a. Shotgun sequencing decades-old lichen specimens to resolve phylogenomic placement of type specimens. Plant and Fungal Systematics 64:xx-xx.

Leavitt, S., F. Fernández-Mendoza, S. Pérez-Ortega, M. Sohrabi, P. Divakar, T. Lumbsch, and L. St. Clair. 2013a. DNA barcode identification of lichen-forming fungal species in the *Rhizoplaca melanophthalma* species-complex (Lecanorales, Lecanoraceae), including five new species. MycoKeys 7:1-22.

Leavitt, S. D., J. D. Fankhauser, D. H. Leavitt, L. D. Porter, L. A. Johnson, and L. L. St Clair. 2011a. Complex patterns of speciation in cosmopolitan "rock posy" lichens--discovering and delimiting

cryptic fungal species in the lichen-forming *Rhizoplaca melanophthalma* species-complex (Lecanoraceae, Ascomycota). Mol Phylogenet Evol 59:587-602.

Leavitt, S. D., F. Fernández-Mendoza, S. Pérez-Ortega, M. Sohrabi, P. K. Divakar, H. T. Lumbsch, and L. L. St. Clair. 2013b. DNA barcode identification of lichen-forming fungal species in the *Rhizoplaca melanophthalma* species-complex (Lecanorales, Lecanoraceae), including five new species MycoKeys 7:1–22.

Leavitt, S. D., F. Fernández-Mendoza, S. Pérez-Ortega, M. Sohrabi, P. K. Divakar, J. Vondrák, H. Thorsten Lumbsch, L. L. S. Clair, and H.-P. Comes. 2013c. Local representation of global diversity in a cosmopolitan lichen-forming fungal species complex (Rhizoplaca, Ascomycota). Journal of Biogeography 40:1792-1806.

Leavitt, S. D., F. Grewe, T. Widhelm, L. Muggia, B. Wray, and H. T. Lumbsch. 2016a. Resolving evolutionary relationships in lichen-forming fungi using diverse phylogenomic datasets and analytical approaches. Sci Rep 6:22262.

Leavitt, S. D., L. A. Johnson, T. Goward, and L. L. St Clair. 2011b. Species delimitation in taxonomically difficult lichen-forming fungi: an example from morphologically and chemically diverse *Xanthoparmelia* (Parmeliaceae) in North America. Mol Phylogenet Evol 60:317-332.

Leavitt, S. D., R. Keuler, C. C. Newberry, R. Rosentreter, and L. L. S. Clair. 2019b. Shotgun sequencing decades-old lichen specimens to resolve phylogenomic placement of type material. Plant and Fungal Systematics 64:237-247.

Leavitt, S. D., P. M. Kirika, G. Amo De Paz, J.-P. Huang, J.-S. Hur, J. A. Elix, F. Grewe, P. K. Divakar, and H. T. Lumbsch. 2018. Assessing phylogeny and historical biogeography of the largest genus of lichen-forming fungi, *Xanthoparmelia* (Parmeliaceae, Ascomycota). The Lichenologist 50:299-312.

Leavitt, S. D., E. Kraichak, J. Vondrak, M. P. Nelsen, M. Sohrabi, S. Perez-Ortega, L. L. St Clair, and H. T. Lumbsch. 2016b. Cryptic diversity and symbiont interactions in rock-posy lichens. Mol Phylogenet Evol 99:261-274.

Leavitt, S. D., H. T. Lumbsch, S. Stenroos, and L. L. St Clair. 2013d. Pleistocene speciation in North American lichenized fungi and the impact of alternative species circumscriptions and rates of molecular evolution on divergence estimates. PLoS One 8:e85240.

Lee, H. Y., J. Y. Chou, L. Cheong, N. H. Chang, S. Y. Yang, and J. Y. Leu. 2008. Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. Cell 135:1065-1073.

Lewontin, R. C. 1966. Hybridization as a new source of variation for adaptation to new environments. Evolution 20:315-336.

Liu, L., S. Wu, and L. Yu. 2015. Coalescent methods for estimating species trees from phylogenomic data. Journal of Systematics and Evolution 53:380-390.

Lodé, T. 2012. Adaptive Significance and Long-Term Survival of Asexual Lineages. Evolutionary Biology 40:450-460.

Lücking, R., B. P. Hodkinson, and S. D. Leavitt. 2017. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. The Bryologist 119.

Lucking, R., S. Huhndorf, D. H. Pfister, E. R. Plata, and H. T. Lumbsch. 2009. Fungi evolved right on track. Mycologia 101:810-822.

Lumbsch, H. T., A. L. Hipp, P. K. Divakar, O. Blanco, and A. Crespo. 2008. Accelerated evolutionary rates in tropical and oceanic parmelioid lichens (Ascomycota). BMC Evol Biol 8:257.

Lumbsch, H. T., and S. D. Leavitt. 2011. Goodbye morphology? A paradigm shift in the delimitation of species in lichenized fungi. Fungal Diversity 50:59-72.

Mack, K. L., and M. W. Nachman. 2017. Gene regulation and speciation. Trends in Genetics 33:68-80.

Maddison, W. P. 1997. Gene trees in species trees. Systematic Biology 46:523-536.

Magain, N., E. Sérusiaux, M. P. Zhurbenko, F. Lutzoni, and J. Miadlikowska. 2016. Disentangling the *Peltigera polydactylon* Species Complex by Recognizing Two New Taxa, *P. polydactylon* subsp. *udeghe* and *P. seneca*. Herzogia 29:514-528.

Mallet, J. 2005. Hybridization as an invasion of the genome. Trends in Ecology & Evolution 20:229-237.

Mallet, J., N. Besansky, and M. W. Hahn. 2016. How reticulated are species? Bioessays 38:140-149.

Marques, D. A., K. Lucek, V. C. Sousa, L. Excoffier, and O. Seehausen. 2019a. Admixture between old lineages facilitated contemporary ecological speciation in Lake Constance stickleback. Nat Commun 10:4240.

Marques, D. A., J. I. Meier, and O. Seehausen. 2019b. A Combinatorial View on Speciation and Adaptive Radiation. Trends Ecol Evol 34:531-544.

Marques, D. A., J. I. Meier, and O. Seehausen. 2019c. A combinatorial view on speciation and adaptive radiation. Trends in Ecology & Evolution.

Martin, S. H., J. W. Davey, and C. D. Jiggins. 2015. Evaluating the use of ABBA-BABA statistics to locate introgressed loci. Mol Biol Evol 32:244-257.

McCune, B., and R. Rosentreter. 2007. Biotic Soil Crust Lichens of the Columbia Basin. Northwest Lichenologists, Corvallis, Oregon.

Meier, J. I., D. A. Marques, S. Mwaiko, C. E. Wagner, L. Excoffier, and O. Seehausen. 2017. Ancient hybridization fuels rapid cichlid fish adaptive radiations. Nat Commun 8:14363.

Merchant, N., E. Lyons, S. Goff, M. Vaughn, D. Ware, D. Micklos, and P. Antin. 2016a. The iPlant Collaborative: Cyberinfrastructure for Enabling Data to Discovery for the Life Sciences. PLoS Biol 14:e1002342.

Merchant, N., E. Lyons, S. Goff, M. Vaughn, D. Ware, D. Micklos, and P. Antin. 2016b. The iPlant Collaborative: cyberinfrastructure for enabling data to discovery for the life sciences. Plos Biology 14.

Meyer, B. S., M. Matschiner, and W. Salzburger. 2017. Disentangling Incomplete Lineage Sorting and Introgression to Refine Species-Tree Estimates for Lake Tanganyika Cichlid Fishes. Syst Biol 66:531-550.

Misof, B., B. Meyer, B. M. von Reumont, P. Kück, K. Misof, and K. Meusemann. 2013. Selecting informative subsets of sparse supermatrices increases the chance to find correct trees. Bmc Bioinformatics 14:348.

Murtagh, G. J., P. S. Dyer, and P. D. Crittenden. 2000. Reproductive systems: Sex and the single lichen. Nature 404:564.

Nguyen, L. T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268-274.

O'Brien, H., J. Miadlikowska, and F. Lutzoni. 2009. Assessing Reproductive Isolation in Highly Diverse Communities of the Lichen-Forming Fungal Genus *Peltigera*. Evolution 63:2076-2086.

Parra, G., K. Bradnam, and I. Korf. 2007. CEGMA: a pipeline to accurately annotate core genes in eukaryotic genomes. Bioinformatics 23:1061-1067.

Philippe, H., H. Brinkmann, D. V. Lavrov, D. T. Littlewood, M. Manuel, G. Worheide, and D. Baurain. 2011. Resolving difficult phylogenetic questions: why more sequences are not enough. PLoS Biol 9:e1000602.

Piercey-Normore, M. D., T. Ahti, and T. Goward. 2010. Phylogenetic and haplotype analyses of four segregates within *Cladonia arbuscula s.l.* Botany 88:397-408.

Pizarro, D., F. Dal Grande, S. D. Leavitt, P. S. Dyer, I. Schmitt, A. Crespo, H. Thorsten Lumbsch, and P. K. Divakar. 2019. Whole-Genome Sequence Data Uncover Widespread Heterothallism in the Largest Group of Lichen-Forming Fungi. Genome Biol Evol 11:721-730.

Pogoda, C. S., K. G. Keepers, A. Y. Nadiadi, D. W. Bailey, J. C. Lendemer, E. A. Tripp, and N. C. Kane. 2019. Genome streamlining via complete loss of introns has occurred multiple times in lichenized fungal mitochondria. Ecol Evol 9:4245-4263.

Prieto, M., and M. Wedin. 2013. Dating the diversification of the major lineages of Ascomycota (Fungi). PLoS One 8.

Rabiee, M., and S. Mirarab. 2021. SODA: Multi-locus species delimitation using quartet frequencies. Bioinformatics.

Reddy, S., R. T. Kimball, A. Pandey, P. A. Hosner, M. J. Braun, S. J. Hackett, K. L. Han, J. Harshman, C. J. Huddleston, S. Kingston, B. D. Marks, K. J. Miglia, W. S. Moore, F. H. Sheldon, C. C. Witt, T.

Yuri, and E. L. Braun. 2017. Why Do Phylogenomic Data Sets Yield Conflicting Trees? Data Type Influences the Avian Tree of Life more than Taxon Sampling. Syst Biol 66:857-879.

Rieseberg, L. H., O. Raymond, D. M. Rosenthal, Z. Lai, K. Livingstone, T. Nakazato, J. L. Durphy, A. E. Schwarzbach, L. A. Donovan, and C. Lexer. 2003. Major ecological transitions in wild sunflowers facilitated by hybridization. Science 301:1211-1216.

Roper, M., C. Ellison, J. W. Taylor, and N. L. Glass. 2011. Nuclear and genome dynamics in multinucleate ascomycete fungi. Curr Biol 21:R786-793.

Rose, J. P., C. A. P. Toledo, E. M. Lemmon, A. R. Lemmon, and K. J. Sytsma. 2020. Out of Sight, Out of Mind: Widespread Nuclear and Plastid-Nuclear Discordance in the Flowering Plant Genus *Polemonium* (Polemoniaceae) Suggests Widespread Historical Gene Flow Despite Limited Nuclear Signal. Syst Biol.

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

Runemark, A., M. Vallejo-Marin, and J. I. Meier. 2019. Eukaryote hybrid genomes. PLoS Genet 15:e1008404.

Salichos, L., A. Stamatakis, and A. Rokas. 2014. Novel information theory-based measures for quantifying incongruence among phylogenetic trees. Mol Biol Evol 31:1261-1271.

Samarasinghe, H., M. You, T. S. Jenkinson, J. Xu, and T. Y. James. 2020. Hybridization Facilitates Adaptive Evolution in Two Major Fungal Pathogens. Genes (Basel) 11.

Sayyari, E., and S. Mirarab. 2016. Fast coalescent-based computation of local branch support from quartet frequencies. Molecular Biology and Evolution 33:1654-1668.

Schardl, C. L., and K. D. Craven. 2003. Interspecific hybridization in plant-associated fungi and oomycetes: a review. Molecular Ecology 12:2861-2873.

Schluter, D., E. A. Clifford, M. Nemethy, and J. S. McKinnon. 2004. Parallel evolution and inheritance of quantitative traits. Am Nat 163:809-822.

Schoch, C. L., K. A. Seifert, S. Huhndorf, V. Robert, J. L. Spouge, C. A. Levesque, W. Chen, and F. B. Consortium. 2012a. 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.

Schoch, C. L., K. A. Seifert, S. Huhndorf, V. Robert, J. L. Spouge, C. A. Levesque, W. Chen, C. Fungal Barcoding, and L. Fungal Barcoding Consortium Author. 2012b. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Natl Acad Sci U S A 109:6241-6246.

Schrempf, D., and G. Szöllösi. 2020. The Sources of Phylogenetic Conflicts.

Schumer, M., G. G. Rosenthal, and P. Andolfatto. 2014. How common is homoploid hybrid speciation? Evolution 68:1553-1560.

Seehausen, O. 2004. Hybridization and adaptive radiation. Trends Ecol Evol 19:198-207.

Seppey, M., M. Manni, and E. M. Zdobnov. 2019. BUSCO: Assessing Genome Assembly and Annotation Completeness. Methods Mol Biol 1962:227-245.

Shen, X.-X., C. T. Hittinger, and A. Rokas. 2017. Contentious relationships in phylogenomic studies can be driven by a handful of genes. Nat Ecol Evol.

Short, D. P., K. O'Donnell, and D. M. Geiser. 2014. Clonality, recombination, and hybridization in the plumbing-inhabiting human pathogen *Fusarium keratoplasticum* inferred from multilocus sequence typing. BMC Evol Biol 14:91.

Silva, D. N., V. Varzea, O. S. Paulo, and D. Batista. 2018. Population genomic footprints of host adaptation, introgression and recombination in coffee leaf rust. Mol Plant Pathol 19:1742-1753.

Simao, F. A., R. M. Waterhouse, P. Ioannidis, E. V. Kriventseva, and E. M. Zdobnov. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210-3212.

Sloan, D. B., J. C. Havird, and J. Sharbrough. 2017. The on-again, off-again relationship between mitochondrial genomes and species boundaries. Mol Ecol 26:2212-2236.

Smith, S. A., J. W. Brown, and J. F. Walker. 2018. So many genes, so little time: A practical approach to divergence-time estimation in the genomic era. PLoS One 13:e0197433.

Smith, S. A., M. J. Moore, J. W. Brown, and Y. Yang. 2015. Analysis of phylogenomic datasets reveals conflict, concordance, and gene duplications with examples from animals and plants. BMC Evol Biol 15:150.

Solis-Lemus, C., and C. Ane. 2016. Inferring Phylogenetic Networks with Maximum Pseudolikelihood under Incomplete Lineage Sorting. PLoS Genet 12:e1005896.

Solis-Lemus, C., P. Bastide, and C. Ane. 2017. PhyloNetworks: A Package for Phylogenetic Networks. Mol Biol Evol **34**:3292-3298.

Solis-Lemus, C., M. Yang, and C. Ane. 2016. Inconsistency of Species Tree Methods under Gene Flow. Syst Biol 65:843-851.

Soltis, D. E., V. A. Albert, V. Savolainen, K. Hilu, Y. L. Qiu, M. W. Chase, J. S. Farris, S. Stefanovic, D. W. Rice, J. D. Palmer, and P. S. Soltis. 2004a. Genome-scale data, angiosperm relationships, and "ending incongruence": a cautionary tale in phylogenetics. Trends Plant Sci 9:477-483.

Soltis, D. E., S. S. Soltis, J. C. Pires, A. Kovarik, J. A. Tate, and E. V. Mavrodiev. 2004b. Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. Biological Journal of the Linnean Society 82:485-501.

Spribille, T. 2018. Relative symbiont input and the lichen symbiotic outcome. Curr Opin Plant Biol 44:57-63.

Stamatakis, A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312-1313.

Stanke, M., R. Steinkamp, S. Waack, and B. Morgenstern. 2004. AUGUSTUS: a web server for gene finding in eukaryotes. Nucleic Acids Res 32:W309-312.

Stankowski, S., and M. A. Streisfeld. 2015. Introgressive hybridization facilitates adaptive divergence in a recent radiation of monkeyflowers. Proc Biol Sci 282.

Steenkamp, E. T., M. J. Wingfield, A. R. McTaggart, and B. D. Wingfield. 2018. Fungal species and their boundaries matter - Definitions, mechanisms and practical implications. Fungal Biology Reviews 32:104-116.

Steinová, J., S. Stenroos, M. Grube, and P. Škaloud. 2013. Genetic diversity and species delimitation of the zeorin-containing red-fruited *Cladonia* species (lichenized Ascomycota) assessed with ITS rDNA and β-tubulin data. The Lichenologist 45:665-684.

Stukenbrock, E. H. 2016. The Role of Hybridization in the Evolution and Emergence of New Fungal Plant Pathogens. Phytopathology 106:104-112.

Stukenbrock, E. H., F. B. Christiansen, T. T. Hansen, J. Y. Dutheil, and M. H. Schierup. 2012. Fusion of two divergent fungal individuals led to the recent emergence of a unique widespread pathogen species. Proc Natl Acad Sci U S A 109:10954-10959.

Sullivan, J., and P. Joyce. 2005. Model Selection in Phylogenetics. Annual Review of Ecology, Evolution, and Systematics 36:445-466.

Svardal, H., F. X. Quah, M. Malinsky, B. P. Ngatunga, E. A. Miska, W. Salzburger, M. J. Genner, G. F. Turner, and R. Durbin. 2020. Ancestral Hybridization Facilitated Species Diversification in the Lake Malawi Cichlid Fish Adaptive Radiation. Mol Biol Evol 37:1100-1113.

Swofford, D. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10.

Tan, G., M. Muffato, C. Ledergerber, J. Herrero, N. Goldman, M. Gil, and C. Dessimoz. 2015. Current Methods for Automated Filtering of Multiple Sequence Alignments Frequently Worsen Single-Gene Phylogenetic Inference. Syst Biol 64:778-791.

Tang, Q., S. V. Edwards, and F. E. Rheindt. 2018. Rapid diversification and hybridization have shaped the dynamic history of the genus *Elaenia*. Mol Phylogenet Evol 127:522-533.

Taylor, J. W., D. J. Jacobson, and M. C. Fisher. 1999. The Evolution of Asexual Fungi: Reproduction, Speciation and Classification. Annu Rev Phytopathol 37:197-246.

Tigano, A., and V. L. Friesen. 2016. Genomics of local adaptation with gene flow. Molecular Ecology 25:2144-2164.

Toews, D. P., and A. Brelsford. 2012. The biogeography of mitochondrial and nuclear discordance in animals. Mol Ecol 21:3907-3930.

Tonini, J., A. Moore, D. Stern, M. Shcheglovitova, and G. Orti. 2015. Concatenation and Species Tree Methods Exhibit Statistically Indistinguishable Accuracy under a Range of Simulated Conditions. PLoS Curr 7.

Townsend, J. P., Z. Su, and Y. I. Tekle. 2012. Phylogenetic signal and noise: predicting the power of a data set to resolve phylogeny. Syst Biol 61:835-849.

Tripp, E. A. 2016. Is asexual reproduction an evolutionary dead end in lichens? The Lichenologist 48:559-580.

Tripp, E. A., and J. C. Lendemer. 2017. Twenty-seven modes of reproduction in the obligate lichen symbiosis. Brittonia 70:1-14.

Wallbank, R. W., S. W. Baxter, C. Pardo-Diaz, J. J. Hanly, S. H. Martin, J. Mallet, K. K. Dasmahapatra, C. Salazar, M. Joron, N. Nadeau, W. O. McMillan, and C. D. Jiggins. 2016. Evolutionary Novelty in a Butterfly Wing Pattern through Enhancer Shuffling. PLoS Biol 14:e1002353.

Warnow, T. 2015. Concatenation Analyses in the Presence of Incomplete Lineage Sorting. PLoS Curr 7.

Wen, D., Y. Yu, J. Zhu, and L. Nakhleh. 2018a. Inferring Phylogenetic Networks Using PhyloNet. Syst Biol 67:735-740.

Wen, D. Q., Y. Yu, J. F. Zhu, and L. Nakhleh. 2018b. Inferring phylogenetic networks using PhyloNet. Systematic Biology 67:735-740.

Whitfield, J. B., S. A. Cameron, D. H. Huson, and M. A. Steel. 2008. Filtered Z-closure supernetworks for extracting and visualizing recurrent signal from incongruent gene trees. Syst Biol 57:939-947.

Widhelm, T. J., F. Grewe, J. P. Huang, J. A. Mercado-Diaz, B. Goffinet, R. Lucking, B. Moncada, R. Mason-Gamer, and H. T. Lumbsch. 2019. Multiple historical processes obscure phylogenetic relationships in a taxonomically difficult group (Lobariaceae, Ascomycota). Sci Rep 9:8968.

Wilson, A. M., P. M. Wilken, M. A. van der Nest, E. T. Steenkamp, M. J. Wingfield, and B. D. Wingfield. 2015. Homothallism: an umbrella term for describing diverse sexual behaviours. IMA Fungus 6:207-214.

Wyman, S. K., R. K. Jansen, and J. L. Boore. 2004. Automatic annotation of organellar genomes with DOGMA. Bioinformatics 20:3252-3255.

Yu, Y., and L. Nakhleh. 2015. A maximum pseudo-likelihood approach for phylogenetic networks. BMC Genomics 16.

Zeng, Q., Z. Cui, J. Wang, K. L. Childs, G. W. Sundin, D. R. Cooley, C. H. Yang, E. Garofalo, A. Eaton, R. B. Huntley, X. Yuan, and N. P. Schultes. 2018. Comparative genomics of Spiraeoideae-infecting Erwinia amylovora strains provides novel insight to genetic diversity and identifies the genetic basis of a low-virulence strain. Mol Plant Pathol 19:1652-1666.

Zhang, C., H. A. Ogilvie, A. J. Drummond, and T. Stadler. 2018a. Bayesian Inference of Species Networks from Multilocus Sequence Data. Mol Biol Evol **35**:504-517.

Zhang, C., M. Rabiee, E. Sayyari, and S. Mirarab. 2018b. ASTRAL-III: polynomial time species tree reconstruction from partially resolved gene trees. BMC Bioinformatics 19:153.

Zhang, D., F. E. Rheindt, H. She, Y. Cheng, G. Song, C. Jia, Y. Qu, P. Alstrom, and F. Lei. 2021. Most genomic loci misrepresent the phylogeny of an avian radiation because of ancient gene flow. Syst Biol.

Zhang, W., K. K. Dasmahapatra, J. Mallet, G. R. Moreira, and M. R. Kronforst. 2016. Genome-wide introgression among distantly related *Heliconius* butterfly species. Genome Biol 17:25.

Zoller, S., F. Lutzoni, and C. Scheidegger. 1999. Genetic variation within and among populations of the threatened lichen Lobaria pulmonaria in Switzerland and implications for its conservation. 8:2049-2059.

Figures and Tables

Figures

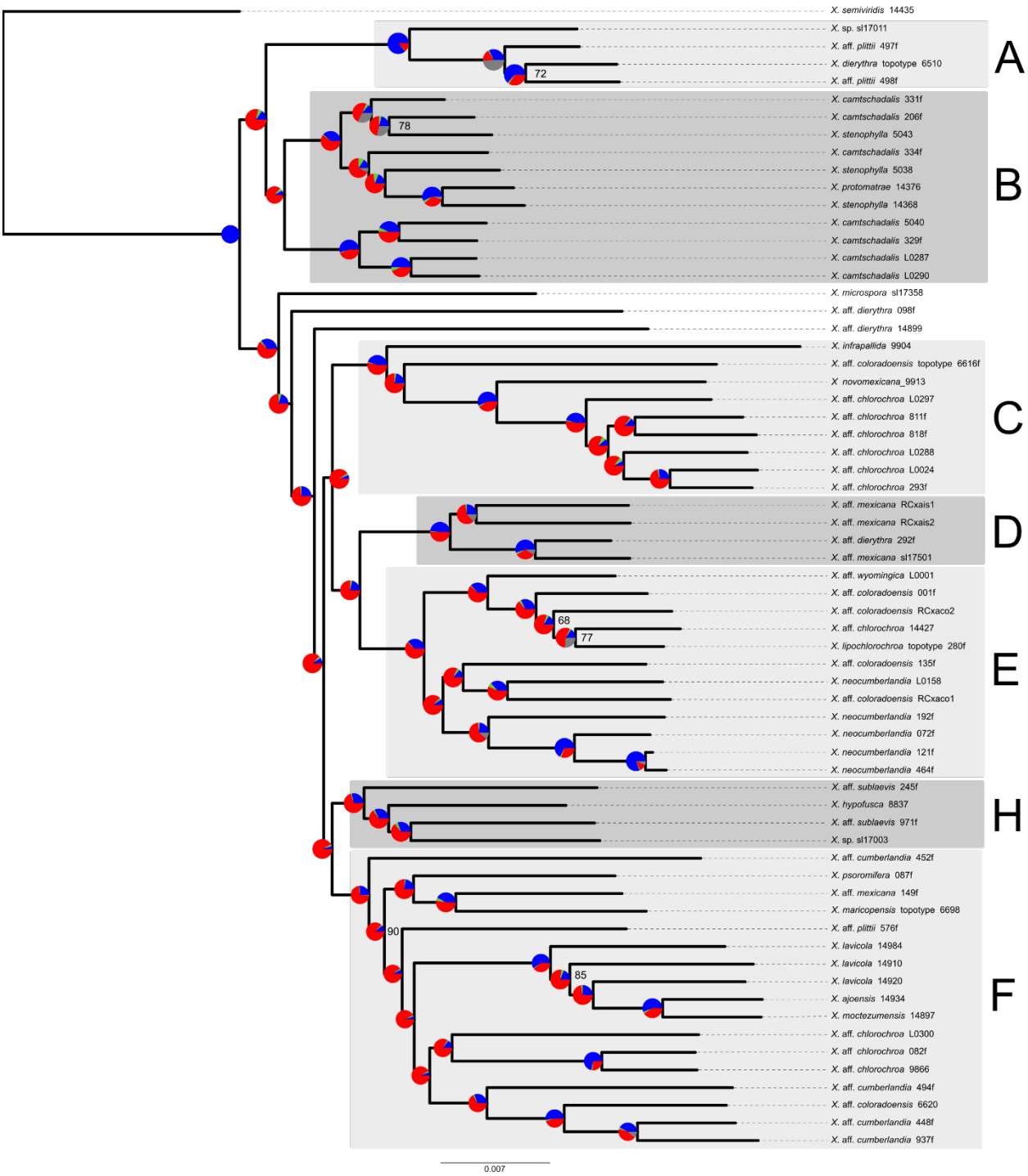

Figure 1: Nuclear IQ-TREE based on 962 BUSCO genes, with nodes with < 100 bootstrap support labeled. At each node is the PhyParts summary visualizing the proportions of gene trees that are:

concordant (blue), support the main alternative (green), support the remaining alternatives (red), and have < 50% bootstrap support (conflict or support; grey).

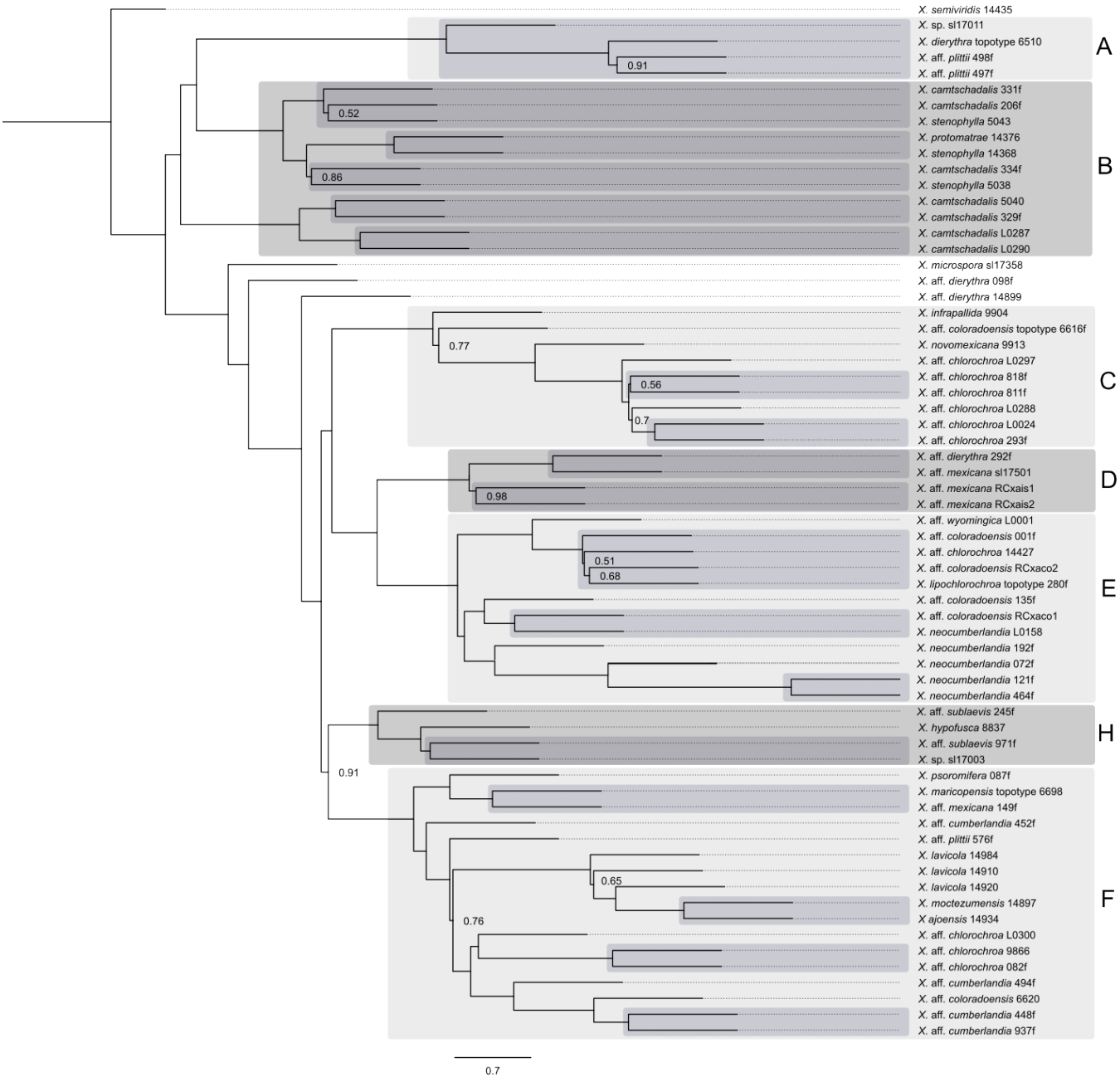

Figure 2: ASTRAL-III tree based on 962 BUSCO genes, with nodes with < 100 posterior probability labeled. Boxes within each clade indicate species delimited by SODA, and taxa that are not in a box represent their own species.

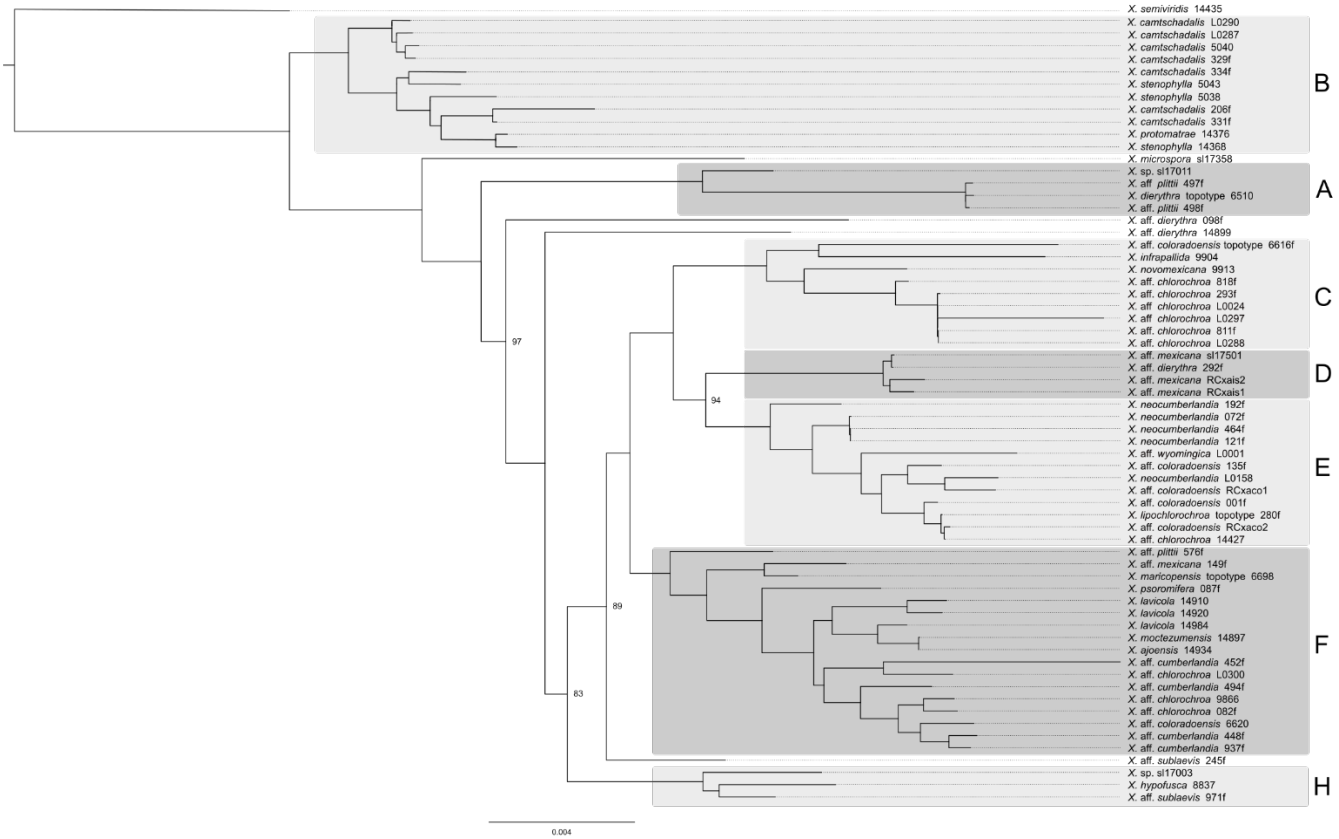

Figure 3: Mitochondrial tree inferred with IQ-TREE. Nodes that have < 100 bootstrap support are labeled.

The seven main sub-clades (A-F, H) are in boxes.

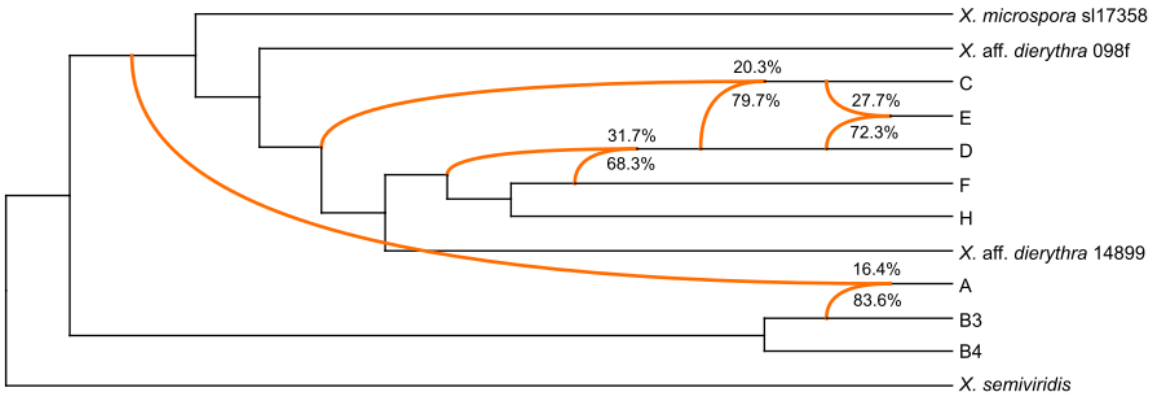

Figure 4: Phylogenetic network inferred by Phylonet using Maximum Pseudolikelihood under a four-reticulation model. Orange branches indicate lineages involved in hybridization, with inheritance probabilities or proportion of genes inherited from that parent for each reticulation.

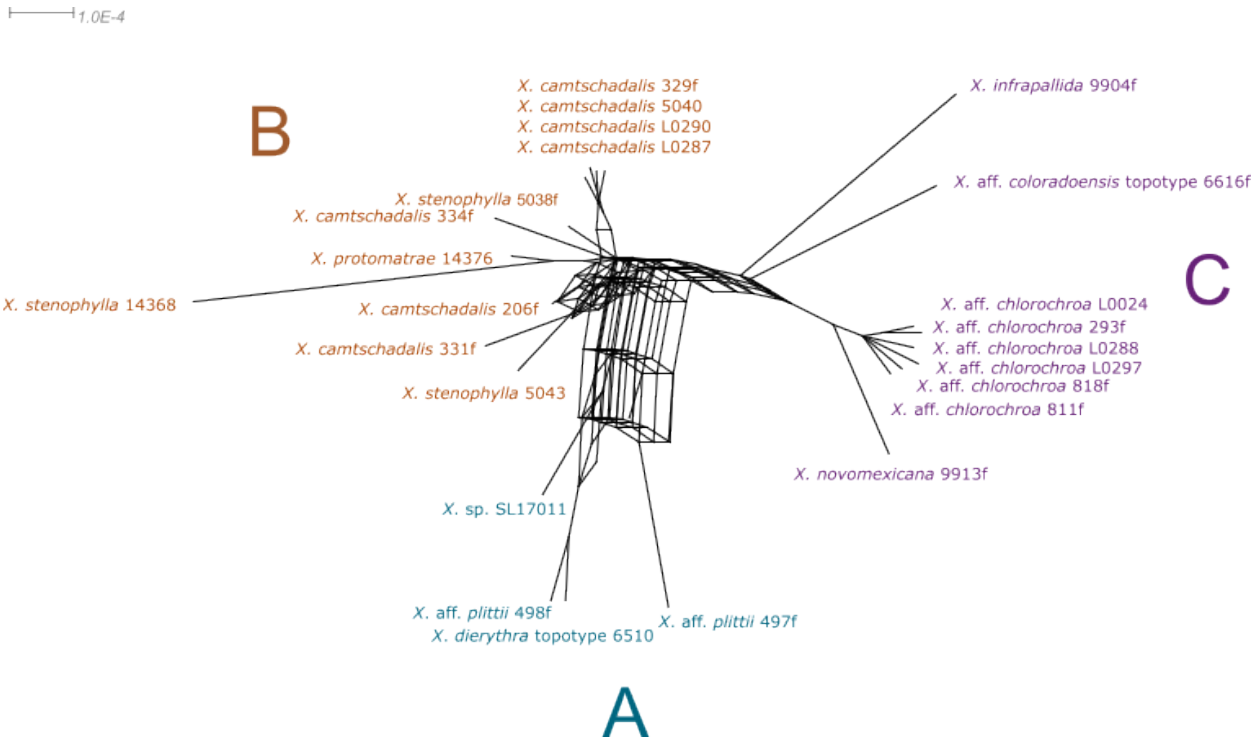

Figure 5: Z-closure supernetwork displaying the splits found in or compatible with a minimum of 25% of the 962 complete and partial gene trees.

Tables

Table 1: AIC calculations to select best-fitting network among Phylonet results. *L* is the likelihood value, and *k* is number of reticulations plus branch lengths (number of parameters used in the AIC calculation). The optimal network is in bold.

# Reticulations	ln*L*	Δln*L*	# Branch Lengths	*k*	AIC	ΔAIC
0	-17281650		12	12	34563324.5	113130.73
1	-17245376	36274.0062	12	13	34490778.4	40584.718
2	-17232066	13309.5653	12	14	34464161.3	13967.5874
3	-17228677	3389.75035	13	16	34457385.8	7192.08673
4	**-17225081**	**3596.04336**	**12**	**16**	**34450193.7**	**0**