HARNESSING GENOMIC AND BIOINFORMATIC TOOLS TO INFORM CONSERVATION DECISIONS OF SPECIES THAT ARE VULNERABLE TO HUMAN-DRIVEN IMPACTS OF CLIMATE CHANGE

by

KYLE GARRETT KEEPERS

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Professor Nolan Kane, Primary Advisor
Professor James Lendemer
Professor Andrew Martin
Professor Stacey Smith
Professor Erin Tripp

ABSTRACT

Keepers, Kyle Garrett (Ph.D. EBIO)

Harnessing Genomic and Bioinformatic Tools to Inform Conservation Decisions of Species that are Vulnerable to Human-Driven Impacts of Climate Change

Thesis directed by Associate Professor Dr. Nolan C. Kane

The field of bioinformatics began late in the 20th century to enable the analysis of proteomic, genetic, and genomic datasets. Since the 1990s and the advent of 'big data', there has been a glut of genomic data, and a dearth of people with the skillsets to analyze them. As of 2019, the world's largest genetic sequence archive, NCBI's Short-Read Archive, was home to over 40 petabytes of genetic data, and that number is growing larger every day. Hidden within those sequences of As, Cs, Ts, and Gs, are the answers to many biological questions, including those pertaining to how we may best conserve species in the face of the existential threat of a drastically changing climate.

One such species is the Warm Springs pupfish, which is endemic to several low-flow springs in the Ash Meadows National Wildlife Refuge in Southwestern Nevada. One population, at South Scruggs Spring, was facing a demographic collapse due to predation by invasive species, declining spring flow, and what may have been an extinction vortex. An extinction vortex is caused when inbreeding in small populations leads to the accumulation of deleterious genotypes, which causes low fecundity, feeding back into the low population size. Once a species enters into one, there is little we know of outside of human-facilitated introgression of novel genetic material that can save the population from extirpation. In 2009, ten individuals from a neighboring spring were added to South Scruggs and the population demographics were monitored over the following three years using mark-recapture combined with microsatellite genotyping. Based on the probability of recapture, I calculated that hybrid offspring of the ten

introgressed individuals had a probability of survival between mark/recapture events that was 20% higher than that of the genetically poor resident individuals. In the process of the study, I sequenced and assembled the nuclear and mitochondrial genomes of the Warm Springs pupfish, which are resources that may be used to monitor the health of these isolated populations of endangered fish.

Another class of organisms that is especially sensitive to changing environmental conditions are lichens, which are visually stunning symbiotic assemblages of a fungus, or mycobiont, and at least one photosynthetic partner, called the photobiont. Their genomes are relatively small, enabling a low cost of sequencing the genomes of both partners in the symbiosis. I was able to sequence and assemble the genomes of over 500 lichen specimens. Many of the mitochondrial genomes of these species were assembled, annotated, and published on NCBI as a result of this study. One of the primary resources to come from these sequences is a formidable database of molecular barcoding sequences–the ribosomal DNA complexes of over 400 of the different lichen species assemblies came together. Using this database, I developed a novel bioinformatic pipeline that was able to detect which lichen propagules are present in environmental metagenomic samples. Such a tool should enable researchers to evaluate factors leading to the ability of a lichen to establish in an area, versus which ones are only able to disperse into it, but not establish.

In addition to the fungal rDNA complexes, algal rDNA complexes also assembled. By aligning metagenomic reads to these algal and cyanobacterial complexes, I was able to calculate the diversity of the photobiont communities within each lichen thallus and test the conditions determining photobiont diversity. I concluded that algal photobiont diversity is highest in the surface-adhering crustose lichens, and lowest in the tufty, three-dimensional fruticose lichens. In lichens that use cyanobacteria as their photobiont, diversity decreases with elevation.

Surprisingly, and contrary to our expectations, lichen photobiont diversity did not differ between sexual and asexual species.

The bioinformatic pipelines and data sets generated in this thesis provide valuable information on understudied and threatened species. These resources will enable adjacent researchers to make better decisions about conservation of these species in the face of habitat loss, pollution, and a changing climate.

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To the close friends I made in graduate school, Sydney, Gabby, Carly, Daniela, Kristin, Silas. Thinking back on all of the good times we've had fill me with the gratitude that I am lucky enough to count you as friends. My life is better for having you all in it. Lets spend more time together once this pandemic is all over.

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CHAPTER 1

INTRODUCTION

The field of biology as a coherent discipline is hundreds of years old, although curious minds throughout history have delved into human biology and natural history using the methodologies available to them at the time. The ancient practice of ayurveda has long been an attempt at understanding the basis of human disease (Patwardhan et al., 2005), however lacking it may be of a rigorous method of rejecting spurious treatments. One of the first recorded natural historians was Aristotle of Greece, whose observations of the conditions under which some animals died young and which flourished have been thought by some as a foundational precedent for later thinkers, such as Charles Darwin and Thomas Huxley (Gotthelf 1999; Gilson 2009). Throughout history, humanity's understanding of the natural world has advanced, to a first approximation, as a ratchet. This applies not only to encyclopedic facts, like what a shark's skin is comprised of, or the succession of development of a tracheophyte from seed to plant, how many species there are. It also applies to the methodologies we apply to learn such information. Systematics, microscopy, histology, dating, cultures, genotyping, PCR, sequencing. The discovery of a new methodology can be seen as a door opening into a wealth of new information.

In the grand scheme of our attempts to understand the world in which we live, the discipline of bioinformatics is relatively new. Bioinformatics began in the 1960s with Margaret Dayhoff's development of computational techniques for analyzing protein sequence variation for her doctoral thesis (Hagen 2000). The invention of DNA sequencing by Ray Wu and R Padmanhaban in 1970 (Bambara et al., 1974; Padmanabhan et al., 1972), which was later improved by Fred Sanger in 1977 (Sanger et al., 1977; Sanger 2001), created a desperate demand for the computational tools, and the computers themselves, to analyze the influx of new genetic

information (Wadman 1999). Ever since, new algorithms, programs and platforms have been in lock-step with advances in computational power, enabling ever-more complex analyses.

One of the specific applications of bioinformatics has been in informing how conservation decisions are made in the face of threats such as habitat loss, invasive species and changing climate pose to native ecosystems. For example, crop development is being accelerated with genomics and bioinformatics to help outpace the changing climate (Batley and Edwards, 2016). In this thesis, I employ a variety of bioinformatic tools, such as genomic sequencing, annotation, and pipeline-development, to two biological systems that are facing hardship due to these pressures. The first system is a rare desert fish that is endemic to several low-flow springs in the desert of southwest Nevada. The second is the biological diversity hotspot for lichens in the southwest Appalachians Mountains.

In Chapter 2, I investigate the fate of a facilitated migration of 10 desert pupfish, *Cyprinodon nevadensis pectoralis*, from one spring to another that was facing a steep decline in its population (Keepers et al., 2018). These endemic pupfish live in several low-flow springs in the Ash Meadows National Wildlife Refuge (Deacon and Williams, 1991), an oasis in the Mojave Desert. Migration of the fish between springs only occurs during rare high rainfall events. As such, the gene pool of each spring is limited, and inbreeding is common. A long-term population demography conducted by Dr. Andrew Martin between 1998 and 2007 (Martin and Wilcox, 2004; Martin 2010) found low effective population size of the fish in one spring named South Scruggs, which was facing declining outflow rate and an invasion of crayfish that predated on the pupfish. When an isolated population dips to a low-enough size, the only mating partners available are relatives, which leads to the negative consequences of inbreeding, such as low fecundity. The low fecundity feeds back into the low population size, which can lead to what is

called an "extinction vortex" (Reed and Stockwell, 2014; Fagan and Holmes, 2006), in which local extirpation of the isolated population is inevitable. Consequently, the decision was made to facilitate the migration of 10 pupfish in 2009, five males and five females, from a neighboring spring, North Indian, into South Scruggs. The intention was to infuse the South Scruggs population with novel genetic material with the hopes of ejecting the population from its extinction vortex. The population of South Scruggs was then monitored and genotyped between 2009 and 2012, for the presence of resident individuals, introduced individuals, and descendant hybrid offspring. The population size quickly rebounded after the admixture event. I calculated the probability of survival of each class of individual and found that admixed individuals had a 20% higher probability of survival than resident individuals. This study joins a small contingent of studies that investigates the fate of facilitated migrations to populations with low effective population size, including the Florida puma (Onorato et al., 2010; Johnson et al., 2010) and the Colorado bighorn sheep (Packard 1946; Buechner 1960; Singer et al., 2000; Lowrey et al., 2019). We also demonstrate the utility of bioinformatics in population demography and its application in managing populations that are vulnerable due to invasive species and changing environmental conditions.

In the course of my analysis of the admixture event in South Scruggs spring, I was fortunate to have the opportunity to sequence the genome of *C. nevadensis pectoralis*. I took tissue from a female sampling mortality and generated a 100 base-pair paired-end whole-genome shotgun (WGS) library, supplemented by a 3 kilobase mate-pair library. From these libraries, I performed *de novo* genome assembly using SOAPdenovo (Li et al., 2010) which resulted in a 1.011 billion base-pair genomic assembly [NCBI Accession JSUU00000000.1].

With few exceptions (Karnakowska et al., 2016), eukaryotes contain mitochondria, which were an ancient alpha-proteobacterium (Margulis 1981) that now resides in most eukaryotic cells as an endosymbiotic organelle that performs oxydative phosphorylation of ATP. Owing to their nature as an erstwhile bacterium, mitochondria contain their own genomes (Burger et al., 2003), although they are highly reduced in size, since many of the autonomous functions needed by bacteria have been made redundant by the machinery of the nuclear genome in their hosts (Andersson and Kurland 1998; Rand et al., 2004). In my third chapter, I present the mitochondrial genome of the Warm Springs pupfish (Keepers et al., 2016), which was assembled into several contigs, or stretches of DNA, that I stitched together into a single contig using bioinformatic techniques. Since the mitochondrial genomes of vertebrates are circular (Clayton 2000), I circularized the contig, then annotated the features contained within the DNA. Mitochondrial genomes contain not only genes encoding proteins (mostly localized to the ribosome complex and the oxidative phosphorylation complexes), but also genes encoding the catalytic transfer RNAs and ribosomal RNAs. Some stretches of DNA within an organism are variable enough in sequence to be used to bioinformatically differentiate their host from other species, leading to their designation as "molecular barcodes". Mitochondria contain several of these barcodes, including the protein-coding cytochrome c oxidase subunit 1 gene (*cox1*), and the genes encoding the ribosomal RNAs, referred to as the 16S ribosomal DNA and 23S ribosomal DNA when they are still encoded as DNA. Moreover, the entire mitochondrial genome itself, all 16,499 bp in the case of *C. n. pectoralis*, may be used as an "ultra" barcode (Kane et al., 2012). Molecular barcodes are useful tools in inferring how species are related to one another, and are often used to detect which species are present in a body of water through the detection of environmental DNA (eDNA; Ficetola et al., 2008; Shokralla et al., 2012). The Warm Springs

pupfish mitochondrial genome is an additional resource for biologists focused on the conservation of critical species in the Ash Meadows refuge.

In my fourth chapter, I had the privilege to take on the genomic and bioinformatic arm of a massive project to catalogue the diversity of lichens in the Southern Appalachian lichen biodiversity hotspot, which is host to a high biodiversity of lichen species. Lichens are a complex symbiosis between a fungus (called the mycobiont), at least one photosynthetic partner (called the photobiont), and often a rich community of commensal microorganisms such as bacteria (Ahmadjian 1993; Lutzoni and Miadlikowska 2009; Bates et al., 2011) and single-celled eukaryotes (De Vera and Ott 2010). They paint nature in a dazzling array of morphologies, chemistries, reproductive modes, colors, substrates and environments, and they cover a significant portion of the land area of the planet (Asplund and Wardle, 2017). Lichens are susceptible to sulfur dioxide (SO_2; Ahmadjian 1995) and thus rarely grow in highly polluted areas, making them a bellwether for pollution. As such, understanding the conditions under which they may exist in an area may help predict when the deleterious effects of pollution may impact other vulnerable species.

Environmental metagenomics is the study of the composition of microbial communities through the detection of molecular barcodes in an environmental sample of DNA, be it a sample of water or a toothbrush swab of a surface of interest (Tripp et al., 2016). The techniques of environmental metagenomics can also be applied to assess which lichens may potentially exist in an environment by detecting their DNA in the form of propagules (reproductive cells such as spores or vegetative, asexual conidia, isidia, and soredia). The traditional metagenomic approach is to dissolve the cells present within an environmental sample and amplify certain DNA barcodes via polymerase chain reaction, or PCR, using primers that specifically target the

barcode of interest. The most common barcode for fungal metagenomics is the ribosomal internally transcribed spacers (ITS1 and ITS2; White et al., 1990). The amplified sequence, or amplicon, is then compared to databases of fungal ITS sequence to identify which fungi were present in the sample.

I developed a novel approach that capitalizes on the small metagenome size of lichens, which is typically less than 100 million base-pairs in length, for the sum of the mycobiont and photobiont genomes. I assembled *de novo* the genomes of over 500 lichen specimens, representing over 400 different species, from tissue collected as a part of a broader study of lichen diversity in the Southern Appalachians. I bioinformatically isolated the ribosomal DNA complex (rDNA) from each assembly, which is a stretch of DNA that is present in all life on Earth, as far as we know. It includes the ITS1 and ITS2 sequences used in traditional metagenomics, as well as the 18S, 5.8S, and 28S sequences, which encode critical structural and catalytic components of the protein-making factories of the cell, the ribosomes. I created a database of these rDNA sequences, each of which contained stretches of sequence that were unique to (or diagnostic of) the species from which it came. I created a bioinformatic pipeline that compared two approaches: 1. aligning un-amplified WGS to the database and 2. aligning ITS1/2 amplicons of the DNA from the same source. I found that the novel approach of aligning the un-amplified WGS sequences to a database of whole rDNA complexes detects more species in environmental samples than the traditional amplicon-based approach. This new bioinformatic tool is being used to evaluate the disparity between how many species have the potential to establish in a region, versus how many species are observed living in the area. This disparity may be an indicator of disturbance and could inform conservation biologists' decisions on where to focus their efforts.

In addition to the ribosomal DNA of lichen mycobionts, I was able to obtain whole rDNA assemblies of common photobionts used by lichens, including those of green coccoid algae (Trebouxiophyceae), green trentepohlioid algae (Trentepohliaceae), and of photosynthetic cyanobacterial photobionts (Nostocaceae). Little is yet known of the composition of photobiont communities that take residence inside of a lichen thallus. Small-scale studies of individual species (Dahlkild et al., 2001; Blaha et al., 2006; Thüs et al., 2011) have revealed that photobiont communities are not a monolithic, clonal photobiont, but rather a diversity of genetically different individuals. For my fifth chapter, I investigated the factors that determine how diverse a lichen photobiont community is, including their growth form, their reproductive mode, and the elevation at which the lichen grew. By aligning metagenomic reads from a lichen DNA library to the database of algal and cyanobacterial reads, I was able to assess the photobiont community diversity using two common metrics, θ_{π} and $\theta_{Watterson}$. I hypothesized before obtaining the results that the most important determinants would be: 1. growth form (that crustose species, which tightly adhere to their substrate, would be the most diverse), 2. reproductive mode (that asexually reproducing species would have lower diversity because they carry their photobionts with them when they propagate) and 3. elevation (that higher elevation lichens would have lower diversity due to there being fewer species at higher elevation). I found that growth form does indeed play a significant role in determining photobiont diversity – crustose species have more diverse communities than both foliose (leafy, lobed) and fruticose (tufty, three-dimensional) lichens.

We found that algal photobiont does not vary according to elevation, but that cyanobacterial photobiont diversity does. This finding may indicate that the availability of suitable cyanobionts becomes scarce at higher elevations. However, the underlying physiological

reason for why this relationship exists in cyanobacteria but not algal photobionts remains to be characterized.

Contrary to our expectations, reproductive mode does not play a strong role in photobiont diversity. Asexual species contain just as much diversity in their photobiont communities as sexually-reproducing species. This implies that not only are asexual species also incorporating suitable photobionts as their thalli grow, as do sexual species, that they don't have any narrower a phylogenetic range of suitable photobionts than sexually reproducing species. This may be evolutionarily adaptive, as any genetic change in an asexual mycobiont that reduces its range of suitable photobionts would poorly weather any environmental change in the availability of those photobionts.

In my final chapter, I summarize the key findings of this thesis. I discuss limitations of each study and suggest future research for the lichen metagenomic data I used in my fourth and fifth chapters.

In the course of developing this thesis, I took special interest in developing a broad bioinformatic skillset that I used to address questions in many other biological systems, including barn swallows, prairie dogs, cutthroat trout, diatoms, *Cannabis*, *Arabidopsis*, *Iochroma*, catfish, and sunflowers. These projects often required novel coding, tools, and applications. Collaborations were a key feature of my tenure as a Kane Lab graduate student, as many researchers in other EBIO labs were working on systems and questions that could be tackled from a bioinformatic standpoint, and those researchers would seek out the specialized skillset of a bioinformatician. I derived much enjoyment in collaborating with these researchers and helping them elevate and elaborate their research by adding an exciting bioinformatic component to their projects.

CHAPTER 2

This chapter was published as a manuscript in *The Southwestern Naturalist* in March 2018. Kyle Keepers conducted survivorship analyses and contributed to the writing of the manuscript. Nolan Kane helped with the survivorship analysis. Andrew Martin conducted the facilitated migration experiment and the mark-recapture study, a few analyses and much of the writing of the manuscript.

Following the Fate of a Facilitated Migration In a Small Desert Spring

2.1 ABSTRACT

Human modification of the environment can result in the fragmentation and isolation of natural populations. If isolated populations are small, they may experience higher probabilities of extirpation from genetic, demographic, and environmental effects. One approach for managing fragmented and isolated populations is facilitated migration in which individuals are moved between habitat fragments. Here we report on a study of a single system in which we followed the genetic and population consequences of facilitated migration. We moved a small number of pupfish (*Cyprinodon nevadensis pectoralis*) from one small spring into another small spring that had become isolated as a consequence of human modification of surface hydrology. We followed the fate of immigrant, resident, and admixed fish over multiple generations using molecular identification of individuals and mark-recapture methods. The mark-recapture data revealed that survival probabilities for admixed individuals were about 20% greater than those for the original resident fish. Furthermore, there was a steady increase in the proportion of admixed individuals, suggesting that immigrant alleles spread through the population consistent with the estimate of

relative fitness. Overall, the results suggest facilitated migration can have restorative effects over the course of very few generations, and these results, in the context of other studies, suggest facilitated migration is likely to be an effective strategy for managing populations that have become isolated as a consequence of human modification of landscapes.

2.2 INTRODUCTION

Human activities have caused the fragmentation and isolation of populations (e.g., Westemeier et al., 1998). For fish, population fragmentation can happen as a consequence of modifications to surface hydrology and connectivity; the most dramatic examples are dams that can severely impact the connectivity of populations (e.g., Nerass and Spruell, 2001). It is often the case that, following fragmentation and isolation, populations are more susceptible to demographic and environment stochasticity (Lande, 1993) and the decline of population viability due to genetic effects (Lande, 1988; Templeton, et al. 1990; Ellstrand and Elam, 1993; Westemeier et al., 1998; Paland and Schmid, 2003; Johnson et al., 2010; Adams et al., 2011). One strategy for reversing the detrimental effects of human-caused population isolation is facilitated migration. Facilitated migration—also called human-assisted migration—involves movement of individuals as a strategy for increasing population persistence in the face of environmental change (Olden et al., 2011), countering the detrimental effects of demographic stochasticity (Burkey, 1989) and increasing individual and population viability through genetic rehabilitation (Whiteley et al., 2015).

In the Ash Meadows National Wildlife Refuge in southwestern Nevada, near the southern end of the Mojave Desert, there are a number of low-flow springs (<100 gallons/min) that support populations of pupfish (*Cyprinodon nevadensis pectoralis*). Historically, the low-flow springs were intermittently connected during periods of exceptional precipitation (Miller and

Deacon, 1973; Martin, 2010; Paulson and Martin, 2014). However, some of the springs have become isolated due to water diversion (Miller and Deacon, 1973). Other factors suspected of decreasing the connectivity of the springs include local and regional groundwater pumping (Deacon et al., 2007) and declines in average precipitation due to the effects of climate change (Seager et al., 2007).

Here we focus on one low-flow spring named South Scruggs. Several factors have influenced the pupfish population in the spring over the last 50 years or more. First, crayfish invaded the spring and there has been a correlated decline in pupfish numbers (Rogowski and Stockwell, 2006; Scoppettone, 2011, 2012). Second, a small stream diversion severed a historical drainage connection between South Scruggs and lower-elevation springs (Martin, 2010), causing isolation of the population to a small section near the spring source (Scoppettone, 2011, 2012). Third, the region has experienced increased groundwater extraction that may explain an apparent decline in spring flow rates, suggesting the extent of available habitat has declined over time (Fig. 2.1). These factors may explain, at least partially, the smaller population size today than in the past (Miller and Deacon, 1973; Martin, 2010; Scoppettone, 2011, 2012).

Isolation of the South Scruggs population and the apparent decline in population size, coupled with low effective population (Martin, 2010), prompted a decision to assess whether assisted migration might provide a means of maintaining the viability of the population. In 2009, we introduced 10 pupfish of the same recognized subspecies into the South Scruggs spring from a nearby spring (North Indian). We followed the fate of individuals and genes for a period of 40 months. Our results suggest facilitated migration may provide a useful and relatively easy strategy for maintaining connectivity across populations that have become isolated as a consequence of human modifications to landscapes.

Figure 2.1. Upper: Estimated volume of groundwater extracted from the regional aquifer for Ash Meadows (Zdon and Associates, Inc., 2014). Lower: Spring flows for South Scruggs, a low-flow spring in the Ash Meadows National Wildlife Refuge in southwestern Nevada (A. Martin, pers. observ.).

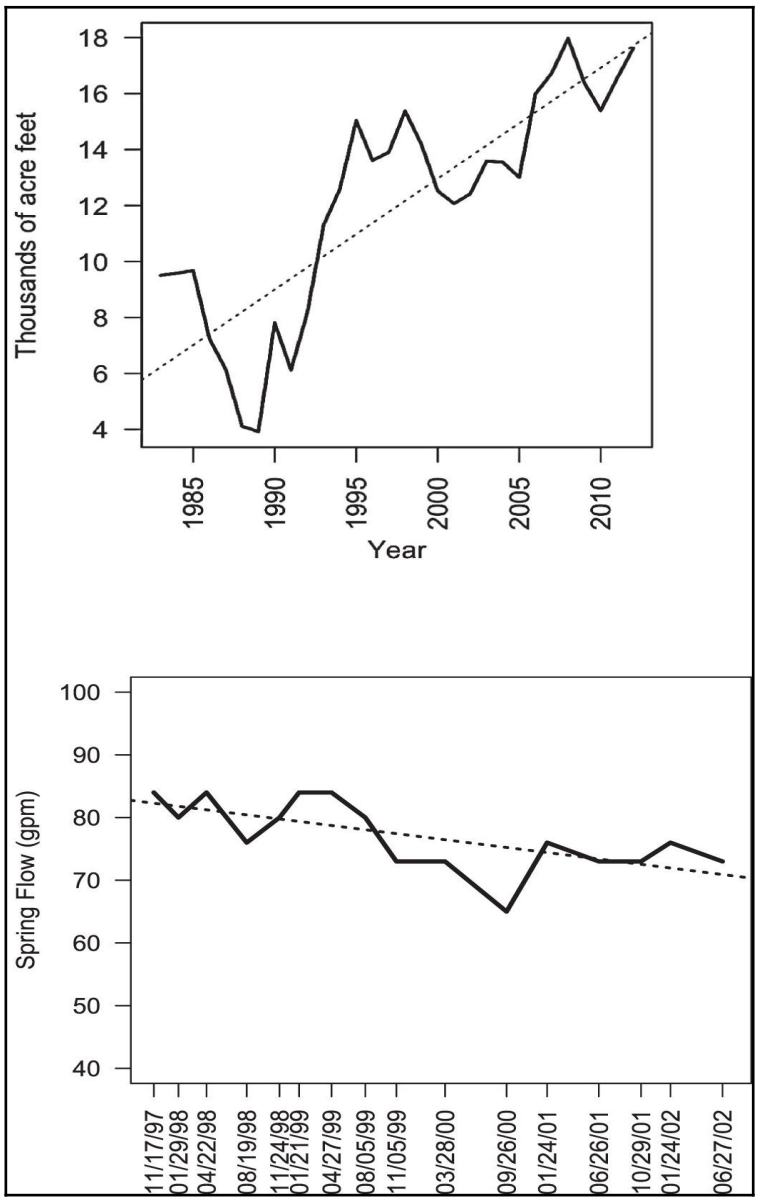
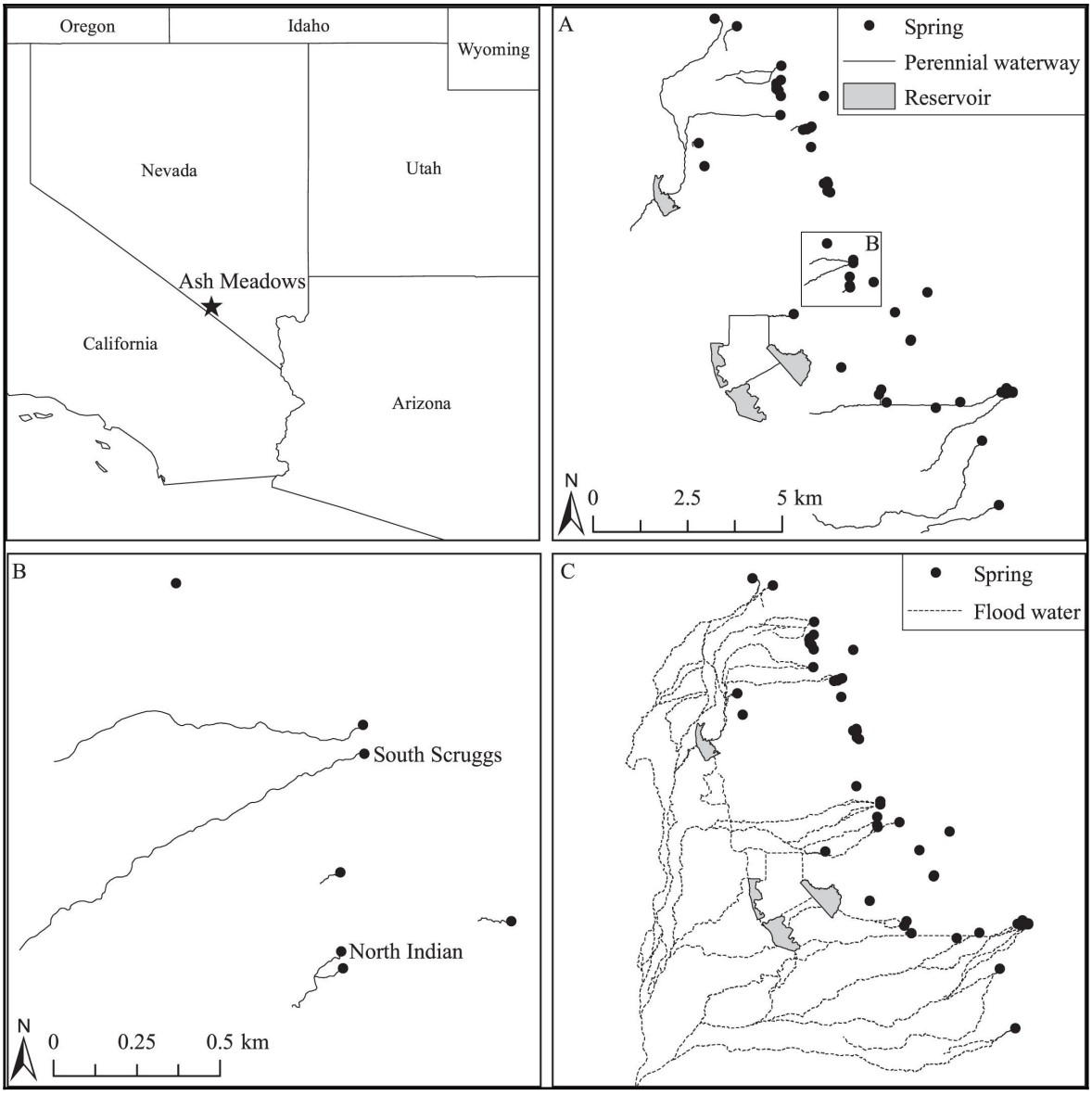


Fig 2.2. Map of the location of Ash Meadows National Wildlife Refuge in southwestern Nevada (upper left), the location of low-flow springs (in the square box in the upper right map), the outflows and locations of South Scruggs and North Indian (lower left), and a picture of the inferred connectivity among species during periods of exceptional precipitation. Note the inferred connectivity reflects some hydrological diversions due to roads so that the inferred patterns do not portray the native historical conditions (see Paulson and Martin, 2014).



2.3 MATERIALS AND METHODS

South Scruggs spring is one of five isolated low-flow springs in a relatively small area of the Ash Meadows National Wildlife Refuge in southwestern Nevada (Fig. 2.2). Each spring and outflow supports a small population of an endemic pupfish (*Cyprinodon nevadensis pectoralis*; Martin, 2010). All of the springs supporting pupfish populations are thermal springs and have a relatively low variation in temperature across seasons, especially near the spring sources where most pupfish are found (Scoppettone, 2011, 2012). Spawning and recruitment occur throughout the year. Historical records indicate the springs probably supported more fish in the past than in 2009 when we began this study (Miller and Deacon, 1973). Previous analyses of mtDNA and microsatellite genotypes revealed South Scruggs was genetically divergent from the other low-flow springs in the immediate area (Martin, 2010).

We captured 10 individuals (five females and five males) in April 2009 from a nearby spring (North Indian). North Indian is another low-flow thermal spring about 0.6 km south of South Scruggs (Fig. 2.2). The North Indian environment is very similar to South Scruggs: several low-flow springs coalesce into a single outflow stream, and spring flow rates are similar, 70 and 50 gallons/min for South Scruggs and North Indian, respectively (A. Martin, pers. observ.). Additionally, pupfish size distributions were similar between the two springs (Scoppettone, 2011). The 10 pupfish were held in an aquarium for approximately 4 months and were treated for parasites prior to their introduction into the South Scruggs spring in July 2009.

We monitored the population in South Scruggs by regularly collecting fish using small minnow traps. For each trapping session, we deployed eight traps at the same regularly spaced positions in a small section of the upper portion of the spring just below an old road. The targeted region spans an area of preferred habitat based on United States Geological Survey (USGS)

survey data (Scoppettone et al., 2011, 2012). We used the same types of traps as those deployed by the USGS (Scoppettone, 2012). We left the traps in the outflow stream for approximately 2 h and then retrieved them. We transferred all captured fish to an aerated bucket and redeployed the traps. We continued this process of trap retrieval, transfer of fish to buckets, and redeployment of traps until all traps failed to capture a single fish for a period of 2 h. We trapped fish every 3–4 months from July 2009 to January 2012 and then once in November 2012. For all trapping episodes, we removed a very small piece of tissue (≈1–2 mm2) from the caudal fin and placed it in 95% ethanol for each fish greater than 18 mm in length. We immediately returned fish smaller than 18 mm to the stream to decrease the likelihood of mortality.

We extracted DNA using Qiagen tissue extraction kits (Qiagen, Germantown, Maryland). We genotyped each individual for five highly polymorphic microsatellite loci: CmD1, CmD16, Gata9, Gata26, and Gata108 (Burg et al., 2002). For each sample episode, we determined the number of alleles, observed and expected heterozygosity, the number of unique genotypes, the number of genotypes shared by different individuals, the number of marked individuals that were recaptured, and the probability of identity (PID) of two randomly sampled individuals (Paetkau and Strobeck, 1994). GenoDive was used for estimating these parameters of genetic variation (Meirmans, 2013; Table S2.1).

We assigned each individual captured to one of three groups—NI (immigrants from North Indian), SS (resident South Scruggs fish), or NI × SS (admixed between these two groups)—using a hybrid index method (Buerkle, 2005) implemented in GenoDive (Meirmans, 2013). We used the NI and SS individuals from July 2009 as reference populations for assigning individuals from subsequent sample dates as migrant, resident, or admixed.

Table S2.1. Highly polymorphic microsatellite genotypes (Gata9, Gata26, Gata109, CmD16, CmD1) of the individuals sampled in this study. Individuals from North Indian Spring begin with NI and resident individuals of South Scruggs are designated with SS. Population ordinals (1-11) refer to the different sampling events in order, except for 1 and 2, which were North Indian and South Scruggs sampled at the same time, April 2009.

South Scruggs experiment					
451	10	5	2	3	
NI					
709					
1109					
22410					
52610					
82510					
112510					
31211					
92211					
12012					
111512					
Population	Individual	Gata9	Gata26	Gata109	CmD16	CmD1	
	1NI709-10	269293	234262	286298	366382	242262	
	1NI709-1	257269	234234	298298	350366	242254	
	1NI709-2	265269	234250	286294	370370	242262	
	1NI709-3	245245	234234	298298	346378	242254	
	1NI709-4	257273	234234	298298	382382	242254	
	1NI709-5	257269	234234	290298	346382	242242	
	1NI709-6	245245	234234	298298	346366	218254	
	1NI709-7	245265	234234	252262	350350	254262	
	1NI709-8	265293	234250	294294	370382	242266	
	1NI709-9	269297	234250	290298	350374	262262	
	2SSc709-10	249249	242246	278278	378394	258258	
	2SSc709-11	249273	246254	270278	378394	226258	
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10SS12012-18	265265	0	274278	0	226226
10SS12012-19	269289	234246	278294	366378	242262
10SS12012-2	269289	234246	278290	378394	242262
10SS12012-20	269289	230250	270282	370394	226242
10SS12012-21	289289	234262	282286	366382	214242
10SS12012-3	289289	246250	286294	318370	226242
10SS12012-4	265289	246250	274294	370378	242258
10SS12012-5	265289	246250	274294	370378	242258
10SS12012-6	289289	262262	282294	378382	242242
10SS12012-7	289293	246246	270270	378394	262262
10SS12012-8	249265	246250	274278	370394	226254
10SS12012-9	289289	246262	270286	378382	214258
11SS11152012-10	249289	230246	278294	378394	214226
11SS11152012-11	289289	246262	278278	370382	214226
11SS11152012-12	289289	246246	270286	378394	258262
11SS11152012-13	269289	246250	278286	382394	242266
11SS11152012-14	273289	250262	286294	370382	214258
11SS11152012-15	289293	246246	286294	378378	214242
11SS11152012-16	289289	246262	270294	378378	226242
11SS11152012-17	249289	250262	278286	370382	242258
11SS11152012-18	269289	246262	294294	378382	214242
11SS11152012-19	249249	0	0	0	214242
11SS11152012-1	289289	234246	274278	366394	226266
11SS11152012-20	265293	246250	286294	366370	242258
11SS11152012-21	269289	246246	286294	318366	242262
11SS11152012-22	289289	250262	274286	370394	214266
11SS11152012-23	289289	246250	278286	370378	258266
11SS11152012-24	269289	250250	282294	370370	226262
11SS11152012-25	249289	246262	278286	366394	258266
11SS11152012-26	289289	246246	286294	366378	214262
11SS11152012-27	269289	246246	294294	378378	226262
11SS11152012-29	249289	246250	274278	370394	254258
11SS11152012-2	289289	246246	278286	366378	214242
11SS11152012-30	289289	250262	278278	366370	214242
11SS11152012-31	289289	246262	282286	378382	214242
11SS11152012-32	269289	250262	274286	370370	226242
11SS11152012-33	289289	250254	274282	370370	226262
11SS11152012-34	265293	250262	286294	366378	258262
11SS11152012-35	289289	246250	270294	318370	214262
11SS11152012-35	289293	246246	286294	378378	214242
11SS11152012-36	289289	246262	278294	382382	226266
11SS11152012-37	265289	246246	278278	318366	262266

11SS11152012-38	269289	246246	278282	318394	214226
11SS11152012-3	289289	246246	270278	318378	262262
11SS11152012-40	289289	246246	286294	378394	214262
11SS11152012-41	269289	234246	274278	318382	214214
11SS11152012-42	265289	262262	278286	366394	226254
11SS11152012-43	289289	246262	278294	318378	226262
11SS11152012-44	289289	246262	278286	378378	214262
11SS11152012-45	269289	246246	286294	318366	214214
11SS11152012-46	289289	246246	278294	318378	242266
11SS11152012-47	289289	234262	278294	366378	214226
11SS11152012-48	249265	250262	278294	366370	242258
11SS11152012-49	273289	246262	278286	366366	242258
11SS11152012-50	285289	262262	270294	366382	258266
11SS11152012-51	269289	246250	294294	318394	214214
11SS11152012-52	289289	246246	294294	378378	214262
11SS11152012-53	265269	246262	278278	366366	214226
11SS11152012-54	269289	246262	270286	318382	226242
11SS11152012-55	289289	246262	278278	318370	242266
11SS11152012-56	289289	262262	274294	366382	214242
11SS11152012-57	249269	246246	278278	366394	242254
11SS11152012-58	289289	246250	274278	318370	254262
11SS11152012-59	289289	246250	278286	382382	214262
11SS11152012-5	245249	234250	274286	370394	226242
11SS11152012-60	269289	246246	274294	378394	226258
11SS11152012-61	289289	246262	282294	378382	242258
11SS11152012-62	289289	246262	278294	366394	258266
11SS11152012-63	249289	250262	274282	370382	242242
11SS11152012-64	269289	246246	274286	378394	214262
11SS11152012-65	269289	246250	278294	370394	214242
11SS11152012-66	265269	246250	294294	378394	226266
11SS11152012-67	249265	246262	278278	366394	214226
11SS11152012-68	269289	246246	286294	370378	258262
11SS11152012-69	265265	246266	274294	366394	214226
11SS11152012-6	289289	262262	282294	378382	242242
11SS11152012-70	289289	246246	294294	378382	226262
11SS11152012-71	265269	234246	278290	366378	226266
11SS11152012-72	289289	246246	286294	378394	214226
11SS11152012-73	269289	234246	286294	366378	242242
11SS11152012-74	269269	246246	278286	370378	214254
11SS11152012-75	269289	246246	286294	366378	214262
11SS11152012-76	289289	246246	278278	378378	226262
11SS11152012-77	289289	234246	278294	366378	226266
11SS11152012-79	265289	234246	278278	366366	214258
11SS11152012-7	269289	246250	278282	318370	226262
11SS11152012-80	289289	246246	0	318318	242262
11SS11152012-81	289293	250262	0	366370	226242
11SS11152012-82	269289	246250	282282	370382	214226
11SS11152012-83	289289	246262	278282	382394	226242
11SS11152012-86	249249	262262	270270	366382	226258
11SS11152012-87	245245	246246	270278	366370	226242
11SS11152012-88	265273	246246	278278	366366	226258
11SS11152012-89	265265	0	0	0	242242
11SS11152012-8	289289	234246	278294	378382	214242
11SS11152012-90	289289	0	0	0	214214
11SS11152012-91	249269	246246	0	0	214214
11SS11152012-92	289289	246262	274294	366378	214266
11SS11152012-93	289289	246246	270282	378378	226242
11SS11152012-94	269289	246262	278278	382394	226226
11SS11152012-95	289289	246262	278282	318382	242262
11SS11152012-96	269269	214214	0	318318	214214
11SS11152012-97	289289	246246	0	0	214214
11SS11152012-9	289289	246246	270294	378378	214262

We included all genetically unique individuals in a mark-recapture analysis of survival and sampling probabilities for the three genotype groups—resident SS, immigrant NI, and SS × NI admixed—with the program MARK (White and Burnham, 1999). We were interested in evaluating two *a priori* hypotheses. The null hypothesis was that there were no differences in survivorship among the three genotypes across all sample episodes. The alternative, biologically relevant hypothesis was that there were differences in survivorship among the three groups. For the latter hypothesis, we imagined two possible scenarios. The first was that immigrant fish would have higher survivorship because they were introduced into South Scruggs after being held in captivity for 4 months, where they were fed and treated for parasites. The second was that individuals with admixed ancestry would have higher survival than the resident individuals, possibly due to the amelioration of inbreeding depression. We evaluated alternative models using Akaike information criterion (AIC; White and Burnham, 1999).

We estimated population size from the estimated capture probabilities that were generated by mark-recapture analysis using MARK (White and Burnham, 1999). We compared the estimated population size estimates with census data from USGS surveys. We estimated effective population size using CoNe (Anderson, 2005). We based the estimation on the genotypes at the beginning and end of the experiment, in July 2009 and November 2012, respectively, and assumed a generation time of 300 days (or four generations during this period). We based the estimate of generation time on the time until the first appearance of an F1 admixed (NI × SS) adult individual following the introduction of immigrant NI individuals into SS.

2.4 RESULTS

We trapped the population and counted and genotyped fish 10 times between July 2009 and November 2012. The number of fish sampled during the study period declined from 82

individuals in July 2009 (this is the sum of the 72 resident fish and 10 immigrant individuals), to a low of 21 individuals in January 2012, and then increased to 107 individuals by November 2012.

We captured and genotyped a total of 413 fish for all five loci. Of these 413 individuals, there were 336 unique genotypes. Across all individuals and loci we discovered 53 alleles. For each sample, we observed from 29 to 39 different alleles across the five polymorphic loci. Expected heterozygosity (HE) of the sampled fish from the South Scruggs spring ranged from 0.64 at the beginning of the experiment to a maximum of 0.76 after about a year and was 0.72 at the end of the experiment. There was an excess of observed heterozygotes (HO) relative to HE for several sample times, especially after the appearance of admixed individuals. The estimated probability of identity based on theory was less than 0.0004 for all sample episodes (Table 2.1). However, we discovered four genotypes that were shared by four pairs of individuals from three of the sample episodes. Based on these data, the proportion of unique individuals that shared genotypes (4/413 = 0.0097) was about two orders of magnitude greater than expected, assuming random assortment of alleles across all five loci (Table 2.1). The discrepancy between theoretical and observed PID observed in this study was similar to other studies (Waits et al., 2001). The observed PID was sufficiently small (<0.01) for robust estimation of population size using mark-recapture methods (Mills et al., 2000).

Sample	N_{ind}	N_{all}	H_O	H_E	G_{IS}[a]	PID	G	G_R	P_S	Re
NI70209	10	28	0.640	0.706	0.093	0.00024	10	0	0	NA
SS70209	68	31	0.700	0.660	−0.061**	0.00030	66	2	0.03	NA
SS112309	57	39	0.656	0.694	0.055**	0.00023	57	0	0	9
SS22410	56	39	0.675	0.693	0.026	0.00025	55	0	0	16
SS52610	38	39	0.737	0.687	−0.072*	0.00031	38	0	0	17
SS82510	11	30	0.764	0.684	−0.117**	0.00037	11	0	0	5
SS112510	36	35	0.789	0.759	−0.039	0.00013	35	1	0.028	7
SS31211	12	30	0.800	0.730	−0.095	0.00021	12	0	0	4
SS92211	24	34	0.842	0.732	−0.150***	0.00015	24	0	0	6
SS12012	18	30	0.800	0.743	−0.077*	0.00022	17	1	0.056	4
SS111512	84	35	0.717	0.722	0.007	0.00020	83	0	0	1
Total	414	53	0.738	0.761	−0.039		336	4		68

[a] $*P < 0.1$, $**P < 0.05$, $***P = 0.001$

Table 2.1. Number of individuals genotyped for all five loci (N_{ind}), number of different alleles detected in the sample (N_{all}), and observed (H_O) and expected (H_E) heterozygosity. G_{IS} is the inbreeding coefficient estimated from all five loci, the probability that two individuals were identical across all five genotypes (PID), the number of unique genotypes (G), the number of repeated genotypes (G_R), the proportion of individuals sharing a genotype (P_S), and the number of recaptured individuals (Re). NA = not applicable. Data were gathered at South Scruggs, a low-flow spring in the Ash Meadows National Wildlife Refuge in southwestern Nevada, beginning in 2009.

Of the 413 individuals genotyped, 331 of them were marked and could have been recaptured. We recaptured 56 individuals: 42 individuals were recaptured once and 13 individuals were recaptured twice (Table 2.1). The minimum and maximum times between marking and recapture were 3 and 22 months, respectively.

We assigned individuals to one of three genotype groups, immigrant, resident, and admixed, based on hybrid index scores (Fig. 2.3). We identified them as immigrant and resident individuals if the lower and upper confidence intervals for the hybrid score included 1 or 0, respectively. We identified them as admixed individuals if the confidence limits for the hybrid score were between 0.05 and 0.95. Overall, using the hybrid index scores from the genotype data, we identified 10 North Indian immigrant individuals, 234 resident South Scruggs individuals, and 96 individuals with admixed (NI × SS) ancestry. Based on a predictive model,

the probability of sampling an admixed individual at the end of the experiment was just over 0.40.

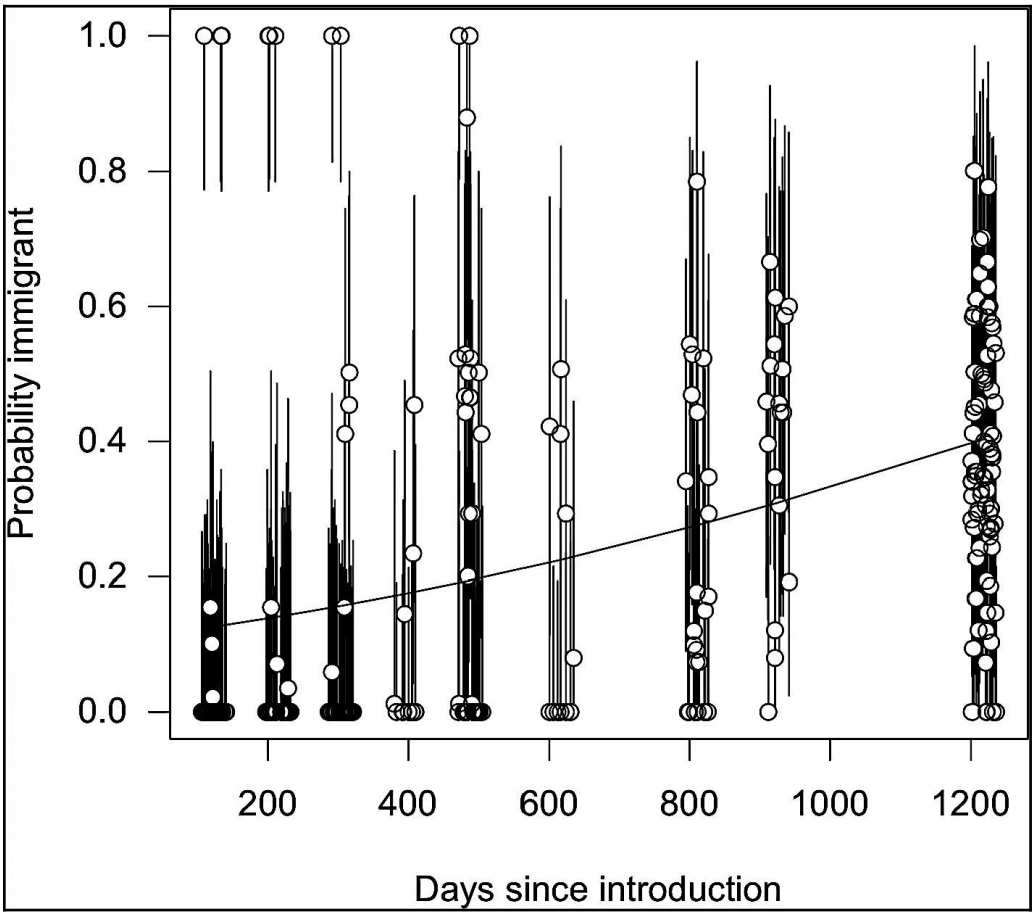


Fig 2.3. The probability that an individual has an immigrant genotype inferred from the hybrid index score. Vertical lines are confidence intervals. Model line was derived from beta regression. Values were jittered slightly along the x axis to better reveal the data.

For the mark-recapture analysis, we first randomly omitted one individual for each of the four pairs of genetically identical but different individuals. Using the program MARK, we compared 17 models that varied with respect to the probabilities of sampling individuals by date and

genotype (immigrant, resident, and admixed) and the probabilities of survival by date and genotype. We were particularly interested in evaluating the fit of the data to two models aligned with an *a priori* biological hypothesis and the null hypothesis. The best model (of the 17 models evaluated) was our *a priori* biological hypothesis; namely that survivorship differed among the three genotype groups, that the probability of sampling was identical across the three genotype groups, and that there was no effect of sample date for each parameter estimate. The *a priori* biological hypothesis was approximately 100 times more likely, given the data, than the model representing the null hypothesis (Table 2.2). Moreover, a likelihood ratio test of the two hypotheses revealed the null hypothesis was an implausible explanation of the data relative to the biological hypothesis ($\chi 2 = 13.175$, $P = 0.0014$). There were two other models that were similar to the best model based on AIC scores (Table 2.2). Likelihood ratio tests revealed these two models were indistinguishable from the best model.

Hypothesis	Model	Parameters	AIC	ΔAIC	AIC_W	Model likelihood
Real world	**Phi(g), p(.)**	**4**	**439.3**	**0.00**	**0.41**	**1.00**
	Phi(g), p(g)	6	440.0	0.72	0.28	0.697
	Phi(.), p(g)	4	440.1	0.80	0.27	0.671
	Phi(g), p(t)	12	444.8	5.49	0.03	0.064
Null	**Phi(.), p(.)**	**2**	**448.4**	**9.10**	**0.00**	**0.011**
	Phi(t), p(g)	12	450.1	10.81	0.00	0.004
	Phi(.), p(t)	10	451.9	12.56	0.00	0.002
	Phi(t), p(i)	10	457.7	18.41	0.00	< 0.001
	Phi(g*t), p(g)	22	460.8	21.50	0.00	< 0.001
	Phi(.), p(g*t)	25	463.1	23.77	0.00	< 0.001

Table 2.2. MARK results for 10 AIC-ranked models. Phi and p are the probabilities of survival and capture, respectively. The notation (.) indicates no difference among genotypes or sample dates, (t) indicates differences among sample dates, (g) indicates differences among genotype groups (resident, immigrant, and admixed), and (g*t) indicates differences across genotypes and times. Our a priori real world and the null hypotheses are indicated in bold.

Parameter estimates for survivorship and probability of sampling each genotype group for the three best models are included in Table 2.3. For the best model, corresponding to the *a priori* biological hypothesis, survivorship estimates of immigrant (NI) and admixed individuals were statistically indistinguishable (0.90 and 0.89, respectively) and were approximately 1.2 times higher than the survivorship of the resident (SS) fish (0.77). Survival values were similar between the two best models (Table 2.3), although the difference between the resident and admixed individuals was slightly less for the second-best 6-parameter model than for the statistically best 4-parameter model (Table 2.3).

Model	G	Parameters	Phi	lwr	upr	p	lwr	upr
Phi(g),p(.)	NI	4	0.900	0.804	0.952	0.344	0.241	0.465
	SS		0.771	0.719	0.816	0.344	0.241	0.465
	Admix		0.892	0.806	0.943	0.344	0.241	0.465
Phi(g),p(g)	NI	6	0.879	0.776	0.939	0.556	0.261	0.816
	SS		0.790	0.732	0.839	0.279	0.177	0.411
	Admix		0.871	0.762	0.934	0.461	0.183	0.766
Phi(.), p(g)	NI	4	0.824	0.779	0.861	0.606	0.319	0.834
	SS		0.824	0.779	0.861	0.226	0.147	0.332
	Admix		0.824	0.779	0.861	0.567	0.288	0.808

Table 2.3. Parameter estimates from MARK for the three best models. Model variables and symbols same as Table 2.2. Lwr and upr are the lower and upper confidence intervals.

The mark-recapture estimate of the probability of sampling an individual was 0.32 (95% confidence interval, 0.226–0.436). When we applied the probability of sampling an individual to the sample sizes in this study, we found the population size estimates declined from approximately 200 to about 60 individuals. These estimated population sizes were similar to the census sizes reported by the USGS (Fig S2.2). In addition, at the end of the experiment biologists exhaustively removed a total of 279 fish from the stream (in November and December of 2012) prior to drying out the spring. The number of fish from exhaustive sampling fell within the 95% confidence interval (245–473) based on the population size estimate from the mark-recapture model.

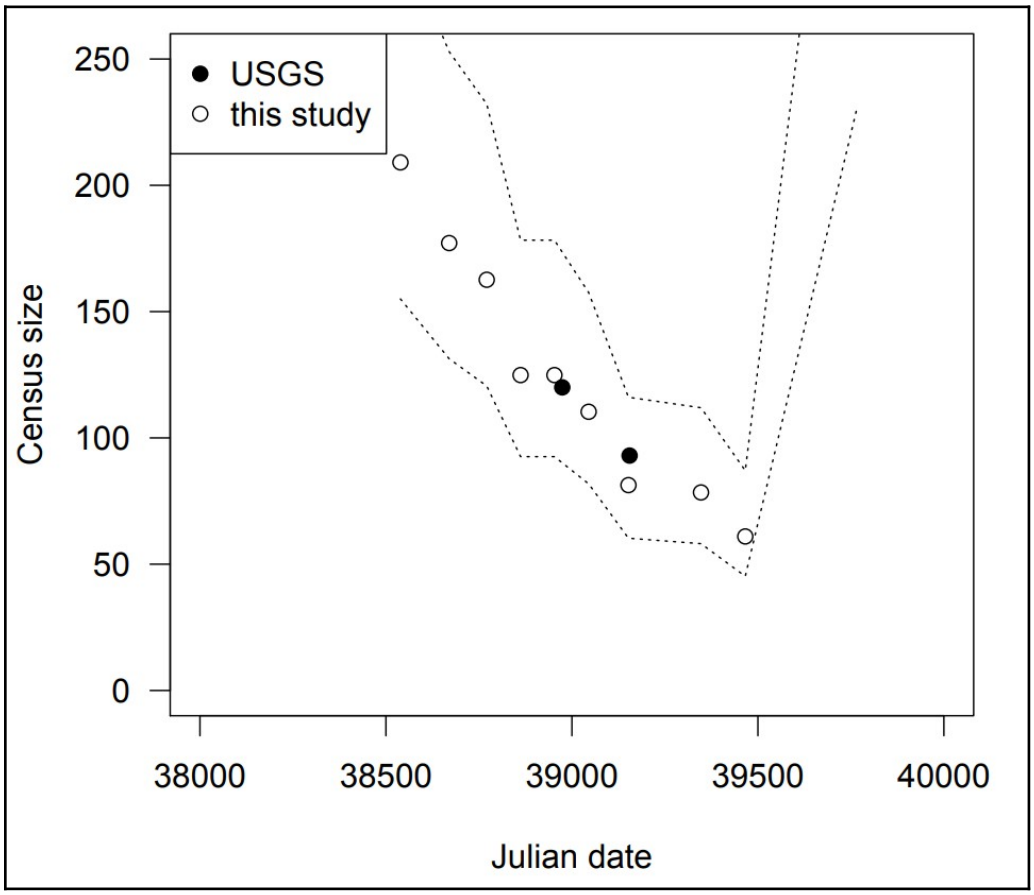


Fig S2.1. USGS estimates of census size (filled symbols) and estimates of population size based on probability of capture parameter estimated from mark recapture analysis (open symbols). Dashed lines are lower and upper confidence intervals for the population size estimates. Importantly, this study used fewer traps, over a smaller portion of the spring (see Supplemental Figure S1), and for less time than the USGS surveys (Scoppettone et al. 2011; 2012). Thus, our overall trapping effort was lower than for the published USGS surveys.

Adult F1 offspring from reproduction between NI and SS adults were first detected in May 2010, 10 months after the introduction of the NI fish. The observation of reproductively mature fish with admixed ancestry suggests the upper boundary on generation time is approximately 300 days. Based on these data, the duration of the experiment encompassed four generations. Using this estimated number of generations, the effective population size, which we estimated by

coalescent-based methods using the changes in allele frequencies between July 2009 and November 2012, was approximately 20 (95% confidence limits = 14, 32).

2.5 DISCUSSION

Natural populations are increasingly faced with prevailing environmental conditions that are without historical precedence (Hobbs et al., 2006). One particularly troubling and expanding problem is the fragmentation and isolation of populations. Because fragmentation and isolation can precipitate declines in population viability and increase the probability of extirpation (e.g., Burkey, 1989; Templeton et al., 1990; Ellstrand and Elam, 1993; Lande, 1993; Westemeier et al., 1998), conservation actions necessarily require adopting strategies that can mitigate these negative effects of human actions (see Frankham, 2015). Facilitated migration in which the connectivity of populations is reestablished through human-mediated movement of individuals can be an effective conservation tool. This study adds to a growing list of cases supporting the claim that facilitated migration can help manage species subjected to fragmentation and isolation into multiple small populations (e.g., Vander Wal et al., 2015).

The main purpose of our study was to assess whether movement of a small number of individuals had a demonstrable effect on the genetic characteristics of a recipient population over the course of multiple generations. We made several important discoveries. First, the monthly survival probability of resident individuals was approximately 20% less than the survival probability of admixed individuals derived from reproduction between resident and immigrant fish. Because the population was increasingly composed of admixed individuals over time, the average viability of individuals in the population increased as a consequence of an infusion of new alleles. Second, once a large fraction of individuals was of admixed ancestry, the population growth rate shifted from an average monthly decline of 3.4% (±0.9 *SE*) to an estimated monthly

increase of 41%. We cannot assert the change in growth rate was due to the infusion of new alleles because there were a number of confounding variables. Nevertheless, the shift from negative to positive population growth rate following the introduction of fish suggests facilitated migration can be an effective conservation tool. Third, there was an increase of genetic diversity in the population due to the establishment and increase in frequency of immigrant alleles. The increased genetic diversity is likely to provide greater fuel for adaptation (see Barrett and Schluter, 2008) in the face of environmental change predicted for this region of the world (Seager et al., 2007).

Evidence of Selection?

There were two lines of evidence suggesting natural selection can explain some of the change in the characteristics of the population following the infusion of new alleles by assisted migration. First, mark-recapture data revealed a 10–20% higher survivorship of admixed individuals relative to resident fish. The range in survivorship estimates reflects parameter values from two different models. Second, there was a consistent excess of observed heterozygotes following the emergence of admixed individuals that lasted until the last sampling episode 40 months after the experiment began. An excess of heterozygotes relative to predictions from theory suggests heterozygous individuals may have a fitness advantage relative to homozygous individuals.

While the data suggest a role for natural selection, it is possible the difference in survival and the excess of heterozygotes may be due to other factors. For instance, the original 10 immigrant fish may have had higher fitness than all of the resident fish because they were in better condition after spending 4 months in captivity. The difference may have transmitted across generations through maternal effects owing to differences in egg quality of females derived from crosses involving immigrant mothers. In addition, it is possible drift and sampling variation may

explain the results. During the period of time when there was an excess of heterozygotes, effective population size was small (approximately 20 individuals) and sample sizes were also small, conditions that can result in a heterozygosity excess in the absence of selection (Luikart and Cornuet, 1998). Finally, relative survival values of the three genotypes were identical for a model that was statistically similar to, but less likely than, the best model. Although this particular model was not the best model based on likelihood scores, it remains possible that survival estimates for the three genotypes were not different.

However, because our *a priori* hypothesis was that there would be a difference in survival among the three genotypes, and we discovered that this model best explained the data, we are inclined to accept the hypothesis that selection explains the establishment of immigrant alleles and the marked increase in individuals with admixed ancestry over the course of four generations. Nevertheless, mechanisms underlying differences in survivorship among the three genotypic groups of pupfish (immigrant, resident, and admixed) remain unknown.

Comparison to Other Studies

We estimated the difference in survivorship between resident and admixed genotypes as a relevant measure of the effect of assisted migration. We discovered three other field studies of animals focused on survivorship following assisted migration. In three cases, the Florida panther (*Puma concolor*) (Johnson et al. 2010), bighorn sheep (*Ovis canadensis*) (Hogg et al., 2006), and this study, there was a marked increase in survivorship of admixed individuals arising from reproduction between immigrant and resident individuals. In one of the studies, on a fish (the Trinidadian guppy, *Poecilia reticulata*), despite evidence of genetic rescue, survivorship was not different between the residents and fish with admixed ancestry (Fitzpatrick et al., 2016). Our

study and these published studies, along with others showing translocation effects on population size, changes in reproductive rate, and changes in phenotypic characters linked to fitness (e.g., Westemeier et al., 1998; see reviews by Carlson et al., 2014; Vander Wal et al., 2015; Whiteley et al., 2015; Fitzpatrick et al., 2016), suggest facilitated migration increases population fitness across a wide variety of species and environmental contexts.

Facilitated Migration

Natural populations of many animals and plants are increasingly subjected to habitat fragmentation and isolation due the combined effects of climate change and human modification of landscapes (Foley et al., 2005). Our study, like others, suggests that facilitated migration of individuals between populations that have become isolated can provide a relatively easy means of reversing predicted and observed declines in population fitness (e.g., Westemeier et al., 1998). So far there are no general rules for the number and frequency of facilitated migration events necessary to maintain high population fitness, and it is likely each natural system will require some type of adaptive management when implementing facilitated migration programs for achieving particular conservation goals. Ideally, migration is sufficiently frequent to maintain population size at historical levels before population fragmentation and isolation occurred. Unfortunately, for most species and populations historical data are lacking; nonetheless, there may be proxy data that can provide some guidance. In the Ash Meadows system, extraordinary precipitation events cause flooding and enable the movement of individuals between populations. For instance, between 1997 and 2007 there was unidirectional migration between two normally isolated springs that probably occurred during a localized flood (Martin, 2010). Thus, we can use the precipitation record as a proxy for calibrating the frequency with which individuals should be moved between currently isolated populations that were historically, but episodically, connected

in the Ash Meadows system (Martin, 2010). Relevant proxies for the frequency of connections between populations may exist for other fragmented systems.

For many systems, habitat fragmentation and the isolation of populations may be irreversible, making reestablishment of historical connectivity among populations impossible. For these systems, a new era of management is upon us in which metapopulation dynamics are managed through facilitated migration (Aitken and Whitlock, 2013). Knowing whether facilitated migration is a viable option, implementing an optimal strategy for manipulating the demography of metapopulations, and engineering evolution through demographic manipulation represent new challenges for conservation biologists. Our case study suggests engineering evolution through facilitated migration is a viable strategy that may aid conservation efforts aimed to stem the tide of extirpation and extinction that often accompanies population fragmentation and isolation.

CHAPTER 3

This chapter was published in *Mitochondrial DNA Part A* in June 2015. Kyle Keepers performed the library prep, sequencing, genome assembly, and annotation of the mitochondrion. Andrew Martin collected the tissue, advised on sequencing and provided feedback on the manuscript. Nolan Kane advised on the library prep, sequencing, and bioinformatics, and helped with writing the manuscript.

The Complete Mitochondrial Genome of the Warm Springs pupfish, *Cyprinodon nevadensis pectoralis.*

3.1 ABSTRACT

In this article we report the complete mitochondrial genome of the Warm Springs pupfish, *Cyprinodon nevadensis pectoralis*. The genomic DNA of a single female individual was extracted and sequenced on the Illumina HiSeq2000 platform. It contains 16,499 bp and a total of 37 genes, divided into 22 tRNA genes, 2 rRNA genes and 13 protein-coding genes. It exhibits 94% sequence similarity with the other published mitochondrion in its genus, *C. rubrofluviatilis*. A Tamura–Nei maximum-likelihood tree constructed from mitochondrial sequences shows expected phylogenetic relationships between *C. nevadensis* and sister taxa.

3.2 INTRODUCTION

In evolutionary biology, *Cyprinodon* has played an important role in understanding hybridization and introgression (Echelle et al., 1997) and adaptive radiation (Martin & Feinstein, 2013). The

desert pupfish *Cyprinodon nevadensis* is subdivided into several subspecies within the Amargosa river basin, including the Warm Springs pupfish, *C. n. pectoralis*. (La Rivers, 1994).

We present here the whole mitochondrion of *C. n. pectoralis* – the second mitochondrion to be assembled from its family, *Cyprinodontidae*. Tissue was collected from a single female sampling mortality from the South Scruggs spring in the Ash Meadows National Wildlife Refuge in Nevada. Genomic DNA was extracted with the Qiagen DNeasy Blood & Tissue purification kit (Germantown, MD). One whole-genome shotgun library (WGS) was constructed at Macrogen, Korea, producing 100 bp paired-end reads with a 200 bp insert. To supplement the WGS library, a 3 kb mate-pair library was also constructed. Each library was sequenced on its own lane of the Illumina HiSeq 2000 platform (San Diego, CA). The mitochondrion was assembled concurrently with the nuclear genome using SOAPdenovo v2.04 (Luo et al., 2012). Mitochondrial genome sequences were identified and ordered based on homology to known mitochondrial sequences. Overlapping contigs were merged and small gaps were filled using our unassembled Illumina reads to create a single contig representing the complete mitochondrial genome. Errors were identified and corrected by aligning our cleaned and trimmed reads to this draft genome. The final, corrected genome was annotated in DOGMA annotation software (Wyman, 2004), and completed with Sequin v12.91 (Bethesda, MD).

The genes for *nad6*, trnQGln, trnMMet, trnAAla, trnNAsn, trnCCys, trnYTyr, trnSSer-CGA, trnSSer-UGA, trnEGlu and trnPPro are encoded on the heavy strand, with every other gene on the light strand. Genes *cytB* and *cox1* undergo RNA-editing to change their GTG start codon to an ATG. Every gene uses a TAA as a stop codon, with the exception of *nad1*, which uses TAG. There is an average of 26 bp between genes, but the majority of this average is accounted for by a large intergenic region of 832 bp between the last gene and the first. This intergenic space is

likely the hypervariable control region (Stoneking, 2000) for the origin of replication, a feature of many metazoan mitochondria. With this feature removed, the average intergenic space drops to 4 bp. A BLAST search shows this mitochondrion exhibits 94% sequence similarity with the other published *Cyprinodontidae* mitochondrion, the Red River pupfish, *C. rubrofluviatilis*.

A maximum likelihood tree was constructed from the mitochondrial sequences available from NCBI (Fig. 3.1). In addition to the other *Cyprinodon* whose mitochondrial genome sequence was available, sequences from eight other taxa within the order *Cyprinodontiformes* were obtained, as well as sequence from *Melanotaenia*, in the sister order *Atherinoformes* were also available. Alignments were made in ClustalW2 using default parameters (Larkin et al., 2007). The tree was constructed from the Tamura–Nei substitution model (Tamura & Nei, 1993), and was boostrapped with 100 replicates. All analyses were performed in MEGA6 (Tamura et al., 2013). The tree shows expected phylogenetic relationships between all taxa.

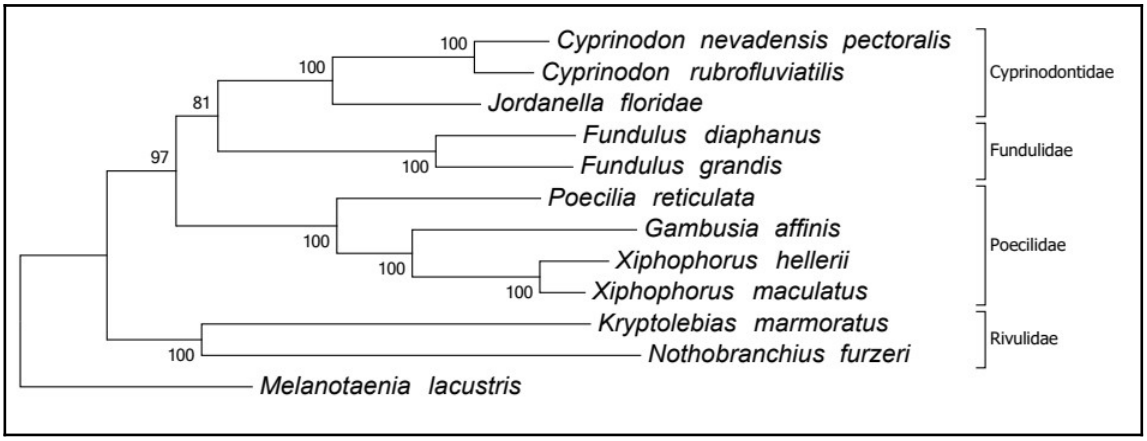


Fig. 3.1. The evolutionary history of Cyprinodontiformes mitochondria was inferred using the Maximum Likelihood method based on the Tamura–Nei model. The tree with the highest log likelihood (−111,802.2510) is shown. Bootstrap values are shown in the figure. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The tree is rooted by Melanotaenia, in the sister order to Cyprinodontiformes, Atherinoformes. Accessions for taxa: C. n. pectoralis (KP064222); C. rubrofluviatilis (NC_009125); J. floridae (NC_011387); F. diaphanus (NC_012361); F. grandis (NC_012377); P. reticulata (NC_024238); G. affinis (NC_004388); X. hellerii (NC_013089); X. maculatus (NC_011379); K. marmoratus (NC_003290); N. furzeri (NC_011814).

CHAPTER 4

This chapter was published in *Frontiers in Ecology and Evolution* in December 2019. Kyle Keepers conducted the research, analyzed the data, and interpreted the data. Kyle Keepers, Cloe Pogoda, Erin Tripp, and James Lendemer wrote the paper. Erin Tripp, James Lendemer, Christy McCain, and Nolan Kane conceptualized the project and secured and managed funding. James Lendemer, Erin Tripp, Kyle Keepers, Kristin White, Carly Anderson-Stewart, and Jordan Hoffman collected and vouchered samples. Processing, curation and archiving of voucher specimens and field data was managed by Ana Maria Ruiz. Kyle Keepers, Kristin White, Cloe Pogoda, and Carly Anderson-Stewart performed the extractions and library preps. Nolan Kane helped develop the bioinformatics pipeline and edited the paper.

Whole genome shotgun sequencing detects greater lichen fungal diversity than amplicon-based methods in environmental samples

4.1 ABSTRACT

In this study we demonstrate the utility of whole genome shotgun (WGS) metagenomics in study organisms with small genomes to improve upon amplicon-based estimates of biodiversity and microbial diversity in environmental samples for the purpose of understanding ecological and evolutionary processes. We generated a database of full-length and near-full-length ribosomal DNA sequence complexes from 273 lichenized fungal species and used this database to facilitate fungal species identification in the southern Appalachian Mountains using low coverage WGS at higher resolution and without the biases of amplicon-based approaches. Using this new database and methods herein developed, we detected between 2.8 and 11 times as many species from

lichen fungal propagules by aligning reads from WGS-sequenced environmental samples compared to a traditional amplicon-based approach. We then conducted complete taxonomic diversity inventories of the lichens in each one-hectare plot to assess overlap between standing taxonomic diversity (TD) and diversity detected based on propagules present in environmental samples (i.e., the "potential" of diversity, or PoD). From the environmental samples, we detected 94 species not observed in organism-level sampling in these ecosystems with high confidence using both WGS and amplicon-based methods. This study highlights the utility of WGS sequence-based approaches in detecting hidden species diversity and demonstrates that amplicon-based methods likely miss important components of fungal diversity. We suggest that the adoption of this method will not only improve understanding of biotic constraints on the distributions of biodiversity but will also help to inform important environmental policy.

4.2 INTRODUCTION

Microbial diversity present in the environment is recognized increasingly for its important and varied roles in the health of ecosystems (Chen et al., 2018; Nottingham et al., 2018; Pike et al., 2018), particularly in the face of a changing climate (Cavicchioli et al., 2019). Unsurprisingly, a great deal of focus has been on quantifying biodiversity—the number, identity, and functions of species (Gotelli & Colwell 2001; Faith 2002; Barlow et al., 2007). It has also helped researchers more fully document the ranges of rare or endangered species through environmental DNA detection strategies (Olson et al, 2012; Spear et al, 2015; Thomsen et al, 2012). Further expanding the impacts of this relatively new field, microbial metagenomics has proven exceptionally useful towards informing remediation strategies of disturbed habitats such as ecologically sensitive biological soil crusts (BSC; Bowker 2007; Steven et al., 2012). Failure to fully understand the microbial (biotic) community can thus dramatically limit understanding of

what structures ecological interactions, species distributions, and environmental sustainability, all of which can, in turn, negatively impact informed conservation decision making (Guisan et al., 2013).

Increased accessibility and affordability of high throughput sequencing, has facilitated broad scale exploration of microbial community structure (Logares et al., 2012; Logares et al., 2014; Chen et al, 2017; Zhang et al., 2018). At present, the majority of broad-scale biotic diversity assessments employ primarily culture-independent, amplicon-based sequencing (Petrosino et al., 2009; Mande et al., 2012; Uyaguari-Diaz et al., 2016). This method relies on sufficiently variable, universally present regions of the genome, or "barcoding loci" (Hebert & Gregory 2005; Kress et al., 2008). Such loci must, first and foremost, be unique enough to yield distinctions between species present in a sample (Kolbert et al., 2004). A proliferation of bioinformatic pipelines developed for barcode sequencing has resulted in widespread capacity to analyze microbial diversity and community structure present in a variety of different environments, ranging from soil to the human body (e.g., QIIME; Caporaso et al., 2010; Kuczynski et al., 2012; Navas-Molina et al., 2013). These pipelines rely primarily on the 16S ribosomal DNA (rDNA) gene as a target for PCR amplification and subsequent sequencing to distinguish species (Winker & Woese 1991; Kolbert et al., 2004; Petrosino et al., 2009). However, shortcomings of such amplicon-based approaches include moderate to extreme amplification bias (Acinas et al., 2005; Wang and Qian, 2009), thus effectively investigating only a fraction of total standing diversity.

Fungi decompose organic litter (Chapin et al., 2002; Osono 2007), produce secondary compounds of tremendous importance to humans such as antibiotics (Keller et al., 2005), are used extensively in food production (e.g. bread, wine, beer; Campbell-Platt & Cook 1989), and

are common agricultural pests (e.g., *Sclerotinia sclerotiorum*; Amselem et al., 2011). Despite their immense ecological (Bever et al., 2001; Pitt & Hocking 2009; Van Der Heijden et al., 2009) and economic importance (Sharma 1989), fungal metagenomics has, on the whole, received less attention relative to bacterial metagenomics. For example, there existed only 360 NCBI Bioprojects studying fungi using amplicon-based molecular barcodes (search conducted on June 21, 2019, in NCBI Bioproject Archive) compared to 5,121 Bioprojects available (same search date) for studies of microbes using amplicon-based molecular barcodes.

Lichens are a species-rich and evolutionarily heterogeneous assemblage of fungi that form obligate symbioses with a minimum of one primary photosynthetic partner, often in addition to other endolichenic fungi, algae, and bacteria (Ahmadjian et al., 1981; Seaward 1997; Brodo et al., 2001; Papazi et al., 2015). Lichens are highly successful and ecologically important, as is evidenced by their abundance and diversity in terrestrial ecosystems around the world (Hawksworth 1991). Along with bryophytes, cyanobacteria, and non-lichenized fungi, lichens are a crucial component of biological soil crust communities and function prominently in ecological restoration processes (Belnap 2001; Belnap & Lange 2001; Thompson et al., 2006; Bowker, 2007). In addition to their pivotal ecological contributions to such communities, lichenized fungi have relatively small metagenome sizes (Armaleo & May, 2009; Tripp et al., 2017), making them ideal targets for cost-effective genomics projects (Allen et al. 2018; Pogoda et al., 2018, 2019; Funk et al., 2018; Brigham et al., 2018). Given the above, lichens serve as an excellent system in which to explore the factors that constrain the establishment and development of obligate symbioses in nature, including those prevalent among soil crust communities. Such factors span the dynamics of propagule dispersal, the distribution and establishment of individual symbionts in the environment, and biotic interactions between extant

symbionts in a given environment. To date, however, few studies have explored such avenues of research, but these have relied entirely on amplicon-based sequencing methods (Banchi et al. 2018; Eaton et al. 2018; but see, e.g., Tripp et al. 2017; Pizarro et al. 2019).

This shortage of fungal genomic resources broadly, and lichen genomic resources more specifically, makes it challenging to investigate key questions about lichen ecology, evolution, genetics, and physiology. Moreover, existing studies that have investigated fungal metagenomic communities have, like bacteria, primarily relied on amplicon-based approaches. Given known complications arising from amplification bias (Acinas et al., 2005; Wang and Qian, 2009), one potential solution is to forego amplification of barcoding loci and instead utilize data from whole-genome shotgun sequencing (WGS). This method avoids classical PCR amplification biases but has been little employed to date, likely as a function of one to several other challenges. In addition to increased costs of WGS relative to amplicon-based methods, the lack of developed, publicly available reference databases (e.g., complete or nearly complete rDNA complexes) as well as a paucity of bioinformatics pipelines have limited the utility of WGS as a primary tool with which to approach fungal and other microbial metagenomic research.

In this study, we construct and then employ a new rDNA database spanning 273 species of lichenized fungi, built from a metagenomic survey of lichens in the southern Appalachian Mountain biodiversity hotspot, which is characterized by stark abiotic gradients and is home to over a thousand species of lichens (Dey 1978; Brodo et al., 2001; Hodkinson 2010; Lendemer et al., 2013; Tripp et al., in press; Tripp and Lendemer, in press a, b). Coupled with development of a new bioinformatic pipeline, we identify lichen fungal symbionts present in WGS metagenomic environmental samples and then compare the efficacy of our approach against traditional ITS1-based amplicon sequencing of the same samples. We place our results in the broader framework

of intensive biodiversity inventories of lichens at the same plots from which environmental samples were taken. Drawing on resulting data, we demonstrate that detection of symbionts using amplicon-free methods, here WGS, detects more species than amplicon-based methods. We introduce a new biodiversity metric, “Potential of Diversity” (hereafter PoD), which refers to the potential for species to occur at a given site in a given study area, regardless of whether the species is actually present at this site as determined by traditional taxonomic inventory. In the present study, PoD specifically refers to the ratio of lichen fungal symbionts detected on bare surfaces in the environment that would serve as biotic partners in subsequent lichen symbiosis (i.e., the potential lichens that could occur in a study plot based on presence of the required fungal symbiont). In this context, PoD is a useful metric in which to fully consider biotic constraints that limit establishment of obligate symbiotic organisms. We compare this metric to the number of lichens detected as established symbioses (i.e., the lichens that visibly occur at a plot, henceforth “TD” for taxonomic diversity).

4.3 MATERIALS AND METHODS

Study Area, Field Plots and Field Sampling – This study was carried out in two one-hectare plots located in Great Smoky Mountains National Park, in the southern Appalachian Mountain Biodiversity Hotspot of eastern North America (Fig. 4.1). The plots were selected to span the two extremes of a stark elevational (one high, one low) and ecological (bottomland hardwood vs. cloud-laden forest dominated by conifers) gradient in the region, so as to maximize the difference in extant lichen communities (i.e., minimize overlap) as well as potential environmental symbiont pools between the plots. The high-elevation (2014 m) plot was located on the summit of Clingman’s Dome in spruce-fir forest along the border of Swain County, North Carolina and Sevier County, Tennessee. The low-elevation (670 m) plot was located at White

Oak Branch in mixed-hardwood forest above the north shore of Fontana Lake, in Swain County, North Carolina.

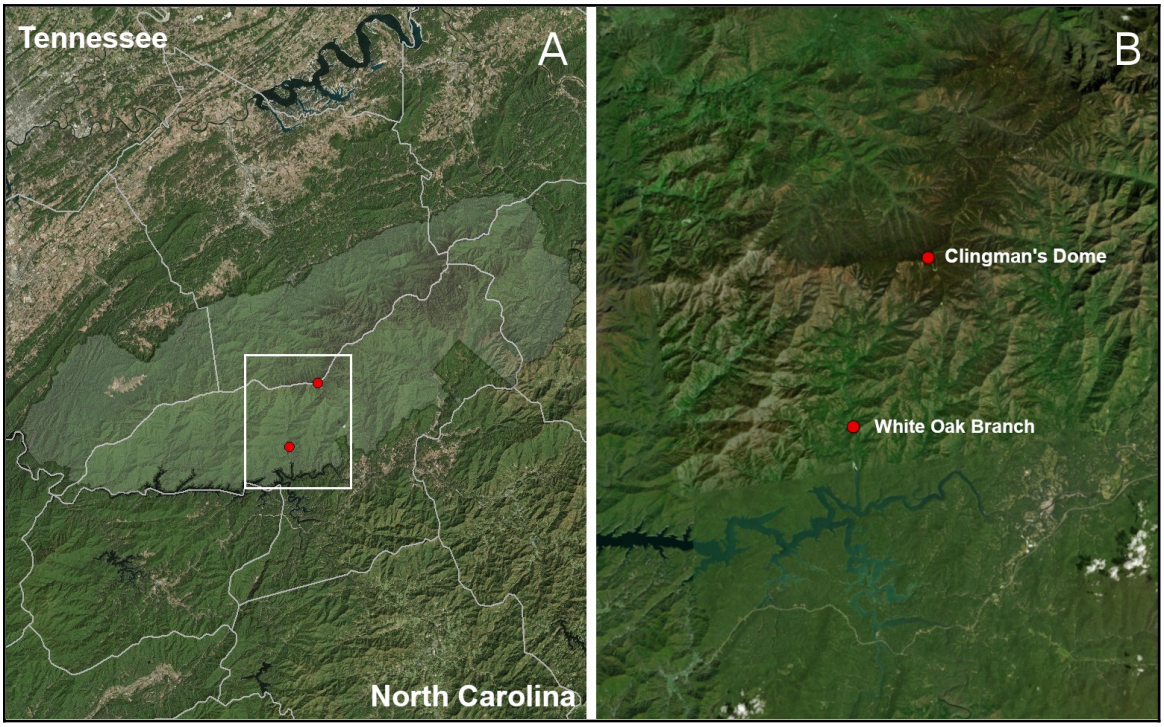


Fig 4.1 Plot map. Area shaded in light green (Panel A) corresponds to Great Smoky Mountains National Park. White Oak Branch is the low-elevation sampling plot, at 670 m elevation, and Clingman's Dome, the highest peak in Tennessee, is at an elevation of 2,014 m (Panel B).

Prior to environmental sampling, data pertaining to several ecological variables (e.g., tree DBH, woody plant inventory, habitat quality assessment, following Tripp et al. 2019) were recorded in order to delimit the plant communities and to ensure maximum difference in lichen communities (see above). Both plots were delimited to be uniform in vegetation type within a plot (i.e., not spanning more than one ecotone). In each plot, a full inventory of lichen species was conducted (carried out by JL, vouchers deposited in the herbaria of the New York Botanical Garden [NY] and University of Colorado, Boulder [COLO]); methods following Tripp et al. 2019). In each plot, 16 environmental samples were obtained by swabbing the surfaces of eight rocks and eight trees (yielding a total of 32 environmental samples) for 30 seconds with a sterile toothbrush (size, aspect, and identification were recorded for each rock and tree type; see below for

additional details). In order to avoid sampling surfaces with artificially inflated propagule counts, such as spore deposits along river beds and bogs, we chose only vertically-oriented, bare surfaces of rocks and trees (i.e. free of any visible growth besides the bark of the tree, when applicable).

Thallus Collection – To facilitate direct comparison of amplicon-based sequencing to WGS-based sequencing of the 32 environmental samples, we first obtained lichen voucher specimens for species present throughout the Southern Appalachian Mountains (Table S1) as part of a system-wide investigation of drivers of lichen biodiversity and distributions in the region, including potential biotic constraints (e.g., presence of symbionts) such as those herein investigated. These samples were collected in order to build a new genomic reference database for lichens of the region (see below). Samples were collected and identified by JCL and EAT between December 2016 and January 2018. All lichen voucher specimens are deposited at NY and COLO (Table S1). Efforts were made to sample only single thalli for both macro- and microlichens. For macrolichens, ca. 1 x 1 cm of thallus was removed, targeting the margins and lobes. For microlichens, thallus was scraped from rock or tree substrates using a sterile razor blade. Samples were air dried in a laminar flow hood for 24 hours then frozen at -20°C until transport to the University of Colorado for DNA extraction and subsequent sequencing.

Metagenomics Sampling Scheme – To quantify the number and identification of lichenized fungi present in the 32 environmental samples as well as assess differences between amplicon- vs. WGS-based sequencing approaches, we collected samples from eight rock plus eight tree surfaces within each hectare (n=16 per plot). Swabs were taken randomly from the available substrates, and only bare surfaces lacking visible bryophyte or lichen thalli were chosen. Standardized stencils of 10 x 10 cm were placed against the substrate and an individually

packaged, sterile toothbrush was used to swab the surface for 30 seconds. The toothbrush containing the sample was then sealed in a sterile plastic bag. To process the samples, the bag was opened, the bristles were cut from the brush using a sterile blade, and a hole was cut into the corner of the bag using sterile scissors. The opened corner of the bag was placed into a sterile 1.5 mL microcentrifuge tube and the bristles were directly transferred into the tube. These samples were stored at -20°C until transport to the University of Colorado for subsequent extraction and sequencing.

Table S4.1 Collection information for the 414 vouchers used in this study.

FEN	Field_Determination	Current_Determination	Collector	Collection Number	Herbarium	Herbarium Barcode
FEN_234_Absconditella_diluta	Absconditella_diluta	Absconditella delutula	Lendemer	47108	NY	2794555
FEN_457_Acanthothecis_fontana	Acanthothecis_fontana	Acanthothecis fontana	Lendemer	49537	NY	3033982
FEN_275_Acarospora_sp2	Acarospora_sp2	Acarospora	Tripp	6053	NY	2858381
FEN_268_Acarospora_sinopica	Acarospora_sinopica	Acarospora sinopica	Tripp	6064	NY	2794310
FEN_227_Acrocordia_megalospora	Acrocordia_megalospora	Acrocordia megalospora	Lendemer	46905	NY	2794733
FEN_363_Ahtiana_aurescens	Ahtiana_aurescens	Ahtiana aurescens	Lendemer	48631	NY	3032963
FEN_246_Alectoria_fallacina	Alectoria_fallacina	Alectoria fallacina	Lendemer	47194	NY	2795449
FEN_266_Anisomeridium_sp1	Anisomeridium_sp1	Anosomeridium sp.	Tripp	6040	COLO	COLO-L-0051344
FEN_236_Anzia_colpodes	Anzia_colpodes	Anzia colpodes	Lendemer	47020	NY	2794613
FEN_99_Arthonia_anglica	Arthonia_anglica	Arthonia anglica	Lendemer	46007	NY	2606942
FEN_292_Arthonia_cupressina	Arthonia_cupressina	Arthonia cupressina	Tripp	5430	NY	2376365
FEN_92_Arthonia_quintaria	Arthonia_quintaria	Arthonia quintaria	Lendemer	46102	NY	2606846
FEN_82_Arthonia_ruana	Arthonia_ruana	Arthonia ruana	Lendemer	46304	NY	2606644
FEN_408_Arthonia_rubella	Arthonia_rubella	Arthonia rubella	Lendemer	49024	NY	3033164
FEN_118_Arthonia_susa	Arthonia_susa	Arthonia susa	Lendemer	45912	NY	2606577
FEN_112_Arthonia_vinosa	Arthonia_vinosa	Arthonia vinosa	Lendemer	46296	NY	2606652
FEN_44_Arthothelium_spectabile	Arthothelium_spectabile	Arthothelium spectabile	Lendemer	45934	NY	2606555
FEN_406_Aspicilia_laevata	Aspicilia_laevata	Aspicilia laevata	Tripp	6120	NY	2796924
FEN_393_Lecanora_nothocaesiella	Lecanora_nothocaesiella	Bacidia	Lendemer	48883	NY	3033306
FEN_116_Bacidia_schweinitzii	Bacidia_schweinitzii	Bacidia schweinitzii	Lendemer	45908	NY	2606582
FEN_387_Bacidia_sorediata	Bacidia_sorediata	Bacidia sorediata	Lendemer	48716	NY	3033024
FEN_468_Bagliettoa_baldensis	Bagliettoa_baldensis	Bagliettoa baldensis	Lendemer	49632	NY	3034319
FEN_448_Bathelium_carolinianum	Bathelium_carolinianum	Bathelium carolinianum	Lendemer	49425	NY	3033920
FEN_197_Biatora_appalachensis	Biatora_appalachensis	Biatora appalachensis	Lendemer	46585	NY	2795063
FEN_241_Biatora_chrysantha	Biatora_chrysantha	Biatora chrysantha	Lendemer	47157	NY	2795372
FEN_378_Biatora_longispora	Biatora_longispora	Biatora longispora	Lendemer	48745	NY	3033000
FEN_376_Biatora_pontica	Biatora_pontica	Biatora pontica	Lendemer	48744	NY	3033001
FEN_293_Botryolepraria_lesdainii	Botryolepraria_lesdainii	Botryolepraria lesdainii	Tripp	5570	NY	2376350
FEN_417_Botryolepraria_lesdainii	Botryolepraria_lesdainii	Botryolepraria lesdainii	Tripp	6161	NY	2796963
FEN_244_Micarea_bauschiana	Micarea_bauschiana	Brianaria bauschiana	Lendemer	47183	NY	2795347
FEN_291_Brigantaea_leucoxantha	Brigantaea_leucoxantha	Brigantaea leucoxantha	Tripp	5000	NY	2359950
FEN_69_Bryoria_bicolor	Bryoria_bicolor	Bryoria bicolor	Lendemer	46154	NY	2606794
FEN_107_Bryoria_nadvornikiana	Bryoria_nadvornikiana	Bryoria nadvornikiana	Lendemer	46133	NY	2606815
FEN_40_Bryoria_tenuis	Bryoria_tenuis	Bryoria tenuis	Lendemer	46176	NY	2606773
FEN_454_Buellia_mamillana	Buellia_mamillana	Buellia mamillana	Lendemer	49467	NY	3033878
FEN_261_Lecidea_tessellata	Lecidea_tessellata	Buellia spuria	Tripp	6057	NY	2792583
FEN_281_Buellia_spuria	Buellia_spuria	Buellia spuria	Tripp	6062	NY	2831250
FEN_507_Buellia_spuria	Buellia_spuria	Buellia spuria	Lendemer	49954	NY	3034834
FEN_65_Buellia_stillingiana	Buellia_stillingiana	Buellia stillingiana	Lendemer	45923	NY	2606566
FEN_98_Buellia_vernicoma	Buellia_vernicoma	Buellia vernicoma	Lendemer	46024	NY	2606923
FEN_440_Bulbothrix_scortella	Bulbothrix_scortella	Bulbothrix scortella	Lendemer	49179	NY	3033664
FEN_506_Byssoloma_meadii	Byssoloma_meadii	Byssoloma meadii	Lendemer	49950	NY	
FEN_222_Byssoloma_subdiscordans	Byssoloma_subdiscordans	Byssoloma subdiscordans	Lendemer	46494-A	NY	2795150
FEN_462_Catillaria_lenticularis	Catillaria_lenticularis	Catillaria lenticularis	Tripp	6389	NY	2796610
FEN_384_Cetrelia_chicitae	Cetrelia_chicitae	Cetrelia chicitae	Lendemer	48677	NY	3033063
FEN_382_Cetrelia_olivetorum	Cetrelia_olivetorum	Cetrelia olivetorum	Lendemer	48757	NY	3033431
FEN_192_Chaenotheca_balsamconensis	Chaenotheca_balsamconensis	Chaenotheca balsamconensis	White	1	NY	3035384
FEN_191_Chaenotheca_furfuracea	Chaenotheca_furfuracea	Chaenotheca furfuracea	White	2	NY	3035385
FEN_416_Chrysothrix_onokoensis	Chrysothrix_onokoensis	Chrysothrix onokoensis	Tripp	6158	NY	2796961
FEN_273_Chrysothrix_susquehannensis	Chrysothrix_susquehannensis	Chrysothrix susquehannensis	Tripp	6065	NY	2794303
FEN_199_Chrysothrix_xanthina	Chrysothrix_xanthina	Chrysothrix xanthina	Lendemer	46750	NY	2794894
FEN_399_Chrysothrix_xanthina	Chrysothrix_xanthina	Chrysothrix xanthina	Lendemer	49059	NY	3033130
FEN_380_Cladonia_apodocarpa	Cladonia_apodocarpa	Cladonia apodocarpa	Lendemer	48789	NY	3033399
FEN_219_Cladonia_arbuscula	Cladonia _arbuscula	Cladonia arbuscula	Lendemer	46390	NY	2795256
FEN_492_Cladonia_caroliniana	Cladonia_caroliniana	Cladonia caroliniana	Lendemer	49894	NY	3034894
FEN_263_Cladonia_coccifera	Cladonia_coccifera	Cladonia coccifera	Tripp	6067	NY	2792576
FEN_75_Cladonia_coniocraea	Cladonia_coniocraea	Cladonia coniocraea	Lendemer	46039	NY	2606908
FEN_284_Cladonia_didyma	Cladonia_didyma	Cladonia didyma	Tripp	6032	NY	2792580
FEN_470_Cladonia_furcata	Cladonia_furcata	Cladonia furcata	Tripp	6423	NY	2796646
FEN_508_Cladonia_furcata	Cladonia_furcata	Cladonia furcata	Lendemer	49961	NY	3034827
FEN_249_Cladonia_grayi	Cladonia_grayi	Cladonia grayi	Lendemer	46385	NY	2795261
FEN_486_Cladonia_leporina	Cladonia_leporina	Cladonia leporina	Tripp	6543	NY	2796674
FEN_247_Cladonia_macilenta	Cladonia_macilenta_bacillaris	Cladonia macilenta v. bacillaris	Lendemer	46386	NY	2795260
FEN_459_Collema_coccophorum	Collema_coccophorum	Collema coccophorum	Tripp	6375	NY	2808530
FEN_365_Collema_conglomeratum	Collema_conglomeratum	Collema conglomeratum	Lendemer	48626	NY	3032968
FEN_430_Collema_furfuraceum	Collema_furfuraceum	Collema furfuraceum	Tripp	6186	NY	2808617
FEN_77_Collema_subflaccidum	Collema_subflaccidum	Collema subflaccidum	Lendemer	46026	NY	2606921
FEN_47_Arthonia_kermesina	Arthonia_kermesina	Coniarthonia kermesina	Lendemer	46280	NY	2606668
FEN_48_Arthonia_kermesina	Arthonia_kermesina	Coniarthonia kermesina	Lendemer	46286	NY	2606662
FEN_377_Conotrema_urceolatum	Conotrema_urceolatum	Conotrema urceolatum	Lendemer	48723	NY	3033017
FEN_453_Canoparmelia_crozalsiana	Crespoa_crozalsiana	Crespoa crozalsiana	Tripp	6332	NY	2808569

FEN	Field_Determination	Current_Determination	Collector	Collection Number	Herbarium	Herbarium Barcode
FEN_234_Absconditella_diluta	Absconditella_diluta	Absconditella delutula	Lendemer	47108	NY	2794555
FEN_502_Cresponea_flava	Cresponea_flava	Cresponea flava	Lendemer	49930	NY	3034858
FEN_79_Pseudocyphellaria_aurata	Pseudocyphellaria_aurata	Crocordia aurata	Lendemer	46027	NY	2606920
FEN_256_Cystocoleus_ebeneus	Cystocoleus_ebeneus	Cystocoleus ebeneus	Lendemer	47369	NY	3032736
FEN_509_Cystocoleus_ebeneus	Cystocoleus_ebeneus	Cystocoleus ebeneus	Lendemer	49962	NY	3034826
FEN_371_Dendriscocaulon_intricatulum	Dendriscocaulon_intricatulum	Dendriscocaulon intricatulum	Lendemer	48702	NY	3033038
FEN_444_Dermatocarpon_luridum	Dermatocarpon_luridum	Dermarocarpon luridum	Tripp	6263	NY	2808575
FEN_423_Dermatocarpon_muhlenbergii	Dermatocarpon_muhlenbergii	Dermatocarpon muhlenbergii	Lendemer	49117	NY	3033554
FEN_366_Dermiscellum_oulocheilum	Dermiscellum_oulocheilum	Dermiscellum oulocheilum	Lendemer	48571	NY	3032933
FEN_426_Dibaeis_absoluta	Dibaeis _absoluta	Dibaeis absoluta	Tripp	6166	NY	2796968
FEN_498_Dibaeis_sorediata	Dibaeis _sorediata	Dibaeis sorediata	Tripp	6574	NY	2796712
FEN_204_Dimelaena_oreina	Dimelaena_oreina	Dimelaena oreina	Lendemer	46354	NY	2795296
FEN_427_Ephebe_solida	Ephebe_solida	Ephebe solida	Tripp	6172	NY	2796978
FEN_412_Fissurina_insidiosa	Fissurina_insidiosa	Fissurina insidiosa	Tripp	6135	NY	2796997
FEN_322_Flakea_papillata	Flakea_papillata	Flakea papillata	Tripp	3955	NY	1887251
FEN_499_Flakea_papillata	Flakea_papillata	Flakea papillata	Tripp	6575	NY	2796711
FEN_260_Flavoparmelia_baltimorensis	Flavoparmelia_baltimorensis	Flavoparmelia baltimorensis	Tripp	6061	NY	2794302
FEN_395_Flavoparmelia_baltimorensis	Flavoparmelia_baltimorensis	Flavoparmelia baltimorensis	Lendemer	48832	NY	3033356
FEN_350_Flavopunctelia_flaventior	Flavopunctelia_flaventior	Flavopunctelia flaventior	Lendemer	48570	NY	3217070
FEN_45_Fuscopannaria_leucosticta	Fuscopannaria_leucosticta	Fuscopannaria leucosticta	Lendemer	45947	NY	2606542
FEN_323_Fuscopannaria_sorediata	Fuscopannaria_sorediata	Fuscopannaria sorediata	Tripp	4948	NY	2359963
FEN_194_????_????	????_????	Gomphillaceae	Lendemer	46730	NY	2794914
FEN_105_Gomphillus_americanus	Gomphillus_americanus	Gomphillus americanus	Lendemer	45954	NY	2606535
FEN_193_Gomphillus_calycioides	Gomphillus_calycioides	Gomphillus calycioides	Lendemer	46470	NY	2795175
FEN_403_Gyalecta_farlowii	Gyalecta_farlowii	Gyalecta farlowii	Lendemer	49035	NY	3033153
FEN_490_Gyalideopsis_ozarkensis	Gyalideopsis_ozarkensis	Gyalideopsis bartramiorum	Tripp	6553	NY	2796833
FEN_429_Halecania_sp	Halecania_sp	Helecania pepegospora	Tripp	6177	NY	2797035
FEN_210_Herteliana_schuyleriana	Herteliana_schuyleriana	Herteliana schuyleriana	Lendemer	46371	NY	2795276
FEN_86_Icmadophila_ericetorum	Icmadophila_ericetorum	Icmadophila ericetorum	Lendemer	46191	NY	2606757
FEN_56_Imshaugia_aleurites	Imshaugia_aleurites	Imshaugia aleurites	Lendemer	46246	NY	2606702
FEN_425_Ionaspis_lacustris	Ionaspis_lacustris	Ionaspis lacustris	Tripp	6164	NY	2796966
FEN_471_Kephartia_crystalligera	Kephartia_crystalligera	Kephartia crystalligera	Lendemer	49633	NY	3034318
FEN_270_Lasallia_papulosa	Lasallia_papulosa	Lasallia papulosa	Tripp	6068A	NY	2794304
FEN_215_Umbilicaria_pensylvanica	Umbilicaria_pensylvanica	Lasallia pensylvanica	Lendemer	46347	NY	2795303
FEN_94_Lecania_croatica	Lecania_croatica	Lecania croatica	Lendemer	46080	NY	2606867
FEN_213_????_????	????_????	Lecidea	Lendemer	46382	NY	2795264
FEN_332_Lecidea_berengeriana	Lecidea_berengeriana	Lecidea berengeriana	Lendemer	44645	NY	2438374
FEN_340_Lecidea_berengeriana	Lecidea_berengeriana	Lecidea berengeriana	Tripp	5297	NY	3721081
FEN_111_Lecidea_nylanderi	Lecidea_nylanderi	Lecidea nylanderi	Lendemer	46290	NY	2606658
FEN_85_Lecidea_roseotincta	Lecidea_roseotincta	Lecidea roseotincta	Lendemer	46189	NY	2606759
FEN_207_Lecidea_tessellata	Lecidea_tessellata	Lecidea tessellata	Lendemer	46384	NY	2795262
FEN_239_Lecidella_sp	Lecidella_sp	Lecidella	Lendemer	46226	NY	2606722
FEN_374_Leptogium_corticola	Leptogium_corticola	Leeptogium corticola	Lendemer	48694	NY	3033046
FEN_121_Lepra_amara	Lepra_amara	Lepra amara	Lendemer	45895	NY	2606595
FEN_392_Lepra_amara	Lepra_amara	Lepra amara	Lendemer	48823	NY	3033365
FEN_30_Lepra_pustulata	Lepra_pustulata	Lepra pustulata	Lendemer	45890	NY	2606601
FEN_357_Lepra_pustulata	Lepra_pustulata	Lepra pustulata	Lendemer	48604	NY	3032990
FEN_87_Lepra_trachythallina	Lepra_trachythallina	Lepra trachythallina	Lendemer	46175	NY	2606772
FEN_354_Lepraria_caesiella	Lepraria_caesiella	Lepraria caesiella	Lendemer	48569	NY	3217071
FEN_465_Lepraria_disjuncta	Lepraria_disjuncta	Lepraria disjuncta	Tripp	6402	NY	2796634
FEN_333_Lepraria_lanata	Lepraria_lanata	Lepraria lanata	Tripp	5092	NY	2359289
FEN_512_Lepraria_finkii	Lepraria_finkii	Lepraria leprolomopsis	Lendemer	49971	NY	3034817
FEN_334_Lepraria_normandinoides	Lepraria_normandinoides	Lepraria normandinoides race protocetraric acid	Tripp	3754	NY	1865620
FEN_390_Lepraria_normandinoides	Lepraria_normandinoides	Lepraria normandinoides race protocetraric acid	Lendemer	48841	NY	3033348
FEN_49_Lepraria_oxybapha	Lepraria_oxybapha	Lepraria oxybapha	Lendemer	46299	NY	2606649
FEN_152_Lepraria_xanthonica	Lepraria_xanthonica	Lepraria xanthonica	Lendemer	46061	NY	2606886
FEN_511_Lepraria_sp	Lepraria_sp	Leprocaulon nicholsiae ined.	Lendemer	49970	NY	3034818
FEN_415_Leucodecton_sp	Leucodecton_sp	Leucodecton	Tripp	6156	NY	2796959
FEN_242_Ionaspis_alba	Ionaspis_alba	Lonaspis alba	Lendemer	47174	NY	2795356
FEN_89_Lopadium_disciforme	Lopadium_disciforme	Lopadium disciforme	Lendemer	46209	NY	2606739
FEN_109_Loxospora_elatina	Loxospora_elatina	Loxospora elatina	Lendemer	46283	NY	2606665
FEN_337_Loxospora_elatina	Loxospora_elatina	Loxospora elatina	Tripp	5040	NY	2358356
FEN_90_Loxospora_ochrophaea	Loxospora_ochrophaea	Loxospora ochrophaea	Lendemer	46150	NY	2606798
FEN_243_Melanelia_culbersonii	Melanelia_culbersonii	Melanelia culbersonii	Lendemer	47178	NY	2795352
FEN_214_Melanelia_stygia	Melanelia_stygia	Melanelia stygia	Lendemer	46383	NY	2795263
FEN_31_Melanohalea_halei	Melanohalea_halei	Melanohalea halei	Lendemer	46188	NY	2606760
FEN_353_Melanohalea_halei	Melanohalea_halei	Melanohalea halei	Lendemer	48557	NY	3217083
FEN_66_Menegazzia_subsimilis	Menegazzia_subsimilis	Menegazzia subsimilis	Lendemer	45892	NY	2606598
FEN_218_Micarea_neostipitata	Micarea_neostipitata	Micarea neostipitata	Lendemer	46798	NY	2794839
FEN_500_Micarea_peliocarpa	Micarea_peliocarpa	Micarea peliocarpa	Tripp	6579	NY	2796697
FEN_339_Micareopsis_irriguata	Micareopsis_irriguata	Micareopsis irriguata	Lendemer	44650	NY	2438369
FEN_483_Micareopsis_irriguata	Micareopsis_irriguata	Micareopsis irriguata	Tripp	6517	NY	2796809
FEN_510_Micareopsis_irriguata	Micareopsis_irriguata	Micareopsis irriguata	Lendemer	49963	NY	3034825
FEN_341_Multiclavula_mucida	Multiclavula_mucida	Multiclavula mucida	Tripp	4936	NY	2359961
FEN_153_Mycobilimbia_sp	Mycobilimbia_sp_nov	Mycoblimbia	Lendemer	46123	NY	2606825

FEN	Field_Determination	Current_Determination	Collector	Collection Number	Herbarium	Herbarium Barcode
FEN_234_Absconditella_diluta	Absconditella_diluta	Absconditella delutula	Lendemer	47108	NY	2794555
FEN_233_Mycocalicium_subtile	Mycocalicium_subtile	Mycocalicium subtile	Lendemer	46974	NY	2794659
FEN_343_Myelochroa_obsessa	Myelochroa_obsessa	Myelochroa obsessa	Tripp	4959	COLO	COLO-L-0050337
FEN_432_Pseudosagedia_chlorotica	Myelochroa_obsessa	Myelochroa obsessa	Tripp	6196	NY	2796940
FEN_466_Nadvornikia_sorediata	Nadvornikia_sorediata	Nadvornikia sorediata	Lendemer	49617	NY	3034332
FEN_2_Nephroma_helveticum	Nephroma_helveticum	Nephroma helveticum	Lendemer	45984	NY	2606966
FEN_254_Nephroma_resupinatum	Nephroma_resupinatum	Nephroma helveticum	Allen	4071	NY	2606415
FEN_211_Ochrolechia_arborea	Ochrolechia_arborea	Ochrolechia arborea	Lendemer	46422	NY	2795225
FEN_389_Ochrolechia_trochophora	Ochrolechia_trochophora	Ochrolechia trochophora	Lendemer	48860	NY	3033329
FEN_245_Ochrolechia_yasudae	Ochrolechia_yasudae	Ochrolechia yasudae	Lendemer	47150	NY	2795379
FEN_345_Opegrapha_corticola	Opegrapha_corticola	Opegrapha corticola	Tripp	4629	COLO	COLO-L-0050140
FEN_419_Opegrapha_moroziana	Opegrapha_moroziana	Opegrapha moroziana	Lendemer	49121	NY	3033550
FEN_41_Opegrapha_varia	Opegrapha_varia	Opegrapha varia	Lendemer	45935	NY	2606554
FEN_226_Opegrapha_viridis	Opegrapha_viridis	Opegrapha viridis	Lendemer	46886	NY	2794753
FEN_117_Opegrapha_vulgata	Opegrapha_vulgata	Opegrapha vulgata	Lendemer	45910	NY	2606579
FEN_347_Pannaria_subfusca	Pannaria_subfusca	Pannaria subfusca	Tripp	3923	NY	2057325
FEN_346_Pannaria_tavaresii	Pannaria_tavaresii	Pannaria tavaresii	Tripp	5299	NY	2376424
FEN_431_Pannaria_tavaresii	Pannaria_tavaresii	Pannaria tavaresii	Tripp	6190	NY	2808619
FEN_361_Parmelia_squarrosa	Parmelia_squarrosa	Parmelia squarosa	Lendemer	48643	NY	3032951
FEN_4_Parmelia_squarrosa	Parmelia_squarrosa	Parmelia squarrosa	Lendemer	46000	NY	2606948
FEN_220_Peltigera_sp	Peltigera_sp	Peltigera	Lendemer	46965	NY	2794668
FEN_503_Peltigera_neckeri	Peltigera_neckeri	Peltigera neckeri	Tripp	6583	NY	2796702
FEN_221_Peltigera_neopolydactylon	Peltigera_neopolydactylon	Peltigera neopolydactyla	Lendemer	46966	NY	2794667
FEN_288_Peltigera_phyllidiosa	Peltigera_phyllidiosa	Peltigera phyllidiosa	Tripp	4939	NY	2359959
FEN_474_Peltigera_phyllidiosa	Peltigera_phyllidiosa	Peltigera phyllidiosa	Tripp	6435	NY	2796656
FEN_67_Peltigera_praetextata	Peltigera_praetextata	Peltigera praetextata	Lendemer	45894	NY	2606596
FEN_469_Peltigera_praetextata	Peltigera_praetextata	Peltigera praetextata	Tripp	6422	NY	2796645
FEN_223_Phaeocalicium_polyporaeum	Phaeocalicium_polyporaeum	Phaeocalicium polyporaeum	Lendemer	46976	NY	2794657
FEN_96_Phlyctis_boliviensis	Phlyctis_boliviensis	Phlyctis boliviensis	Lendemer	45964	NY	2606525
FEN_308_Phlyctis_boliviensis	Phlyctis_boliviensis	Phlyctis boliviensis	Tripp	5526a	NY	2376396
FEN_477_Phlyctis_boliviensis	Phlyctis_boliviensis	Phlyctis boliviensis	Tripp	6443	NY	2796719
FEN_475_Phlyctis_petraea	Phlyctis_petraea	Phlyctis petrae race stictic acid	Lendemer	49648	NY	3034302
FEN_351_Phlyctis_speirea	Phlyctis_speirea	Phlyctis speirea	Lendemer	48563	NY	3217076
FEN_97_Phyllopsora_corallina	Phyllopsora_corallina	Phyllopsora corallina	Lendemer	45963	NY	2606526
FEN_424_Phyllopsora_corallina	Phyllopsora_corallina	Phyllopsora corallina	Lendemer	49103	NY	3033568
FEN_421_Phyllopsora_parvifolia	Phyllopsora_parvifolia	Phyllopsora parvifolia	Lendemer	49142	NY	3033529
FEN_297_Pilophorus_fibula	Pilophorus_fibula	Pilophorus fibula	Tripp	4988	COLO	COLO-L-0050397
FEN_274_Placidium_arboreum	Placidium_arboreum	Placidium arboreum	Tripp	6044	NY	2794318
FEN_456_Placidium_arboreum	Placidium_arboreum	Placidium arboreum	Tripp	6367	NY	2808524
FEN_481_Placynthium_petersii	Placynthium_petersii	Placynthium petersii	Tripp	6508	NY	2796802
FEN_505_Polymeridium_proponens	Polymeridium_proponens	Polymerdium proponens	Lendemer	49947	NY	3034842
FEN_298_Polysporina_simplex	Polysporina_simplex	Polysporina simplex	Tripp	5016	NY	2358352
FEN_195_Porina_heterospora	Porina _heterospora	Porina heterospora	Lendemer	46723	NY	2794921
FEN_414_Porina_heterospora	Porina_heterospora	Porina heterospora	Tripp	6153	NY	2796980
FEN_272_Porina_scabrida	Porina_scabrida	Porina scabrida	Tripp	6035	NY	2794315
FEN_410_Porina_scabrida	Porina_scabrida	Porina scabrida	Lendemer	49016	NY	3033172
FEN_434_Porpidia_albocaerulescens	Porpidia_albocaerulescens	Porpidia albocaerulescens	Lendemer	49203	NY	3033640
FEN_299_Porpidia_contraponenda	Porpidia_contraponenda	Porpidia contraponenda	Tripp	5025	NY	2358395
FEN_310_Porpidia_crustatula	Porpidia_crustatula	Porpidia crustulata	Tripp	5428	NY	3721092
FEN_311_Porpidia_macrocarpa	Porpidia_macrocarpa	Porpidia macrocarpa	Tripp	4984	NY	2359939
FEN_441_Porpidia_subsimplex	Porpidia_subsimplex	Porpidia subsimplex	Lendemer	49216	NY	3033627
FEN_476_Protoblastenia_rupestris	Protoblastenia_rupestris	Protoblastenia rupestris	Lendemer	49650	NY	3034301
FEN_39_Pseudevernia_cladonia	Pseudevernia_cladonia	Pseudevernia cladonia	Lendemer	46177	NY	2606771
FEN_358_Pseudevernia_consocians	Pseudevernia_consocians	Pseudevernia consocians	Lendemer	48609	NY	3032985
FEN_409_Pseudosagedia_cestrensis	Pseudosagedia_cestrensis	Pseudosagedia cestrensis	Lendemer	49020	NY	3033168
FEN_100_Pseudosagedia_isidiata	Pseudosagedia_isidiata	Pseudosagedia isidiata	Lendemer	46012	NY	2606936
FEN_43_Pseudosagedia_rhaphidospermum	Pseudosagedia_rhaphidospermum	Pseudosagedia rhaphidosperma	Lendemer	45919	NY	2606570
FEN_202_Punctelia_appalachensis	Punctelia_appalachensis	Punctelia appalachensis	Lendemer	46661	NY	2794983
FEN_487_Punctelia_caseana	Punctelia_caseana	Punctelia caseana	Tripp	6545	NY	2796676
FEN_312_Pyrenula_subelliptica	Pyrenula_subelliptica	Pyrenula subelliptica	Tripp	4986	NY	2359938
FEN_285_Pyrrhospora_varians	Pyrrhospora_varians	Pyrrhospora varians	Tripp	6041	NY	2794319
FEN_438_Pyrrhospora_varians	Pyrrhospora_varians	Pyrrhospora varians	Lendemer	48973	NY	3033725
FEN_405_Pyxine_albovirens	Pyxine_albovirens	Pyxine albovirens	Lendemer	49050	NY	3033138
FEN_81_Pyxine_sorediata	Pyxine_sorediata	Pyxine sorediata	Lendemer	46021	NY	2606926
FEN_397_Myelochroa_obsessa	Myelochroa_obsessa	Pyxine sorediata	Tripp	6093	NY	2796942
FEN_91_Ropalospora_chlorantha	Ropalospora_chlorantha	Ropalospora chlorantha	Lendemer	46159	NY	2606789
FEN_217_Sarea_resinae	Sarea_resinae	Sarea resinae	Lendemer	46787	NY	2794850
FEN_196_Schismatomma_glaucescens	Schismatomma_glaucescens	Schismatomma glaucescens	Lendemer	46716	NY	2794928
FEN_318_Scoliciosporum_umbrinum	Scoliciosporum_umbrinum	Scoliciosporum umbrinum	Tripp	4949	COLO	COLO-L-0050352
FEN_279_????_ssc2	????_ssc2	ssc	Tripp	6058	COLO	COLO-L-0051364
FEN_394_???_???	???_???	ssc	Lendemer	48835	NY	3720155
FEN_319_Stereocaulon_dactylophyllum	Stereocaulon_dactylophyllum	Stereocaulon dactylophyllum	Tripp	5027	NY	2358398
FEN_404_???_???	???_???	Sterile sorediate crust	Lendemer	49042	NY	3033146
FEN_479_ssc_3	ssc_3	Sterile sorediate crust	Tripp	6296	NY	2808629
FEN_255_Sticta_sp	Sticta_sp_nov	Sticta	Lendemer	47364	NY	2795562

FEN	Field_Determination	Current_Determination	Collector	Collection Number	Herbarium	Herbarium Barcode
FEN_234_Absconditella_diluta	Absconditella_diluta	Absconditella delutula	Lendemer	47108	NY	2794555
FEN_76_Sticta_beauvoisii	Sticta_beauvoisii	Sticta beauvoisii	Lendemer	46028	NY	2606919
FEN_413_Sticta_caroliniana	Sticta_caroliniana	Sticta carolinensis	Tripp	6143	NY	2796954
FEN_381_Sticta_fragillinata	Sticta_fragillinata	Sticta fragilinata	Lendemer	48759	NY	3033429
FEN_208_Tephromela_atra	Tephromela_atra	Tephromela atra	Lendemer	46366	NY	2795284
FEN_436_Thelotrema_defectum	Thelotrema_defectum	Thelotrema defectum	Lendemer	49172	NY	3033671
FEN_449_Thelotrema_subtile	Thelotrema_subtile	Thelotrema subtile	Lendemer	49423	NY	3033922
FEN_18_Trapelia_coarctata	Trapelia_coarctata	Trapelia coarctata	Lendemer	46059	NY	2606888
FEN_437_Trapelia_placodioides	Trapelia_placodioides	Trapelia placodioides	Lendemer	49282	NY	3033716
FEN_320_Trapeliopsis_flexuosa	Trapeliopsis_flexuosa	Trapeliopsis flexuosa	Tripp	5065	NY	2358384
FEN_113_Trapeliopsis_viridescens	Trapeliopsis_viridescens	Trapeliopsis viridescens	Lendemer	46250	NY	2606698
FEN_467_Trentepohlia_sp	Trentepohlia_sp	Trentepohlia	Tripp	6417	NY	2796640
FEN_33_Usnocetraria_oakesiana	Usnocetraria_oakesiana	Usnocetraria oakesiana	Lendemer	46223	NY	2606725
FEN_480_Willeya_diffractella	Willeya_diffractella	Willeya diffractella	Tripp	6489	NY	2796759
FEN_240_Xanthoparmelia_mexicana	Xanthoparmelia_mexicana	Xanthoparmelia mexicana	Lendemer	46370	NY	2795279
FEN_46_Xylographa_truncigena	Xylographa_truncigena	Xylographa trunciseda	Lendemer	46240	NY	2606708
FEN_136_Xylographa_vitiligo	Xylographa_vitiligo	Xylographa vitiligo	Lendemer	46195	NY	2606753
FEN_73_Anaptychia_palmulata	Anaptychia_palmulata	Anaptychia palmulata	Lendemer	46036	NY	2606911
FEN_443_Anaptychia_palmulata	Anaptychia_palmulata	Anaptychia palmulata	Tripp	6240	NY	2796990
FEN_103_Caloplaca_camptidia	Caloplaca_camptidia	Caloplaca camptidia	Lendemer	45991	NY	2606957
FEN_264_Caloplaca_chrysopthalma	Caloplaca_chrysopthalma	Caloplaca chrysophthalma	Tripp	6034	NY	2794317
FEN_198_Caloplaca_feracissima	Caloplaca_feracissima	Caloplaca feracissima	Lendemer	46741	NY	2794903
FEN_250_Cladonia_mateocyatha	Cladonia_mateocyatha	Cladonia mateocyatha	Lendemer	46387	NY	2795259
FEN_282_Cladonia_mateocyathea	Cladonia_mateocyathea	Cladonia mateocyatha	Tripp	6055	NY	2831247
FEN_9_Cladonia_ochrochlora	Cladonia_ochrochlora	Cladonia ochrochlora	Lendemer	46049	NY	2606898
FEN_321_Cladonia_petrophila	Cladonia_petrophila	Cladonia petrophila	Tripp	3984	COLO	COLO-L-0050026
FEN_420_Cladonia_petrophila	Cladonia_petrophila	Cladonia petrophila	Lendemer	49138	NY	3033533
FEN_445_Cladonia_peziziformis	Cladonia_peziziformis	Cladonia peziziformis	Tripp	6281	NY	2808573
FEN_442_Cladonia_polycarpoides	Cladonia_polycarpoides	Cladonia polycarpoides	Tripp	6226	NY	2797047
FEN_267_Cladonia_pyxidata	Cladonia_pyxidata	Cladonia pyxidata	Tripp	6047	NY	2792579
FEN_201_Cladonia_rangiferina	Cladonia _rangiferina	Cladonia rangiferina	Lendemer	46392	NY	2795254
FEN_494_Cladonia_rangiferina	Cladonia_rangiferina	Cladonia rangiferina	Lendemer	49896	NY	3034892
FEN_491_Cladonia_ravenelii	Cladonia_ravenelii	Cladonia ravenelii	Lendemer	49892	NY	3034896
FEN_496_Cladonia_robbinsii	Cladonia_robbinsii	Cladonia robbinsii	Lendemer	49897	NY	3034891
FEN_280_Cladonia_squamosa	Cladonia_squamosa	Cladonia squamosa	Tripp	6038	NY	2794313
FEN_304_Cladonia_squamosa	Cladonia_squamosa	Cladonia squamosa	Tripp	4968a	COLO	COLO-L-0050330
FEN_488_Cladonia_squamosa	Cladonia_squamosa	Cladonia squamosa	Tripp	6547	NY	2796859
FEN_253_Cladonia_stipitata	Cladonia_stipitata	Cladonia stipitata	Lendemer	46375	NY	2795271
FEN_278_Cladonia_stipitata	Cladonia_stipitata	Cladonia stipitata	Tripp	6060	NY	2831246
FEN_265_Cladonia_strepsilis	Cladonia_strepsilis	Cladonia strepsilis	Tripp	6049	NY	2794306
FEN_493_Cladonia_subtenuis	Cladonia_subtenuis	Cladonia subtenuis	Lendemer	49895	NY	3034893
FEN_200_Cladonia_uncialis	Cladonia _uncialis	Cladonia uncialis	Lendemer	46391	NY	2795255
FEN_277_Cladonia_uncialis	Cladonia_uncialis	Cladonia uncialis	Tripp	6056	NY	2794305
FEN_489_Cladonia_uncialis	Cladonia_uncialis	Cladonia uncialis	Tripp	6550	NY	2808460
FEN_20_Coccocarpia_palmicola	Coccocarpia_palmicola	Coccocarpia palmicola	Lendemer	45953	NY	2606536
FEN_276_Diploschistes_scruposus	Diploschistes_scruposus	Diploschistes scriposus	Tripp	6048	NY	2794307
FEN_401_Dirinaria_frostii	Dirinaria_frostii	Dirinaria frostii	Lendemer	49049	NY	3033139
FEN_42_Graphis_scripta	Graphis_scripta	Graphis scripta	Lendemer	45918	NY	2606571
FEN_458_Heterodermia_albicans	Heterodermia_albicans	Heterodermia albicans	Lendemer	49538	NY	3033981
FEN_225_Heterodermia_appalachensis	Heterodermia_appalachensis	Heterodermia appalachensis	Lendemer	46928	NY	2794705
FEN_283_Heterodermia_appalachensis	Heterodermia_appalachensis	Heterodermia appalachensis	Tripp	6039	NY	2794320
FEN_472_Heterodermia_echinata	Heterodermia_echinata	Heterodermia echinata	Tripp	6430	NY	2796652
FEN_398_Heterodermia_granulifera	Heterodermia_granulifera	Heterodermia granulifera	Tripp	6114	NY	2796930
FEN_235_Heterodermia_hypoleuca	Heterodermia_hypoleuca	Heterodermia hypoleuca	Lendemer	46982	NY	2794651
FEN_74_Heterodermia_casarettiana	Heterodermia_casarettiana	Heterodermia langdoniana	Lendemer	46038	NY	2606909
FEN_482_Heterodermia_casarettiana	Heterodermia_casarettiana	Heterodermia langdoniana	Tripp	6512	NY	2796807
FEN_313_Heterodermia_leucomela	Ramalina_intermedia	Heterodermia leucomela	Lendemer	44717	COLO	COLO-L-0051017
FEN_324_Heterodermia_neglecta	Heterodermia_neglecta	Heterodermia neglecta	Tripp	5284	COLO	COLO-L-0050623
FEN_383_Heterodermia_neglecta	Heterodermia_neglecta	Heterodermia neglecta	Lendemer	48750	NY	3033439
FEN_230_Heterodermia_speciosa	Heterodermia_speciosa	Heterodermia speciosa	Lendemer	46925	NY	2794708
FEN_455_Heterodermia_speciosa	Heterodermia_speciosa	Heterodermia speciosa	Lendemer	49524	NY	3033995
FEN_325_Heterodermia_squamulosa	Heterodermia_squamulosa	Heterodermia squamulosa	Tripp	5516	NY	2376517
FEN_372_Heterodermia_squamulosa	Heterodermia_squamulosa	Heterodermia squamulosa	Lendemer	48701	NY	3033039
FEN_110_Hypocenomyce_scalaris	Hypocenomyce_scalaris	Hypocenomyce scalaris	Lendemer	46289	NY	2606659
FEN_32_Hypogymnia_incurvoides	Hypogymnia_incurvoides	Hypogymnia incurvoides	Lendemer	46197	NY	2606751
FEN_70_Hypogymnia_krogiae	Hypogymnia_krogiae	Hypogymnia krogiae	Lendemer	46157	NY	2606791
FEN_55_Hypogymnia_vittata	Hypogymnia_vittata	Hypogymnia vittata	Lendemer	46170	NY	2606778
FEN_326_Hypotrachyna_afrorevoluta	Hypotrachyna_afrorevoluta	Hypotrachyna afrorevoluta	Tripp	5034	NY	2358388
FEN_57_Everniastrum_catawbiense	Everniastrum_catawbiense	Hypotrachyna catawbiensis	Lendemer	46273	NY	2606675
FEN_386_Hypotrachyna_lividescens	Hypotrachyna_lividescens	Hypotrachyna lividescens	Lendemer	48639	NY	3032955
FEN_21_Hypotrachyna_minarum	Hypotrachyna_minarum	Hypotrachyna minarum	Lendemer	45940	NY	2606549
FEN_375_Hypotrachyna_minarum	Hypotrachyna_minarum	Hypotrachyna minarum	Lendemer	48693	NY	3033048
FEN_485_Hypotrachyna_osseoalba	Hypotrachyna_osseoalba	Hypotrachyna osseoalba	Lendemer	49836	NY	3217052
FEN_327_Hypotrachyna_revoluta	Hypotrachyna_revoluta	Hypotrachyna revoluta	Tripp	5069	NY	2358400
FEN_328_Hypotrachyna_taylorensis	Hypotrachyna_taylorensis	Hypotrachyna taylorensis	Tripp	4933	NY	2359957
FEN_212_Lecanora_albella	Lecanora_albella var. rubescens	Lecanora albella v. rubescens	Lendemer	46417	NY	2795230
FEN_329_Lecanora_appalachensis	Lecanora_appalachensis	Lecanora appalachensis	Lendemer	44579	NY	2438440

FEN	Field_Determination	Current_Determination	Collector	Collection Number	Herbarium	Herbarium Barcode
FEN_234_Absconditella_diluta	Absconditella_diluta	Absconditella delutula	Lendemer	47108	NY	2794555
FEN_114_Lecanora_cinereofusca	Lecanora_cinereofusca	Lecanora cinereofusca	Lendemer	45937	NY	2606552
FEN_186_Lecanora_hybocarpa	Lecanora_hybocarpa	Lecanora hybocarpa	Lendemer	46600	NY	2795046
FEN_84_Lecanora_masana	Lecanora_masana	Lecanora masana	Lendemer	46185	NY	2606763
FEN_286_Lecanora_orienoides	Lecanora_orienoides	Lecanora oreinoides	Tripp	6054	NY	2831233
FEN_433_Lecanora_orienoides	Lecanora_orienoides	Lecanora oreinoides	Tripp	6209	NY	2808624
FEN_330_Lecanora_polytropa	Lecanora_polytropa	Lecanora polytropa	Tripp	5019	NY	2358362
FEN_355_Lecanora_pseudistera	Lecanora_pseudistera	Lecanora pseudistera	Lendemer	48566	NY	3217073
FEN_83_Lecanora_rugosella	Lecanora_rugosella	Lecanora rugosella	Lendemer	46180	NY	2606768
FEN_216_Lecanora_strobilina	Lecanora_strobilina	Lecanora strobilina	Lendemer	46780	NY	2794857
FEN_400_Lecanora_saxigena	Lecanora_saxigena	Lecanora subimmergens	Lendemer	49057	NY	3033132
FEN_331_Lecanora_subpallens	Lecanora_subpallens	Lecanora subpallens	Tripp	3981	NY	2057301
FEN_119_Lecanora_thysanophora	Lecanora_thysanophora	Lecanora thysanophora	Lendemer	45887	NY	2606603
FEN_209_Leptogium_austroamericanum	Leptogium_austroamericanum	Leptogium austroamericanum	Lendemer	46734	NY	2794910
FEN_224_Leptogium_chloromelum	Leptogium_chloromelum	Leptogium chloromelum	Lendemer	46938	NY	2794695
FEN_29_Leptogium_corticola	Leptogium_corticola	Leptogium corticola	Lendemer	45903	NY	2606586
FEN_451_Leptogium_corticola	Leptogium_corticola	Leptogium corticola	Tripp	6316	NY	2808550
FEN_80_Leptogium_cyanescens	Leptogium_cyanescens	Leptogium cyanescens	Lendemer	46018	NY	2606929
FEN_411_Leptogium_cyanescens	Leptogium_cyanescens	Leptogium cyanescens	Tripp	6136	NY	2796952
FEN_335_Leptogium_dactylinum	Leptogium_dactylinum	Leptogium dactylinum	Tripp	5452	NY	2376459
FEN_501_Leptogium_dactylinum	Leptogium_dactylinum	Leptogium dactylinum	Tripp	6581	NY	2796699
FEN_231_Leptogium_hirsutum	Leptogium_hirsutum	Leptogium hirsutum	Lendemer	46937	NY	2794696
FEN_452_Leptogium_hirsutum	Leptogium_hirsutum	Leptogium hirsutum	Lendemer	49444	NY	3033901
FEN_473_Leptogium_lichenoides	Leptogium_lichenoides	Leptogium lichenoides	Tripp	6431	NY	2796653
FEN_78_Lobaria_pulmonaria	Lobaria_pulmonaria	Lobaria pulmonaria	Lendemer	46025	NY	2606922
FEN_24_Lobaria_quercizans	Lobaria_quercizans	Lobaria quercizans	Lendemer	46093	NY	2606856
FEN_336_Lobaria_scrobiculata	Lobaria_scrobiculata	Lobaria scrobiculata	Tripp	5055	NY	2358382
FEN_93_Meglaospora_porphyritis	Meglaospora_porphyritis	Megalospora porphyritis	Lendemer	46114	NY	2606834
FEN_338_Megalospora_porphyritis	Megalospora_porphyritis	Megalospora porphyritis	Tripp	5562	NY	2441380
FEN_88_Mycoblastus_sanguinarioides	Mycoblastus_sanguinarioides	Mycoblastus sanguinarioides	Lendemer	46137	NY	2606811
FEN_342_Mycoblastus_sanguinarius	Mycoblastus_sanguinarius	Mycoblastus sanguinarioides	Tripp	5068	NY	2358383
FEN_344_Normandina_pulchella	Normandina_pulchella	Normandina pulchella	Tripp	5296	COLO	COLO-L-0050636
FEN_463_Parmotrema_arnoldii	Parmotrema_arnoldii	Parmotrema arnoldii	Lendemer	49572	NY	3034379
FEN_478_Parmotrema_austrosinense	Parmotrema_austrosinense	Parmotrema austrosinense	Lendemer	49663	NY	3034287
FEN_229_Parmotrema_cetratum	Parmotrema_cetratum	Parmotrema cetratum	Lendemer	46914	NY	2794724
FEN_461_Parmotrema_cetratum	Parmotrema_cetratum	Parmotrema cetratum	Lendemer	49550	NY	3033969
FEN_108_Parmotrema_crinitum	Parmotrema_crinitum	Parmotrema crinitum	Lendemer	46319	NY	2606628
FEN_349_Parmotrema_critinum	Parmotrema_critinum	Parmotrema crinitum	Tripp	5527	NY	2376403
FEN_370_Parmotrema_critinum	Parmotrema_critinum	Parmotrema crinitum	Lendemer	48703	NY	3033037
FEN_3_Parmotrema_diffractaicum	Parmotrema_diffractaicum	Parmotrema diffractaicum	Lendemer	45986	NY	2606964
FEN_237_Parmotrema_gardneri	Parmotrema_gardneri	Parmotrema gardneri	Lendemer	46788	NY	2794849
FEN_238_Parmotrema_hypotropum	Parmotrema_hypotropum	Parmotrema hypotropum	Lendemer	46803	NY	2794834
FEN_262_Parmotrema_hypotropum	Parmotrema_hypotropum	Parmotrema hypotropum	Tripp	6046	NY	2792578
FEN_464_Parmotrema_internexum	Parmotrema_internexum	Parmotrema internexum	Lendemer	49587	NY	3034363
FEN_289_Parmotrema_margaritatum	Parmotrema_margaritatum	Parmotrema margaritatum	Lendemer	44568	NY	2438298
FEN_290_Parmotrema_mellissii	Parmotrema_mellissii	Parmotrema mellissii	Tripp	3909	NY	1886959
FEN_388_Parmotrema_mellissii	Parmotrema_mellissii	Parmotrema mellissii	Lendemer	48896	NY	3033293
FEN_295_Parmotrema_neotropicum	Parmotrema_neotropicum	Parmotrema neotropicum	Lendemer	44839	NY	2438598
FEN_439_Parmotrema_neotropicum	Parmotrema_neotropicum	Parmotrema neotropicum	Lendemer	49188	NY	3033655
FEN_369_Parmotrema_perlatum	Parmotrema_perlatum	Parmotrema perlatum	Lendemer	48705	NY	3033035
FEN_497_Parmotrema_rampoddense	Parmotrema_rampoddense	Parmotrema rampoddense	Lendemer	49908	NY	3034880
FEN_287_Parmotrema_reticulatum	Parmotrema_reticulatum	Parmotrema reticulatum	Tripp	5303	NY	2376314
FEN_348_Parmelia_sulcata	Parmelia_sulcata	Parmotrema reticulatum	Tripp	5523	NY	2441386
FEN_362_Parmotrema_reticulatum	Parmotrema_reticulatum	Parmotrema reticulatum	Lendemer	48628	NY	3032966
FEN_391_Parmotrema_simulans	Parmotrema_simulans	Parmotrema simulans	Lendemer	48891	NY	3033298
FEN_203_Parmotrema_stuppeum	Parmotrema_stuppeum	Parmotrema stuppeum	Lendemer	46669	NY	2794975
FEN_294_Parmotrema_subsidiosum	Parmotrema_subsidiosum	Parmotrema subisidiosum	Tripp	4010	NY	2057320
FEN_446_Parmotrema_submarginale	Parmotrema_submarginale	Parmotrema submarginale	Tripp	6293	NY	2797008
FEN_259_Parmotrema_subsumptum	Parmotrema_subsumptum	Parmotrema subsumptum	Tripp	6036	NY	2792577
FEN_407_Parmotrema_tinctorum	Parmotrema_tinctorum	Parmotrema tinctorum	Lendemer	49041	NY	3033150
FEN_428_Parmotrema_ultralucens	Parmotrema_ultralucens	Parmotrema ultralucens	Tripp	6176	NY	2796974
FEN_205_Parmotrema_xanthinum	Parmotrema_xanthinum	Parmotrema xanthinum	Lendemer	46754	NY	2794890
FEN_258_Pertusaria_andersoniae	Lepra_andersoniae	Pertusaria andersoniae	Lendemer	48277	NY	2700266
FEN_296_Pertusaria_epixantha	Pertusaria_epixantha	Pertusaria epixantha	Tripp	4024	NY	1886970
FEN_120_Pertusaria_macounii	Pertusaria_macounii	Pertusaria macounii	Lendemer	45893	NY	2606597
FEN_460_Pertusaria_obruta	Pertusaria_obruta	Pertusaria obruta	Lendemer	49548	NY	3033971
FEN_232_Pertusaria_ostiolata	Pertusaria_ostiolata	Pertusaria ostiolata	Lendemer	46873	NY	2794766
FEN_101_Pertusaria_paratuberculifera	Pertusaria_paratuberculifera	Pertusaria paratuberculifera	Lendemer	45980	NY	2606971
FEN_305_Pertusaria_plittiana	Pertusaria_plittiana	Pertusaria plittiana	Tripp	5465	NY	2441374
FEN_402_Pertusaria_plittiana	Pertusaria_plittiana	Pertusaria plittiana	Lendemer	49058	NY	3033131
FEN_379_Pertusaria_propinqua	Pertusaria_propinqua	Pertusaria propinqua	Lendemer	48825	NY	3033363
FEN_306_Pertusaria_rubefacta	Pertusaria_rubefacta	Pertusaria rubefacta	Tripp	5482	NY	2376334
FEN_102_Pertusaria_subpertusa	Pertusaria_subpertusa	Pertusaria subpertusa	Lendemer	45987	NY	2606962
FEN_418_Phaeophyscia_adiastola	Phaeophyscia_adiastola	Phaeophyscia adiastola	Lendemer	49114	NY	3033557
FEN_352_Phaeophyscia_hispidula	Phaeophyscia_hispidula	Phaeophyscia hispidula	Lendemer	48558	NY	3217082
FEN_307_Phaeophyscia_rubropulchra	Phaeophyscia_rubropulchra	Phaeophyscia rubropulchra	Tripp	5525	NY	2376404
FEN_450_Phaeophyscia_rubropulchra	Phaeophyscia_rubropulchra	Phaeophyscia rubropulchra	Tripp	6314	NY	2808548

FEN	Field_Determination	Current_Determination	Collector	Collection Number	Herbarium	Herbarium Barcode
FEN_234_Absconditella_diluta	Absconditella_diluta	Absconditella delutula	Lendemer	47108	NY	2794555
FEN_228_Phaeophyscia_squarrosa	Phaeophyscia_squarrosa	Phaeophyscia squarrosa	Lendemer	46920	NY	2794718
FEN_269_Physcia_americana	Physcia_americana	Physcia americana	Tripp	6037	NY	2794314
FEN_309_Physconia_leucoleiptes	Physconia_leucoleiptes	Physconia leucoleiptes	Lendemer	44557	NY	2438309
FEN_257_Physconia_subpallida	Physconia_subpallida	Physconia subpallida	Lendemer	48001	NY	2700627
FEN_54_Platismatia_glauca	Platismatia_glauca	Platismatia glauca	Lendemer	46171	NY	2606777
FEN_38_Platismatia_tuckermanii	Platismatia_tuckermanii	Platismatia tuckermanii	Lendemer	46172	NY	2606776
FEN_356_Platismatia_tuckermanii	Platismatia_tuckermanii	Platismatia tuckermanii	Lendemer	48642	NY	3032952
FEN_62_Pseudocyphellaria_perpetua	Pseudocyphellaria_perpetua	Pseudocyphellaria holarctica	Lendemer	46117	NY	2606831
FEN_122_Pyrenula_leucostoma	Pyrenula_leucostoma	Pyrenula leucostoma	Lendemer	45896	NY	2606594
FEN_115_Pyrenula_pseudobufonia	Pyrenula_pseudobufonia	Pyrenula pseudobufonia	Lendemer	45901	NY	2606588
FEN_300_Pyrenula_pseudobufonia	Pyrenula_pseudobufonia	Pyrenula pseudobufonia	Tripp	4991	NY	2359936
FEN_360_Ramalina_americana	Ramalina_americana	Ramalina americana	Lendemer	48621	NY	3032973
FEN_385_Ramalina_culbersoniorum	Ramalina_culbersoniorum	Ramalina culbersoniorum	Lendemer	48735	NY	3033005
FEN_422_Ramalina_intermedia	Ramalina_intermedia	Ramalina petrina	Lendemer	49122	NY	3033549
FEN_314_Rhizocarpon_geographicum	Rhizocarpon_geographicum	Rhizocarpon geographicum	Tripp	5097	NY	2359284
FEN_206_Rhizocarpon_subgeminatum	Rhizocarpon_subgeminatum	Rhizocarpon subgeminatum	Lendemer	46368	NY	2795281
FEN_106_Rinodina_adirondackii	Rinodina_adirondackii	Rinodina adirondackii	Lendemer	45957	NY	2606532
FEN_315_Rinodina_ascocisana	Rinodina_ascocisana	Rinodina ascociscana	Tripp	4925	NY	2359914
FEN_104_Rinodina_buckii	Rinodina_buckii	Rinodina buckii	Lendemer	45999	NY	2606949
FEN_316_Rinodina_chrysidata	Rinodina_chrysidata	Rinodina chrysidiata	Tripp	5292	NY	2376307
FEN_484_ssc_Plate6	ssc_	Rinodina colobinoides	Lendemer	49833	NY	3217055
FEN_396_Rinodina_sp	Rinodina_sp	Rinodina dolichospora	Lendemer	48902	NY	3033287
FEN_317_Rinodina_tephraspis	Rinodina_tephraspis	Rinodina tephraspis	Tripp	5334	NY	2441365
FEN_301_Trypethelium_tropicum	Trypethelium_tropicum	Trypethelium tropicum	Tripp	3976A	NY	1886980
FEN_95_Trypethelium_virens	Trypethelium_virens	Trypethelium virens	Lendemer	46047	NY	2606900
FEN_504_Trypethelium_virens	Trypethelium_virens	Trypethelium virens	Lendemer	49946	NY	3034843
FEN_302_Tuckermanopsis_ciliaris	Tuckermanopsis_ciliaris	Tuckermanopsis ciliaris	Tripp	4953	COLO	COLO-L-0050342
FEN_364_Tuckermanopsis_ciliaris	Tuckermanopsis_ciliaris	Tuckermanopsis ciliaris	Lendemer	48635	NY	3032959
FEN_495_Tuckermanopsis_ciliaris	Tuckermanopsis_ciliaris	Tuckermanopsis ciliaris	Tripp	6558	NY	2796682
FEN_447_Tylophoron_americanum	Tylophoron_americanum	Tylophoron americanum	Tripp	6302	NY	2797012
FEN_303_Umbilicaria_mammulata	Umbilicaria_mammulata	Umbilicaria mammulata	Tripp	5479	NY	2376333
FEN_373_Umbilicaria_mammulata	Umbilicaria_mammulata	Umbilicaria mammulata	Lendemer	48697	NY	3033043
FEN_64_Usnea_ceratina	Usnea_ceratina	Usnea ceratina	Lendemer	46119	NY	2606829
FEN_367_Usnea_ceratina	Usnea_ceratina	Usnea ceratina	Lendemer	48740	NY	3032997
FEN_63_Usnea_corntua	Usnea_corntua	Usnea cornuta	Lendemer	46118	NY	2606830
FEN_359_Usnea_fulvoreagens	Usnea_fulvoreagens	Usnea fulvoeagens	Lendemer	48610	NY	3032984
FEN_252_Usnea_halei	Usnea_halei	Usnea halei	Lendemer	46374	NY	2795272
FEN_248_Usnea_merrillii	Usnea_merrillii	Usnea merrillii	Lendemer	46379	NY	2795267
FEN_435_Usnea_mutabilis	Usnea_mutabilis	Usnea mutabilis	Lendemer	49260	NY	3033738
FEN_271_Usnea_strigosa	Usnea_strigosa	Usnea strigosa	Tripp	6069	NY	2831249
FEN_68_Usnea_subfusca	Usnea_subfusca	Usnea subfusca	Lendemer	46309	NY	2606639
FEN_368_Usnea_subgracilis	Usnea_subgracilis	Usnea subgracilis	Lendemer	48717	NY	3033023
FEN_251_Usnea_subscabrosa	Usnea_subscabrosa	Usnea subscabrosa	Lendemer	46747	NY	2794897

DNA Extraction and Whole Genome Shotgun Sequencing – For both the 32 environmental samples as well as lichen species vouchered in order to build a new reference genomic database, dried samples were pulverized using tungsten carbide bearings in a Qiagen 96-well plate shaker. Genomic DNA (gDNA) was extracted from lichen thallus samples and toothbrush bristles using a Qiagen DNeasy 96 plant kit. Individual samples were transferred from 1.5 mL microcentrifuge tubes into 96 well plates used in the Qiagen kit. The manufacturer's protocol was modified to include a 10 minute 65°C incubation step for ground material in lysis buffer as well as a 100% ethanol wash before final drying of the membrane prior to elution. Preliminary study found that these modifications improved lichen gDNA concentration and purity (Pogoda et al., 2018). Extracted samples were stored at -20°C prior to subsequent library preparation.

Whole genome shotgun sequencing was conducted on a total of 494 lichen thallus libraries (these collected throughout the southern Appalachian study area [Fig. 4.1]) and 32 environmental samples on the Illumina NextSeq®. Each of the gDNA samples was prepared using the Nextera® XT DNA library prep kit, which is optimized for 1 ng of total input DNA. Each sample was uniquely tagged using the dual index adapters, Nextera® i5 and i7. Libraries prepared for sequencing on the NextSeq® utilized Illumina PhiX v.3 as a control and samples that passed QC were processed for paired-end 151 base pair reads on an Illumina NextSeq® sequencer at the University of Colorado's BioFrontiers Institute (Boulder, Colorado).

ITS1 Sequencing – To compare results of WGS-based sequencing to amplicon-based sequencing, amplification by PCR was performed on the 32 environmental samples using the ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA) (Gardes & Bruns, 1993) and ITS2 (5'-GCT-GCGTTCTTCATCGATGC) (White et al, 1990) primers. Libraries were prepared for sequencing on the MiSeq® utilized Illumina PhiX v.3 as a control. Samples that passed QC were then pro-

cessed for single end 251 base pair reads on the Illumina MiSeq® sequencer at the University of Texas' Genomics Sequencing and Analysis Facility (Austin, Texas).

Genome Assembly and Reference Genomic Database – Libraries were filtered with Trimmomatic-0.36 to trim adapters from reads, and with parameters "LEADING:3 TRAILING:3 MINLEN:100" (Bolger et al., 2014). Filtered reads were then assembled using SPAdes 3.9.0 with parameters "--careful -k 21,33,65,81" (Bankevich et al., 2012). This study utilized the whole nuclear ribosomal DNA (rDNA) complexes that were obtained from the *de novo* genome assembly of lichens that were collected as part of a broader study of lichen diversity in the southern Appalachian study region (Keepers et al., unpub. data). The rDNA complex is easily assembled due to its high copy number in the nuclear genome, and is long (i.e., > 5,000 bp, in comparison to amplicon-based sequencing of ITS1, which is < 500 bp), providing a larger target onto which sequenced reads may map. Moreover, the high-copy nature of the locus provides many opportunities per propagule to be counted in the downstream analyses. The rDNA complexes for each sample were identified by conducting a BLAST search of the rDNA complex of the trebouxioid algal photobiont from *Cladonia uncialis* against each of the assemblies. The algal rDNA was used in the search rather than a mycobiont sequence to avoid bias in BLAST hit length due to phylogenetic similarity. To identify complete or mostly complete rDNA complexes from the BLAST tables, contigs were required to have two or greater distinct hits and the span of these hits were required to be greater than 1000 bp in length. Sequences were parsed from the assemblies based on the nucleotide positions of the BLAST hits and oriented with the 18S in the 5' direction.

Phylogenetic Methods – Each putative rDNA contig parsed from the assemblies was BLAST searched against the NCBI non-redundant database. Any sequences whose best BLAST results mapped to non-lichenized fungi or to non-fungal species were excluded. To further vet the identities of the contigs in the database, sequences were aligned using MUSCLE aligner v3.8.31 (Edgar, 2004) for downstream phylogenetic analysis. The resulting preliminary alignment was trimmed to contain only highly homologous regions, then further trimmed to only sites for which >90% of taxa in the dataset contained sequence data. A best estimate phylogenetic tree was inferred under a model of GTR+I+Γ using MrBayes (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) with 10,000,000 MCMC generations and gaps treated as missing data. The first 25% trees were treated as burn-in and excluded from further analyses; eight runs were implemented and 32 chains were utilized. All other parameters were left at default values and the average standard deviation of split frequencies had converged to less than the recommended 0.01 by the end of the analysis. The trees from the posterior distribution were used to generate a majority consensus tree, upon which we mapped posterior probabilities.

This preliminary consensus tree was visualized using FigTree v.1.4.3, upon which we determined that 34 samples were likely misplaced based on disagreement with established large-scale phylogenic reconstructions of lichenized fungi (e.g., Miadlikowska et al., 2014). As such, these 34 samples were excluded from the alignment, which was then further pruned of duplicated taxon sequences such that the final matrix contained only one representative per species (and in each case, the longest rDNA contig was retained). The resulting final alignment included a total of 273 sequences and, after visual inspection following the same phylogenetic methods described above, was used as the reference database for subsequent queries of sequences obtained from sequencing of the environmental samples.

Read Mapping – The 251bp amplicon reads were truncated to 151 bp to ensure comparability of amplicon-based sequences to WGS sequences. To exclude PCR primer sequence regions, amplicon reads were truncated to include only positions 50-200, representing the highly variable ITS1 sequence. Sequences from both the WGS and amplicon-based approaches were then aligned to the newly constructed reference rDNA database to generate short-read alignment maps. To ensure that reads uniquely mapped to a single locus in the database, several filtering steps were employed. First, only reads that mapped with a CIGAR score between 145-151 matches, corresponding to a mapping identity of between 96-100%, were retained. Second, only read-pairs for which both the left and right read mapped to the same contig were kept, and then only read-pairs for which both reads mapped with a SAM mapping quality of 31 or greater (out of a maximum of 60) were retained. Due to the highly conserved nature of portions of the coding regions of the ribosomal DNA complex, many reads belonging to species for which multiple congeners were represented in the database mapped almost equally well to multiple species. In these instances, the mapping score was bolstered by the estimated read separation. Thus, a higher SAM CIGAR score of 150 or 151 was required to retain reads that also mapped well to a member of the same genus.

Species Accumulation Curves – To facilitate comparison of species diversity and accumulation as assessed from environmental samples, species richness rarefaction curves for each of the 32 samples falling under four sampling regimes (i.e., eight each from high elevation rock, high elevation tree, low elevation rock, low elevation tree) were generated using the Diversity Stats calculator in EstimateS 9.1.0 (Colwell 2013). Rarefaction curves were bootstrapped by randomizing the sample order 100 times.

Comparison of Environmental Sampling Methods and Expert-Based Inventory – To assess the congruence between the two metagenomic methods (i.e., WGS vs. amplicon) of detecting symbionts in environmental samples (potential of diversity, or "PoD") as well as congruence of both methods to expert-based inventory (Coddington et al., 1991; Sorensen et al., 2012) of species found growing in each plot (taxonomic diversity, or "TD"), we pooled the taxa detected in all samples collected in each of the four sampling regimes (high rock, high tree, low rock, low tree) to produce a single list of species detected through a given method) and calculated Jaccard indices in each inventory. These were derived from a presence/absence matrix (Tables S3, S4) that consisted of the species present in the rDNA reference database that were found at each of the two elevation extremes via (1) the expert-based lichen biodiversity inventory, (2) the lichenized fungi detected in environmental samples using amplicon-based sequencing, and (3) the lichenized fungi detected in environmental samples using WGS sequencing. Similarity between the lichens detected with the different methods is reported below as $J_{Treatment,Treatment}$ (e.g., $J_{WGS,Vouchered}$ is similarity of lichenized fungi detected by WGS of environmental samples compared to that detected by expert-based inventory).

4.4 RESULTS

Ribosomal DNA Database – The final reference genomic database of lichen rDNA contained complete or nearly complete ribosomal DNA complexes (NTS, ETS, 18S, ITS1, 5.8S, ITS2, 26/28S) for 273 unique species of lichenized fungi within Pezizomycotina, representing 25 orders and 57 families. The database spanned 1,770,139 bp of sequence, with an average contig length of 6,484 bp.

Detection of Lichenized Fungi Using Expert, WGS, & Amplicon-Based Inventory – Taxonomic diversity (TD: the number of vouchered species) of lichens found growing in the study plots totaled 57 species in the high-elevation plot and 83 species in the low-elevation plot, yielding a total of 136 species in both (n=4 species occurred in both). Of these 136 species, 78 (~60%) were represented in the rDNA database generated in this study and thus could potentially be matched to sequences from the environmental samples. Note that the additional species in the database were vouchered from other plots in the study area and were used to ascertain whether this method detected species that were not vouchered in the expert-based field inventory.

Mapping of paired-end WGS reads to the rDNA reference database resulted in the detection of a total of 94 lichenized fungi present in both plots: 43 species from the high-elevation plot and 71 from the low-elevation plot (Table 4.1). Conversely, using the traditional amplicon-based approach of mapping ITS1 reads to the rDNA reference database resulted in the detection of a total of 34 lichenized fungi present in both plots: 21 species from the high-elevation plot and 18 species from the low-elevation plot (Table 4.1). Species detection accumulated consistently faster by sample using WGS in all four sampling regimes (Fig. 4.2).

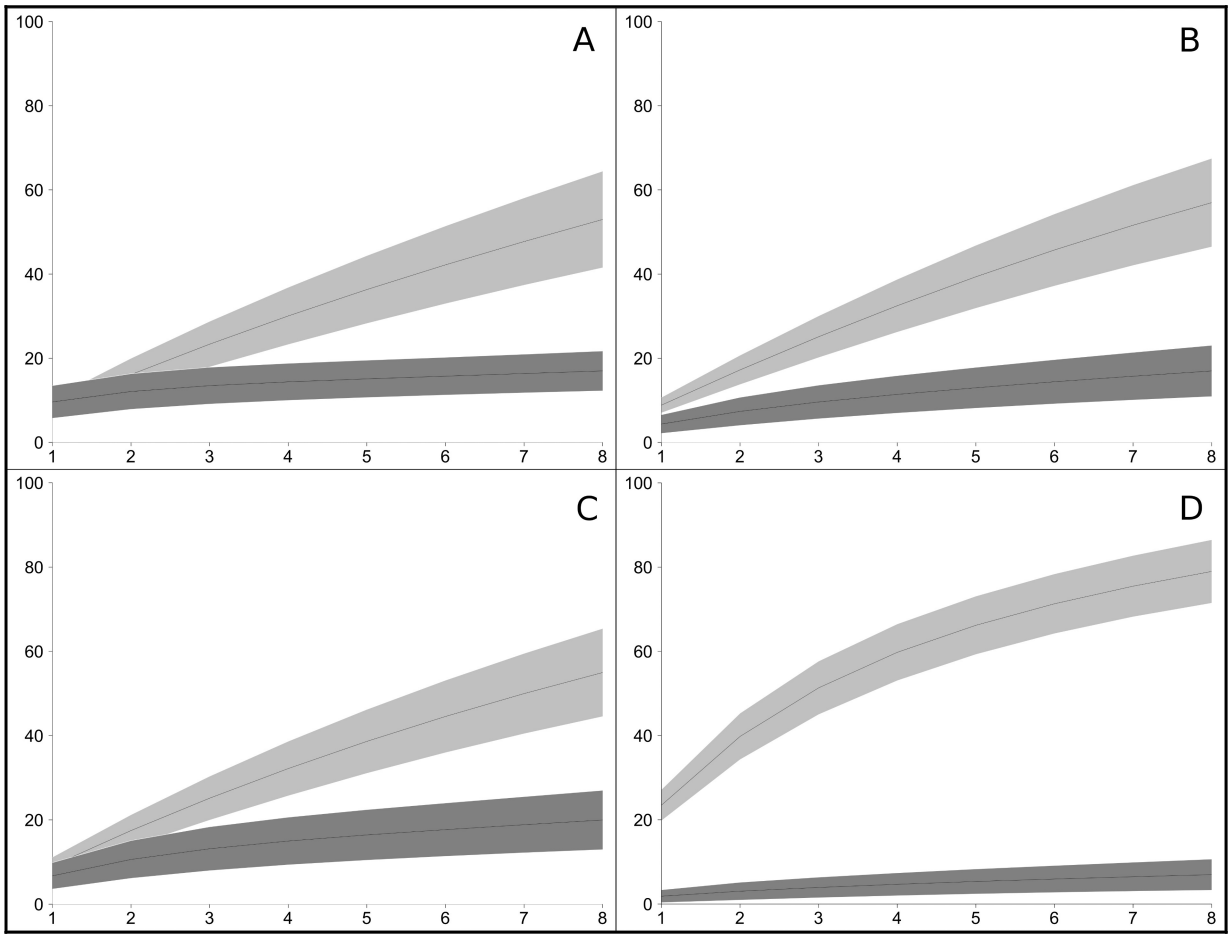


Fig 4.2. Bootstrapped rarefaction curves of the accumulation of species of lichenized fungi, comparing WGS-based rDNA approach (light gray bands) vs. amplicon-based ITS1 approach (dark gray bands), in the four sampling regimes in two plots: A) high-elevation rock samples; B) low-elevation rock samples; C) high-elevation tree samples; D) low-elevation tree sample (x-axis: number of environmental samples; y-axis: number of species of lichenized fungi detected). In all four sampling regimes, the WGS-based approached developed in this study detected greater species diversity than the amplicon-based approach.

Of the 57 species vouchered during the inventory of the high-elevation plot, 13 were detected using both sequencing methods although only seven of these species represented the same species between the two plots (Fig. 4.3; Table 4.1). Conversely, of the 83 species that were vouchered during the inventory of the low-elevation plot, 17 were detected using either sequencing method, although only 4 were detected by both. (Fig. 4.3; Table 4.1).

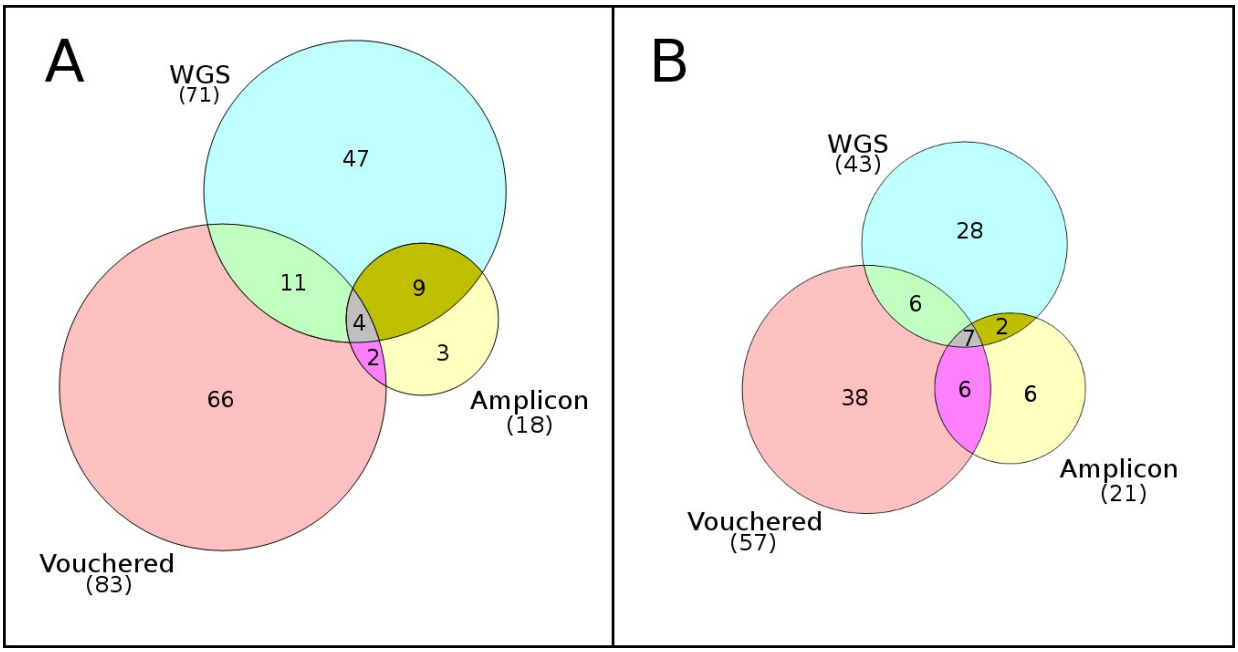
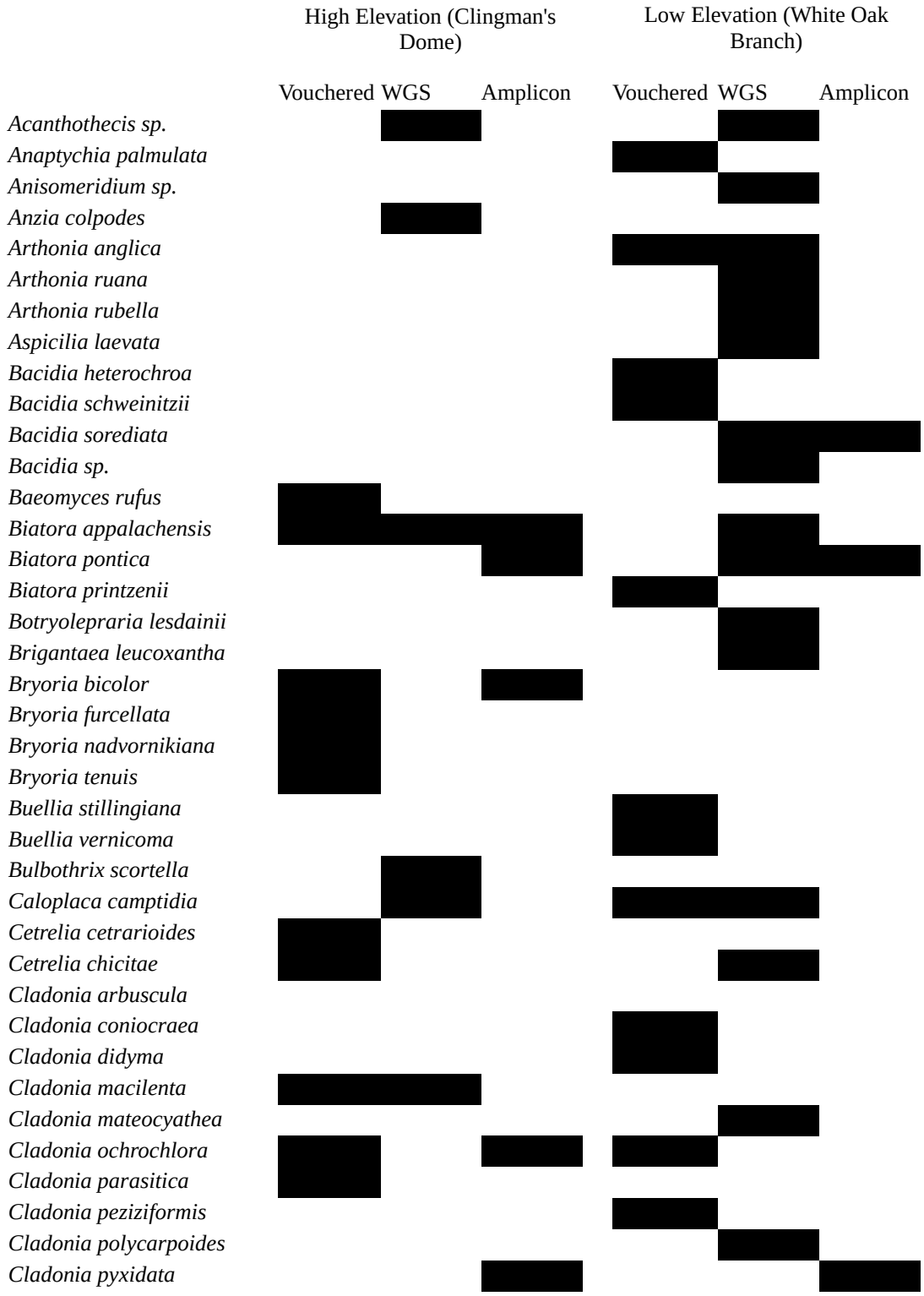


Fig 4.3. Venn diagrams comparing the numbers of species of lichenized fungi detected at A) the low-elevation White Oak Branch plot and B) the high-elevation summit of Clingman's Dome. Diagrams show an overall low overlap of detected species using the WGS approach (PoD), the amplicon approach (PoD), and the expert-based inventory approach (TD).

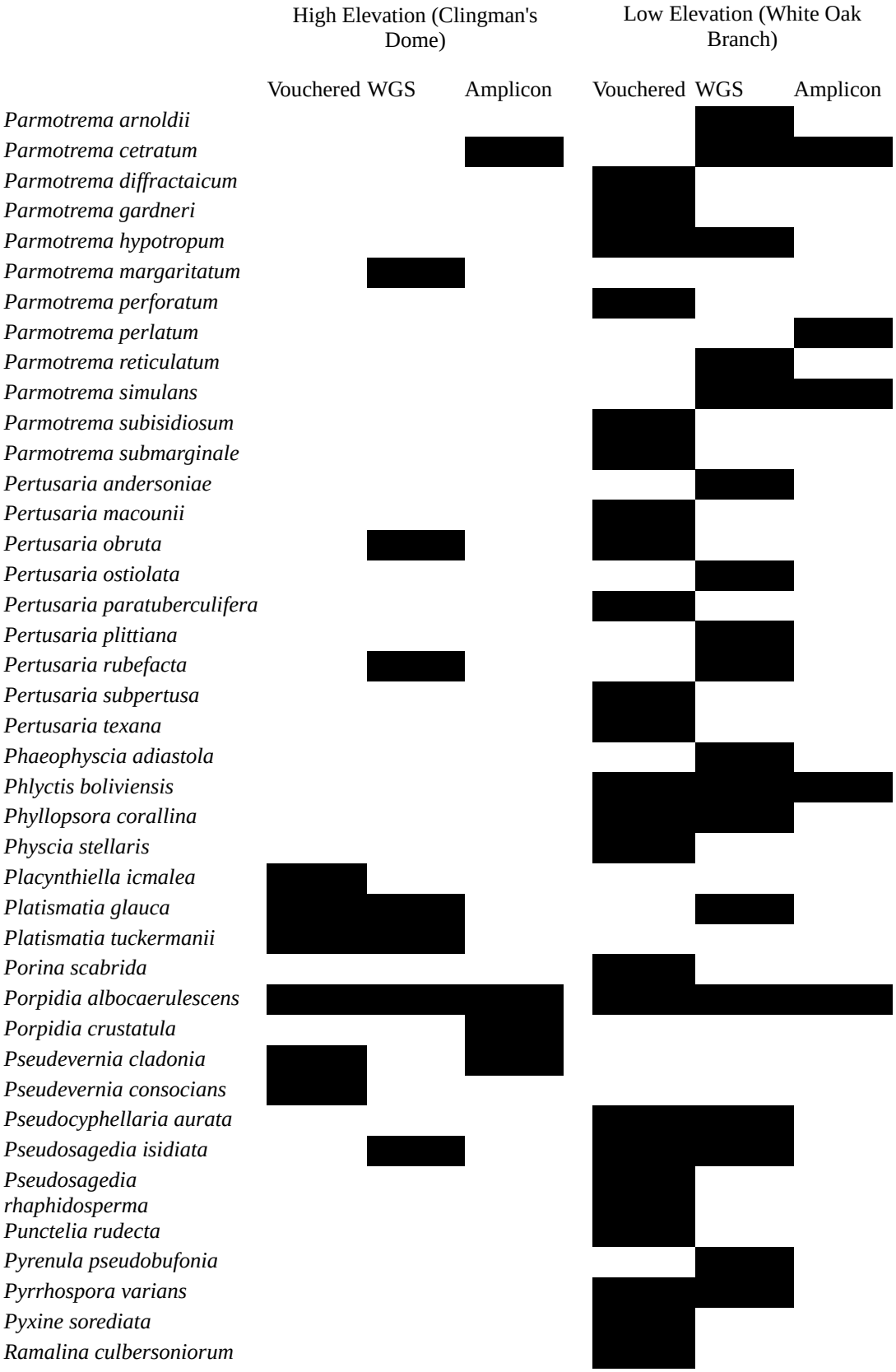
The similarity between the community of lichens detected growing at the plots via expert-based inventory versus the pools of lichen fungal symbionts species detected in environmental samples using WGS or amplicon sequencing are reported as Jaccard indices in Table 4.2. The similarity values for the assemblages of lichenized fungi detected in the environment at the high-elevation plot compared to those vouchered in the expert-based inventory ($J_{WGS,Vouchered}$=0.148, $J_{AMP,Vouchered}$=0.200) were roughly twice as high as their respective indices at the low-elevation plot ($J_{WGS,Vouchered}$=0.108, $J_{AMP,Vouchered}$=0.063). This result conveys a greater congruence between the PoD inventory based on WGS sequencing to the TD inventory, than the PoD inventory based on amplicon sequencing to the TD inventory.

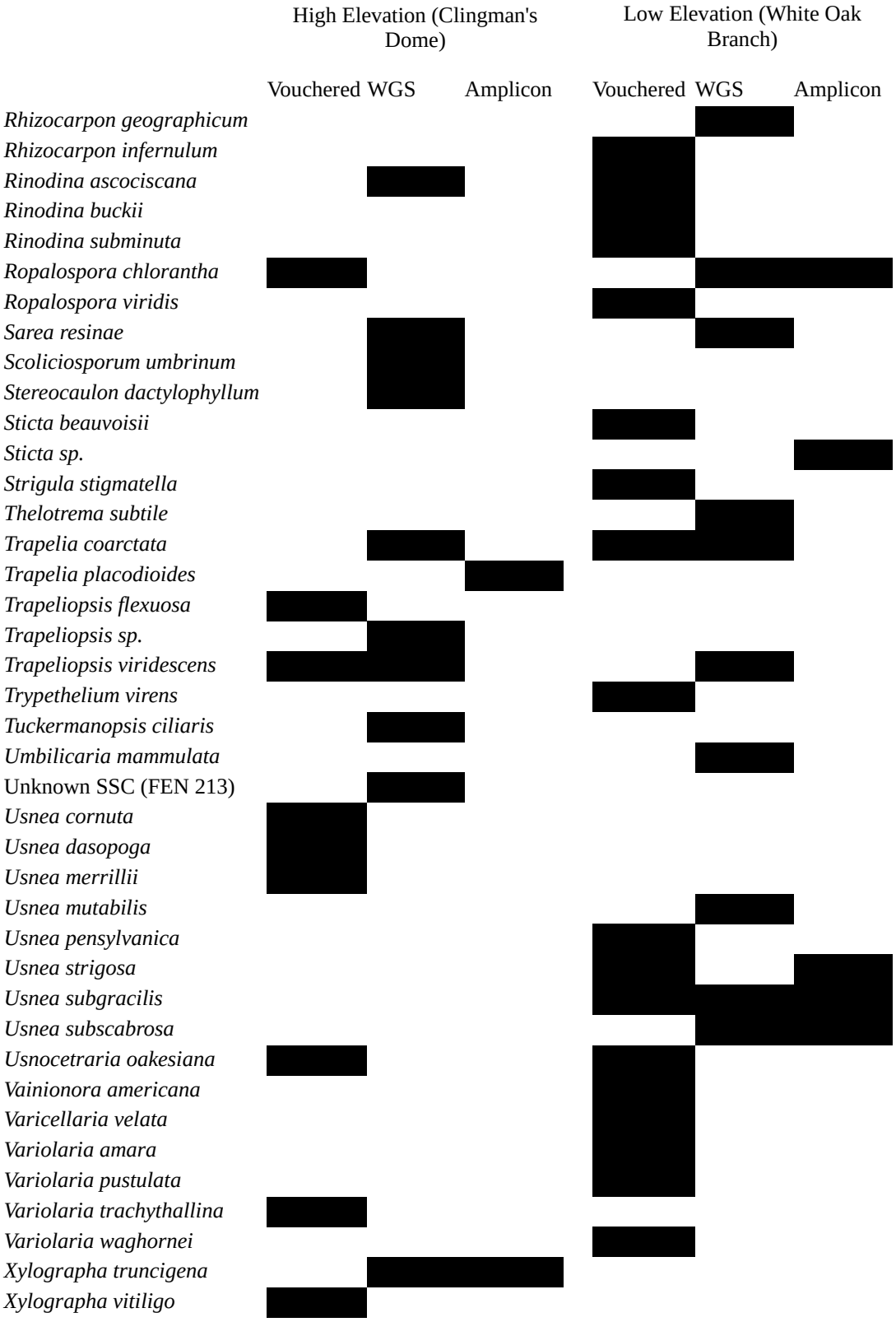
Table 4.1. Inventory of species of lichenized fungi collected at the high-elevation Clingman's Dome and low-elevation White Oak Branch plots.




High Elevation (Clingman's Dome)
Low Elevation (White Oak Branch)
Vouchered
WGS
Amplicon
Vouchered
WGS
Amplicon
Cladonia robbinsii
Cladonia squamosa
Cladonia strepsilis
Cladonia subtenuis
Coccocarpia palmicola
Collema subflaccidum
Dendriscocaulon intricatulum
Everniastrum catawbiense
Flavoparmelia baltimorensis
Flavoparmelia caperata
Fuscopannaria leucosticta
Graphis scripta
Gyalideopsis ozarkensis
Gyalideopsis piceicola
Heterodermia casarettiana
Heterodermia hypoleuca
Heterodermia obscurata
Heterodermia squamulosa
Hypogymnia incurvoides
Hypogymnia krogiae
Hypogymnia vittata
Hypotrachyna afrorevoluta
Hypotrachyna croceopustulata
Hypotrachyna gondylophora
Hypotrachyna horrescens
Hypotrachyna imbricatula
Hypotrachyna livida
Hypotrachyna minarum
Hypotrachyna oostingii
Hypotrachyna prolongata
Hypotrachyna showmanii
Hypotrachyna thysanota
Hypotrachyna virginica
Icmadophila ericetorum
Imshaugia aleurites
Ionaspis alba
Lecanora appalachensis
Lecanora cinereofusca
Lecanora hybocarpa
Lecanora imshaugii


High Elevation (Clingman's Dome)
Low Elevation (White Oak Branch)
Vouchered
WGS
Amplicon
Vouchered
WGS
Amplicon
Lecanora masana
Lecanora nothocaesiella
Lecanora rugosella
Lecanora saxigena
Lecanora symmicta
Lecanora thysanophora
Lecidea berengeriana
Lecidea roseotincta
Lecidea tessellata
Lecidella sp.
Lepra pustulata
Lepraria oxybapha
Lepraria sp.
Lepraria vouauxii
Lepraria xanthonica
Lepra trachythallina
Leptogium corticola
Leptogium cyanescens
Leptogium dactylinum
Leptogium hirsutum
Lobaria pulmonaria
Lopadium disciforme
Loxospora elatina
Loxospora ochrophaea
Megalospora porphyritis
Melanohalea halei
Menegazzia subsimilis
Micarea neostipitata
Micarea peliocarpa
Multiclavula mucida
Mycoblastus caesius
Mycoblastus sanguinarioides
Mycocalicium subtile
Myelochroa aurulenta
Myelochroa galbina
Nephroma helveticum
Ochrolechia trochophora
Opegrapha viridis
Opegrapha vulgata
Pannaria tavaresii
Parmelia saxatilis
Parmelia squarrosa


High Elevation (Clingman's Dome)
Low Elevation (White Oak Branch)
Vouchered
WGS
Amplicon
Vouchered
WGS
Amplicon
Parmotrema arnoldii
Parmotrema cetratum
Parmotrema diffractaicum
Parmotrema gardneri
Parmotrema hypotropum
Parmotrema margaritatum
Parmotrema perforatum
Parmotrema perlatum
Parmotrema reticulatum
Parmotrema simulans
Parmotrema subisidiosum
Parmotrema submarginale
Pertusaria andersoniae
Pertusaria macounii
Pertusaria obruta
Pertusaria ostiolata
Pertusaria paratuberculifera
Pertusaria plittiana
Pertusaria rubefacta
Pertusaria subpertusa
Pertusaria texana
Phaeophyscia adiastola
Phlyctis boliviensis
Phyllopsora corallina
Physcia stellaris
Placynthiella icmalea
Platismatia glauca
Platismatia tuckermanii
Porina scabrida
Porpidia albocaerulescens
Porpidia crustatula
Pseudevernia cladonia
Pseudevernia consocians
Pseudocyphellaria aurata
Pseudosagedia isidiata
Pseudosagedia rhaphidosperma
Punctelia rudecta
Pyrenula pseudobufonia
Pyrrhospora varians
Pyxine sorediata
Ramalina culbersoniorum


High Elevation (Clingman's Dome)
Low Elevation (White Oak Branch)
Vouchered
WGS
Amplicon
Vouchered
WGS
Amplicon
Rhizocarpon geographicum
Rhizocarpon infernulum
Rinodina ascociscana
Rinodina buckii
Rinodina subminuta
Ropalospora chlorantha
Ropalospora viridis
Sarea resinae
Scoliciosporum umbrinum
Stereocaulon dactylophyllum
Sticta beauvoisii
Sticta sp.
Strigula stigmatella
Thelotrema subtile
Trapelia coarctata
Trapelia placodioides
Trapeliopsis flexuosa
Trapeliopsis sp.
Trapeliopsis viridescens
Trypethelium virens
Tuckermanopsis ciliaris
Umbilicaria mammulata
Unknown SSC (FEN 213)
Usnea cornuta
Usnea dasopoga
Usnea merrillii
Usnea mutabilis
Usnea pensylvanica
Usnea strigosa
Usnea subgracilis
Usnea subscabrosa
Usnocetraria oakesiana
Vainionora americana
Varicellaria velata
Variolaria amara
Variolaria pustulata
Variolaria trachythallina
Variolaria waghornei
Xylographa truncigena
Xylographa vitiligo

4.5 DISCUSSION

Our results demonstrate that, in comparison to amplicon sequencing, the WGS approach detects a greater number of species that exist within an environment as propagules. When analyzed in tandem with data from expert-based taxonomic inventories of the same locations, there is an added capability to compare the number and community composition of extant, fully formed symbioses that occur in nature, to those that could potentially occur based on the pool of symbionts in the environment. In turn, this PoD-to-TD comparison helps unravel biotic constraints on the distributions of biodiversity. The estimation of species counts and taxonomic diversity using WGS metagenome sequencing has been conceptually validated in planktonic microbial communities (Poretsky et al., 2014). However, to our knowledge, the application of this method to macro-eukaryotes is only just emerging (Donovan et al., 2018). This study provides a substantial increase in the number of rDNA sequences from lichenized fungi now made available for future research. At the time of writing (April 2019), there were 779 complete (>3000 bp) ribosomal DNA sequences within the Pezizomycotina (if “18S”, “complete” and either “26S” or “28S” are required in the search) publicly available on GenBank. Accounting for the 49 complete lichenized fungal ribosomal DNA sequences that had already been submitted from the database curated here, our additional 224 new sequences represent a 29% increase in genomic resources for this locus for lichens.

Our workflow presented herein adds to the growing toolset of molecular contributions to biodiversity science. We have demonstrated the utility of using WGS metagenomic libraries to estimate the pool of available symbionts that are present in an environment. Given the expected ongoing decreases in sequencing cost (Schuster 2007), we anticipate that WGS will be readily adopted in many study systems and organisms.

Understanding the Distributions of Lichens and Their Propagules

At both sampling plots (high-elevation and low-elevation), species were detected based on environmental (PoD) sampling that were not present based on TD sampling. In other words, lichen mycobionts were detected in the environmental samples but were not detected by the inventory of lichens growing at the site. There are two plausible explanations for these results. First, and likely applicable to most such instances, species detected in the environmental samples but not in the inventories were present only as propagules derived from other locations. It has been shown that the gametes of sexually reproducing lichens can disperse several kilometers (Ronnås et al, 2017), and even clonal propagules are capable of dispersing over a kilometer (Gjerde et al. 2015, Eaton et al. 2018), although these studies suggest that such long-distance dispersal events are rare relative to the total reproductive propagule output of any given individual lichen thallus. It is thus not unexpected that the bank of lichen propagules on a given surface could include representatives of a more diverse pool of lichen species, these derived from a broader geographic neighborhood, regardless of the suitability of the substrate for colonization by those species. Indeed, similar patterns of have been recovered from amplicon sequencing of lichen propagules from indoor dust samples in the United States (Tripp et al., 2016).

The presence of a lichenized fungal propagule alone represents only one element of the total biotic community needed for a lichen species to grow into a mature individual, with the presence or absence of other algal, bacterial, and or other fungal species potentially representing constraints to development of a given lichen thallus (see Tripp et al. 2019). Other constraints include abiotic factors such as elevational or precipitation regimes selected for by different lichen species. One example of this phenomenon in our dataset is *Bulbothrix scortella*, which was detected only in WGS sequencing of the environmental samples from the high-elevation plot.

Bulbothrix scortella is a subtropical species that occurs only at low-elevations in the southern Appalachian Mountains (Hale 1976, Lendemer et al. 2013). As such, the detection of the species with only WGS sequencing likely reflects the dispersal of a lichenized fungal propagule to the high-elevation plot (from nearby low-elevation habitats), where it is unlikely to become established and undergo further development owing to abiotic and/or biotic constraints.

A second and less likely explanation for our results is failure to detect the presence of a lichen using expert-based TD inventories. Lichens are widely recognized as microhabitat specialists with many species present as small or spatially restricted populations in a given geographic area (Peck et al. 2004, Belinchón et al. 2015, Bosh et al. 2016, Dymytrova et al. 2016). Examples from our own work have demonstrated that in some instances, inventories of the same area by more than one collector will yield < 50% overlap in the cohorts of documented species (Lendemer et al. 2016). In the present study, *Loxospora elatina* is a species known to have a distribution restricted to temperate boreal forests (i.e., high elevation habitats in the southern Appalachian Mountains) in North America and Europe (Tønsberg 1992, Lendemer 2013). This species was detected by both sequencing methods in the high-elevation plot, but not found by the TD inventory. Thus, it is possible that in a limited number of instances, the species detected in the environmental samples but not in TD inventories were present in low abundance and not collected. However, our expert-based inventories for the broader research project under which the current study falls (i.e., investigating drivers of diversity and distributions of lichens in the southern Appalachian Mountains) target exhaustive sampling until full vouchering of the entire pool of species diversity has been accomplished (Tripp et al. 2019).

Although replicate sampling of additional high- and low-elevation plots was not carried out in this study, we nonetheless detected clear differences in the cohorts of species between the

two elevational extremes with all methods herein employed. High- and low-elevations in the southern Appalachians host different lichen communities that share in common only a small number of species (Lendemer & Tripp 2008, Allen & Lendemer 2016, Muscavitch & Lendemer 2016, Lendemer et al. 2017; Tripp & Lendemer in press a, b; Tripp et al. 2019). Thus, differences between the inventories of the two plots are to be expected. Some of the differences in the cohorts of species detected through environmental sampling may reflect the overall dispersal limitation of lichen propagules that leads the propagule pool to be dominated by locally occurring species. We hypothesize that at higher elevations one would expect the detection of fewer "long distance dispersal" events such as is described for *B. scortella* above, when compared to lower elevations. The ability of a propagule to disperse is influenced in part by gravity (Ronnås et al, 2017), and thus propagules will face an uphill battle in dispersing upward.

Benefits and Limitations of WGS vs. Amplicon Sequencing

Our analyses detected approximately four times as many species using the WGS approach compared to the amplicon-based method in the low-elevation plot, and twice as many in the high-elevation plot. The higher number of species detected by WGS affords a stricter threshold for what counts as a detected species in the SAM file (e.g., by only counting species as detected if they occur at least a certain number of times throughout the SAM file, or by requiring a higher SAM mapping quality score before counting the species as detected).

The community present in a metagenomics sample contains the DNA of species that are represented by many cells in some cases, and potentially by a single cell in other cases. Such stark differences in input quantity from different species in the sample leads to potential issues for both WGS and amplicon environmental sequencing methods. While both methods rely on low abundance DNA being extracted in sufficient quality and quantity to be detected in

downstream analysis, amplicon-based metagenomics suffers from difficulties in primer design, including issues with the use of universal primers (Acinas et al., 2005; Wang et al., 2009). Moreover, unequal (biased) amplification is of wide concern in skewing the distribution of amplicons in the PCR product (Acinas et al., 2005; Sipos et al., 2010). Conversely, one potential issue with WGS metagenomics is that for species with low abundance in the sample, the probability of sampling that species in the prepared library will scale inversely with the proportion of the prepared library that is sequenced. For these reasons, the cohorts of species detected in the community may differ minimally to substantially between the two methods.

One complication of our approach is the presence of group I introns (DePriest & Been 1992; DePriest 1993; Gargas et al., 1995) in the coding sequences (18S, 5.8S and 28S) of many lichenized fungal ribosomal complexes. These introns may be horizontally transferred between species (Hibbett 1996; Fitzpatrick 2012; Roy & Irimia 2012). Thus, it is possible that a read originating from a group I intron in the rDNA complex of a species that is not present in our dataset may appear to map to the database to a species that recently received a horizontal transfer of that intron. However, this will occur only very rarely, and even less-so as the database improves, for several reasons. First, our heuristics for read filtering are fairly strict, requiring read-pairs to uniquely map with high percent-identity to members of the database. Unless the intron transferred very recently, the rapid evolution of such introns (Dujon 1989; Gargas et al., 1995, Roy & Irimia 2012) would prevent the false detection of a species within the database. Moreover, these introns may facilitate a higher rate of species detection, due to their rapid evolution relative to the coding regions that are too conserved to uniquely identify species in many cases.

Moving forward, we aim to implement the approach presented here to measure the congruence between the observed TD of lichens in the southern Appalachians and the PoD measured in the propagule bank, as one step forward in understanding potential biotic constraints on the distributions of lichen biodiversity. The environmental covariates that predict this congruence may enable the development of cost-effective conservation measures in ecologically sensitive keystone systems such as biological soil crusts (Eldridge 2000; Belnap 2003; Belnap and Lange, 2013). We propose that the WGS method presented here should be added to the toolset used by molecular taxonomists in pursuit of conservation and restoration efforts, in addition to facilitating more general understanding on what structures the distributions of species. The datasets and bioinformatic resources presented here represent an important set of new tools and approaches that can be used to address a broad range of questions.

Data availability – All genomic libraries used in this study are available on NCBIs SRA database, at Bioproject https://www.ncbi.nlm.nih.gov/bioproject/PRJNA731936.

CHAPTER 5

This chapter is being prepared for submission to Molecular Ecology. Nolan Kane and Cloe Pogoda contributed with the writing and bioinformatics. Cloe Pogoda headed the genomic library preparation for sequencing. Erin Tripp and James Lendemer curated the samples, helped conceptualize this aspect of the project, performed the covariate scoring, and consulted on the biological assumptions on the project. Kyle Keepers developed the bulk of the bioinformatics pipelines, performed the analyses, and wrote the manuscript.

Growth Form is Strongest Predictor of Algal Photobiont Community Diversity in Lichens

5.1 ABSTRACT

Lichens are a symbiotic assemblage containing primarily a fungal species in association with at least one photosynthetic partner. Lichens exist in a variety of growth forms and may reproduce sexually or asexually. We investigated how these variables contribute to the community diversity within the thalli of 405 lichens across a broad taxonomic range. We found that reproductive mode is not a predictor for community diversity of lichens using only algal photobionts, but that growth form is a strong predictor. Crustose lichens contain the highest photobiont diversity, foliose lichens contain an intermediate level of diversity, and fruticose lichens have the least diverse photobiont communities. We also found that lichens using cyanobacteria as photobionts decrease in diversity at higher elevations.

5.2 INTRODUCTION

Lichens are a widespread, highly diverse group of organisms predicted to occupy up to 8% of the Earth's surface (Asplund and Wardle 2016). They are defined as a symbiosis (or commensalism, and even a parasitism) between a fungus, called the mycobiont, and a photosynthetic partner, called a photobiont, which can be an alga, a cyanobacterium, or sometimes both (Ahmadjian 1993). The fungal partner can sometimes survive and grow axenically (del Carmen Molino and Crespo, 2000; e.g. in the absence of a photobiont), but grows much more slowly, leading to the designation of the symbiosis as effectively 'obligate'. They grow on many different substrates, including rocks, tree bark, and soil (Brodo 1973; Garty and Galun, 1974). Their morphologies are highly variable, and most commonly take on the tufty, branched fruticose, the surface-adhering crustose, and sprawling, lobed foliose forms (Bokhorst et al., 2015), among several others. They vary highly in their modes of reproduction. Lineages can reproduce sexually, or asexually, either as a lichenized propagule containing a package of both fungal and algal/cyanobacterial cells, or simply as a propagule containing fungal cells (Bowler and Rundel, 1975). Some species reproduce in both modes (Lutzoni and Miadlikowska, 2009).

The remarkable diversity of reproductive mode and growth form in lichens leads to questions of how the communities of photobionts are structured among lineages. For a long time, a dogma of "one thallus, one mycobiont, one photobiont" was thought to apply to lichens – that is, the thallus (or body) of lichens were thought to have a simple, clonal community of mycobionts and photobionts. Researchers have long known that photobionts *between* species was variable (Sadowska-Deś et al., 2014), but only recently have the technologies existed that enable a look into the community structure *within* the thallus of a lichen. We present the first large-scale, taxonomically-broad study into the photobiont community diversity within lichens.

Within the thallus of a lichen, the photobionts may exist as a monolithic population of clones of a progenitor photobiont that was present at the establishment of the thallus (Ahmadjian 1967). Alternatively, they may be incorporated opportunistically as the thallus grows outward and encounters suitable free-living photobionts (Rikkinen et al., 2002; Nelsen and Gargas, 2008). We assumed the latter to be a possibility in this study, and consequently treated the corpus of photobiont genomic reads obtained from a lichen sample to be a community, for which community diversity metrics of θ_{π} and $\theta_{Watterson}$ suitably represent the diversity. The metric θ_{π}, sometimes referred to as π, is a common measure of the overall sequence variation within a population at a particular genetic locus. The $\theta_{Watterson}$ metric similarly measures the number of segregating sites (single nucleotide variants, in this case) scaled by a factor that makes it comparable to θ_{π} when the population is in Hardy-Weinberg equilibrium. If deviations from HW equilibrium are expected due to demographic factors such as a recent population bottleneck or a population expansion, care should be taken in using θ_{π}.

The nascent field of bioinformatics is an automation workhorse that has enabled this look into such a taxonomically broad set of lichens, representing 250 million years of evolution (Lutzoni et al., 2018). Such a broad taxonomic sampling provides the biggest picture of how fecund certain species of lichen mycobionts may be in a future in which changing climate conditions (Jackson et al., 2006; Deduke et al., 2014) may affect the availability of suitable photobionts for those species. We foresee these findings enabling better-informed decisions that maximize the utility of often-small conservation budgets.

The biodiversity of organisms in an environment is a key ecological parameter, with important effects on community stability and functioning (Tilman and Downing 1994; Tilman 1996). There are many factors that affect how diverse a community is, such as climate (García-

Palacios et al., 2018), soil chemistry (Lal 1991; Pankhurst 1997), disturbance levels (Angelstom 1998; Thom and Seidl 2016), among many others. Within small geographic regions, one factor that can have important effects ondiversity is elevation. One pattern we might expect is that the lowest diversity would be observed at high-elevations, where there is less contiguous habitat for species to inhabit (Sergio and Pedrini 2007). Another pattern often observed in plants and animals is the "intermediate elevation" hypothesis, wherein the highest diversity of communities is seen at intermediate elevations (McCain 2005; McCain 2009). The reasons for this pattern are complex, but one factor contributing to it is that intermediate elevations are the zone where lower-elevation generalist species that exhibit plasticity in their habitat range meet with specialists that live in marginal high-elevation habitat which also have plasticity in their range.

In this study, we bioinformatically isolated whole genome shotgun reads from the ribosomal DNA (rDNA) sequences of the photobiont communities contained within the thalli of 405 lichen species. We then aligned the reads to a rDNA reference appropriate to each species (cyanobacterial 16S/23S, and/or the rDNA of either green coccoid Trebouxioid sequence or green Trentepohlioid sequence, depending on which type the species uses). Next, we calculated the sliding-window θ_{π} and $\theta_{Watterson}$ values across the sequence, and averaged across each sliding window for which we obtained a value. We formulated three hypotheses for how the photobiont community diversity varies among the covariates of 1. growth form, 2. primary reproductive mode, and 3. elevation:

Hypothesis 1: Photobiont communities will be more diverse in the thalli of crustose lichens than they will be in fruticose thalli. Foliose thalli will have an intermediate range of diversity values.	Justification: Lichens that tightly adhere to their substrate, such as crustose lichens, will have more diverse photobiont communities than the 3-dimensional fruticose lichens that only attach to their substrate at a single point, due to their ability to opportunistically incorporate suitable free-living photobionts.

Hypothesis 2: Photobiont diversity will be higher in lichens that utilize a sexual reproductive mode.	Justification: Sexually-reproducing lichens that propagate via fungal spores need to encounter suitable, free-living algae as photobionts. They will thus have evolved a tolerance for a broader range of suitable photobionts than lichenized-asexual species that bring their photobionts with them when they propagate.
Hypothesis 3A: Lichens will have higher photobiont diversity at lower elevations	Justification: The photobiont diversity will decrease along an elevational gradient, where there is more surface area for photobionts to speciate and develop sequence variation.
Hypothesis 3B: Lichens will have higher photobiont diversity at intermediate elevations	Justification: A pattern frequently observed in nature is that plants and animals have the highest level of diversity at intermediate elevations

An additional question that was more exploratory was whether substrate was a factor that could affect the results we were obtaining. Specifically, substrate could have affected our analysis because it changes the environmental growing conditions of the lichens in a way that changed the composition of the photobiont communities living within them. Additionally, any effects of environmental algal reads that made it into our genomic libraries and incorporated into the analyses should be clear from this analysis, helping us understand this factor we wanted to preclude from our results. Both of these factors were important to assess, thus we included substrate as a covariate in our analysis.

5.3. METHODS AND MATERIALS

DNA Extraction and Whole Genome Shotgun Sequencing – Genomic libraries were prepared according to Pogoda et al. 2018. In short, roughly 1cm x 1cm of tissue from 494 lichen vouchers were pulverized using tungsten carbide beads and DNA was extracted using the Qiagen DNeasy Plant extraction kit. The elution was then prepared for sequencing with the Nextera® XT DNA library prep kit. Libraries that passed QC were processed for paired-end 151 base pair reads on

an Illumina NextSeq® sequencer at the University of Colorado's BioFrontiers Institute (Boulder, Colorado).

De novo Genomic Assembly – Libraries were filtered with Trimmomatic-0.36 to trim adapters from reads, and with parameters "LEADING:3 TRAILING:3 MINLEN:100" (Bolger et al., 2014). Filtered reads were then assembled using SPAdes 3.9.0 with parameters "--careful -k 21,33,65,81" (Bankevich et al., 2012). We then bioinformatically isolated the complete or partial ribosomal DNA complexes of 60 algal photobionts within the dataset. These rDNA sequences were then queried with web BLAST to confirm their identities as either green coccoid (Trebouxioid) or green trentepohlia (Trentepohlioid) algae. We also obtained the rDNA complexes from 43 assemblies containing cyanobionts. We verified the identities of these sequences as Nostocaceae (the group of cyanobacteria used as photobionts by cyanolichens) using web BLAST.

Photobiont Diversity Calculation Pipeline – Our process for identifying the diversity of photobiont communities within our genomic libraries involved two steps. The first step was to map the libraries to algal and cyanobacterial (when applicable, respectively) databases of rDNA to isolate only reads that plausibly sourced from a photobiont of the lichen. The process for ensuring that only photobiont reads were obtained from this step is described in its own section below (see *Masking Non-diagnostic Regions of the rDNA Databases*). The collection of photobiont rDNA reads were then aligned to one of two algal references (the Trebouxioid photobiont of *Usnea ceratina* [NCBI Accession KY033354] and the Trentepohlioid photobiont of *Opegrapha moroziana* [unpublished data]. In the case of lichens with cyanobionts, we mapped cyanobiont reads to a reference from *Nostoc sphaeroides* [NCBI Accession CP031941]. Pileup

files were generated from the alignments, which were then passed to PoPoolation v. 1.2.2 (Kofler et al., 2011) with the parameters "--measure theta –input <input.mpileup> --fastq-type sanger --min-qual 20 --max-coverage 200 --pool-size 500 --window-size 25 --step-size 25 --output <output.theta>" to measure $\theta_{Watterson}$ in a sliding window of 25 bp across the length of the pileup against each reference. A similar command with "--measure pi" was used to calculate θ_{π}. The average of the sliding window values was calculated for each output file, and averages were discarded if the number of windows in an output file containing a calculated value was less than 10.

Comparing θ_{π} *and* $\theta_{Watterson}$ – To ascertain the appropriateness of $\theta_{Watterson}$ for phylogenetic comparisons, (see "Correlation Analyses"), we compared them to the values of θ_{π} in a simple linear regression, to confirm that both of these related metrics show similar patterns within this data set.

Masking Non-diagnostic Regions of the rDNA Databases – Due to the highly conserved nature of ribosomal DNA, we found that non-photobiont reads were introgressing into our analyses. To remediate this, we masked regions of our databases that were non-diagnostic of our intended targets. For the algal database, we mapped all genomic reads to the database, filtered the hits by a SAM CIGAR score of 148-151M or higher (described in Keepers et al. 2019) then web BLAST-queried the resultant reads to identify highly conserved regions to which non-algal reads, such as fungal or bryophyte, mapped. The spans of these regions were then masked with Ns throughout all of the contigs in the database.

To mask non-diagnostic cyanobacterial reads, we were able to take a less labor-intensive approach. We mapped reads to the *Nostoc sphaeroides* cyanobacterial reference in two classes –

libraries from lichens that only used cyanobacteria as a photobiont, and libraries that should have entirely lacked significant numbers of cyanobacteria. We called SNP variants in vcftools across all of these alignments, and calculated the proportion of variants in each class. The difference between variant proportions enabled us to identify regions in the reference to which large numbers of non-cyanobacterial reads, which were typically proteobacterial, were mapping. We then restricted our $\theta_{Watterson}$ and θ_{π} analyses to outside of those non-diagnostic regions.

Covariate Information – The specimens, from which each genomic library used in this study was derived, are vouchered at the New York Botanical Gardens' Sweetgum Database (Thiers et al., 2016). We derived covariate values for each lichen, comprising of: photobiont type, growth form, taxonomic family, reproductive mode, and the elevation at which the specimen was collected. For reproductive mode, we coded a species as "Sexual" if it reproduces exclusively sexually, as "Asexual" if exclusively asexual, and "Both" if it utilizes a combination of both. To evaluate bias in our estimates attributed to the substrate on which a lichen grows, which may have varying availability of suitable photobionts, we also broke down our estimates according to substrate, which fell into 5 categories: bark, wood, humus, calcareous rock, and non-calcareous rock.

Correlation Analyses – To test for differences in the photobiont diversity distributions of the categorical covariates of growth form, dominant reproductive mode, photobiont identity (trebouxioid or trentepohlioid), and substrate, we conducted pairwise two-tailed Student t-tests between each category. To test if photobiont diversity varied significantly with elevation, we performed separate linear regressions of each photobiont type, algal and cyanobacterial, against the elevation at which the lichen was collected. To evaluate the "intermediate diversity"

hypothesis, that diversity should be highest at middle elevations, we fit a quadratic regression to the data.

To account for the possibility that photobiont community diversity could be driven by a shared evolutionary history, we conducted a phylogenetic generalized least squares (PGLS; Grafen 1989) analysis. We fit two models of trait evolution, the Brownian motion (BM; Felsenstein 1985) model, and the Ornstein-Uhlenbeck (OU; Martins and Hansen 1997) model, and compared the results to a non-phylogenetic "TIPS" analysis. As required for PGLS and other comparative methods the phylogenetic tree we generate (see the section "Phylogenetic Tree") was ultrametricized using the chronopl function in the R package APE (Paradis and Shliep 2019), and the PGLS tests were conducted using the R package nlme (Pinheiro et al., 2021). Goodness of fit was evaluated using AIC.

Phylogenetic Tree – The sequences in the ribosomal DNA database described in Keepers et al., 2019 were used to generate an alignment to infer phylogenetic relatedness. First, any sequences in the database that were shorter than 3,000 base-pairs were discarded. The remaining 297 sequences were aligned using MUSCLE v.3.8.31 (Edgar 2004) using default parameters. The highly variable internal transcribed spacers 1 and 2 were identified using the conserved ITS primer regions ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA) (Gardes and Bruns, 1993) and ITS2 (5'-GCTGCGTTCTTCATCGATGC) (White et al., 1990), and removed by from the alignment to reduce the possibility analogous characters in this region from obfuscating phylogenetic signal. The best nucleotide substitution model was inferred to be GTR+Γ+I using ModelGenerator v.0.85 (Keane et al., 2006). We used RAxML v.8.0.0 (Stamatakis 2014) to infer a phylogeny using "-m GTRGAMMAI".

5.4 RESULTS

Phylogenetic Tree – The sequence alignment and phylogenetic tree used for statistical comparative analyses are deposited on Dryad (DOI:https://doi.org/10.5061/dryad.3n5tb2rhp). The tree recovers expected relationships (Figure 5.S2).

Phylogenetic Correlation Models – We evaluated three statistical models in comparing the photobiont communities in lichens exhibiting different growth forms. For both the algal and cyanobacterial communities, the best statistical model was the OU model that takes phylogenetic relatedness into account. The OU model estimates a parameter "α" representing the strength of selection along branches of the tree. Small values of α (<<1) are equivalent to the evolution under Brownian motion, whereas large values (>>1) are approximate to a non-phylogenetic model (TIPS). We estimated α=5804.8 for algal diversity estimates, which suggests the influence of phylogenetic history on those communities is small, but relevant. The AIC values from the PGLS tests and the non-phylogenetic ANOVA tests are reported in Table 5.3. The best model for the patterns of photobiont diversity in both the algal communities and the cyanobacterial communities accounted for phylogenetic similarity under the OU model (AIC_{OU} = -1144.99 versus AIC_{TIPS}=-1138.147). After accounting for phylogenetic signal, both fruticose and foliose growth forms were significantly less diverse than crustose communities. The correlation coefficient in the algal OU model comparing crustose species to foliose species is -0.0066 (p=0.006) and the coefficient comparing crustose to fruticose species is -0.0133 (p=0.004), indicating that these species contain significantly less diverse photobiont communities than crustose species. These effects remain significant after a conservative Bonferroni correction (α = 0.05/6 = 0.008).

Nostoc rDNA Reference– The regions of the *Nostoc* reference that are diagnostic to cyanobacteria are shown in Figure 5.S1. Variants only present in libraries using cyanobionts are negative values in the graph. We restricted our $\theta_{Watterson}$ and θ_{π} calculations to these regions.

Reproductive Mode – There was no significant difference in algal (Fig 5.1) or cyanobacterial (Fig. 5.2) community diversity between sexually- and asexually-reproducing species (algal p=0.1355; cyanobacterial p=0.1131), sexually-reproducing and those that use a mixture of both modes (algal p=0.4068; cyanobacteria p=0.06159), or asexually-reproducing and those that use a mixture of both (algal p=0.5652; cyanobacterial p=0.8096).

Substrate – There were no significant differences in the algal photobiont diversity of any lichens in this study depending on the substrate occupied by the lichens (Table 5.2; Figure 5.4). However, among cyanobacterial photobionts, there were significant differences (α=0.05) between lichens living on calcareous rock and bark, calcareous rock and humus, and non-calcareous rock and humus, as well as a highly significant difference (α=0.01) between the diversity of non-calcareous rock and bark.

Elevation – The diversity of algal community diversity in lichens does not vary significantly with elevation when the data are fit with a linear regression, but the quadratic regression model we fit was statistically significant (p=0.0109) with a negative leading quadratic coefficient $-1.609*10^{-5}*(\text{elevation})^2$, There was a significant inverse correlation between the elevation of a cyanolichen and its photobiont diversity (p=0.01093).

Figure 5.1. Algal photobiont diversity values $\theta_{Watterson}$ plotted according to the (top) reproductive mode and (bottom) growth form.

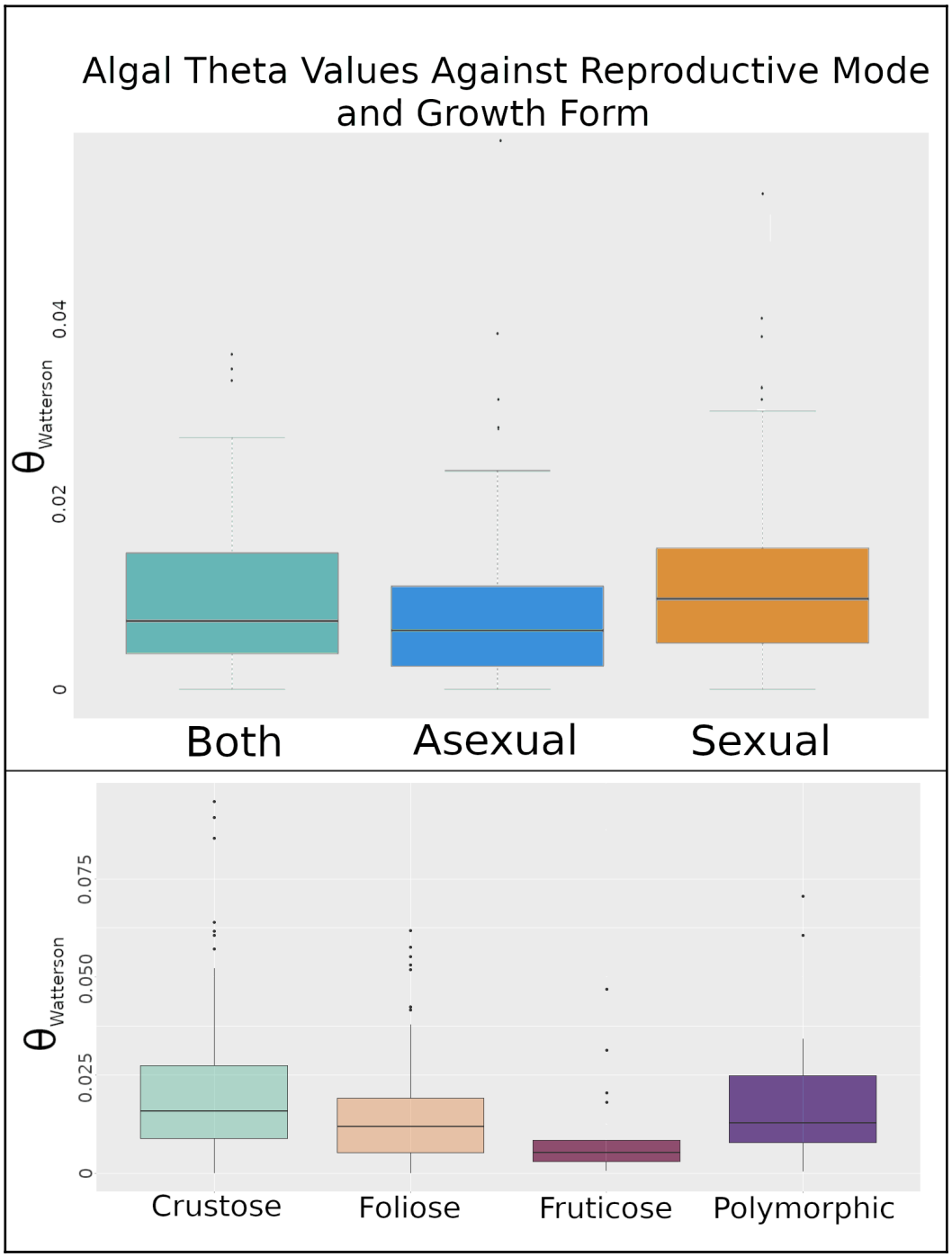


Figure 5.2. Cyanobacterial photobiont diversity values $\theta_{Watterson}$ plotted according to the (top) reproductive mode and (bottom) growth form.

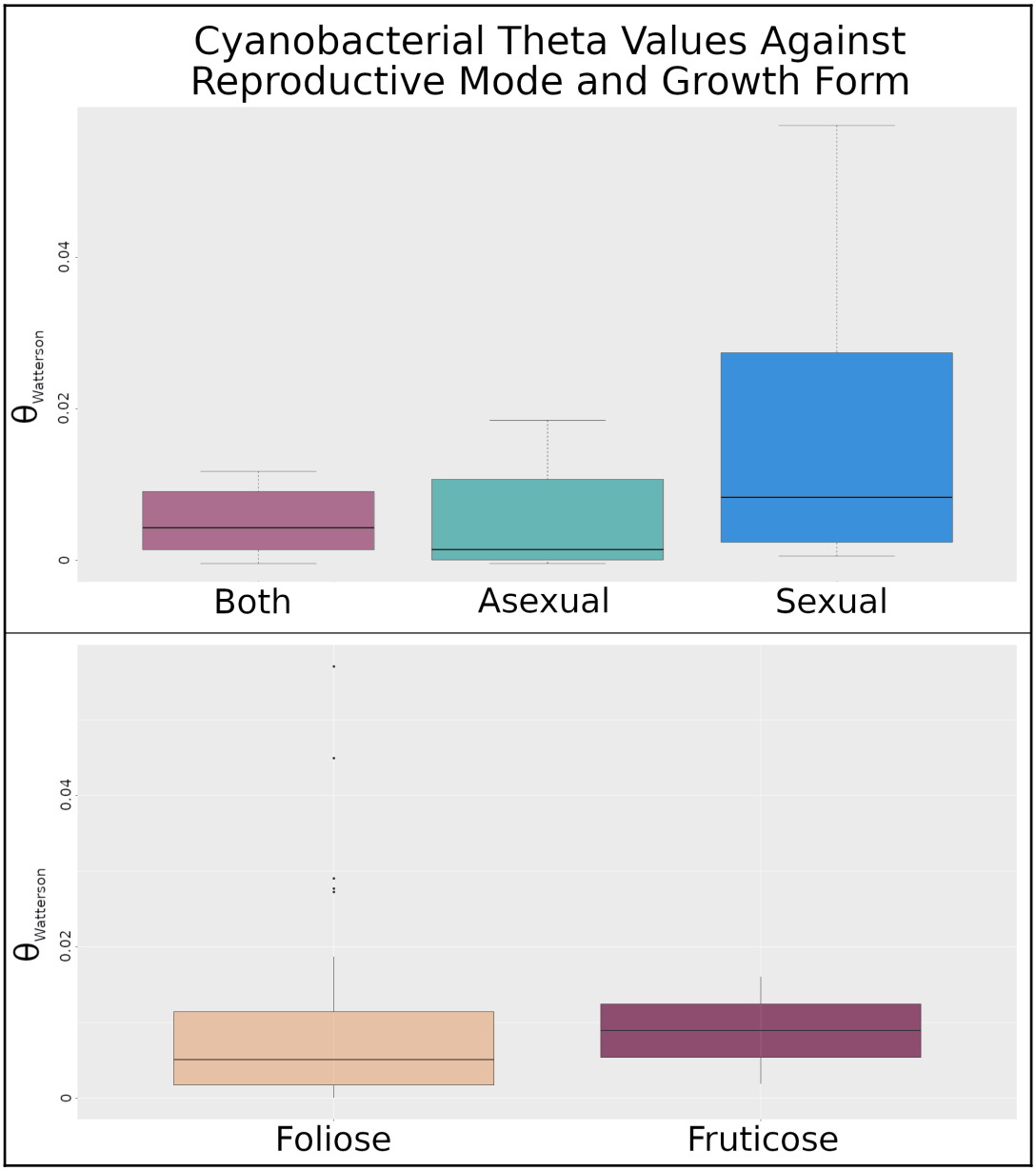


Figure 5.3. Elevation values plotted against $\theta_{Watterson}$ diversity of algal photobionts (A) and cyanobacterial photobionts (B).

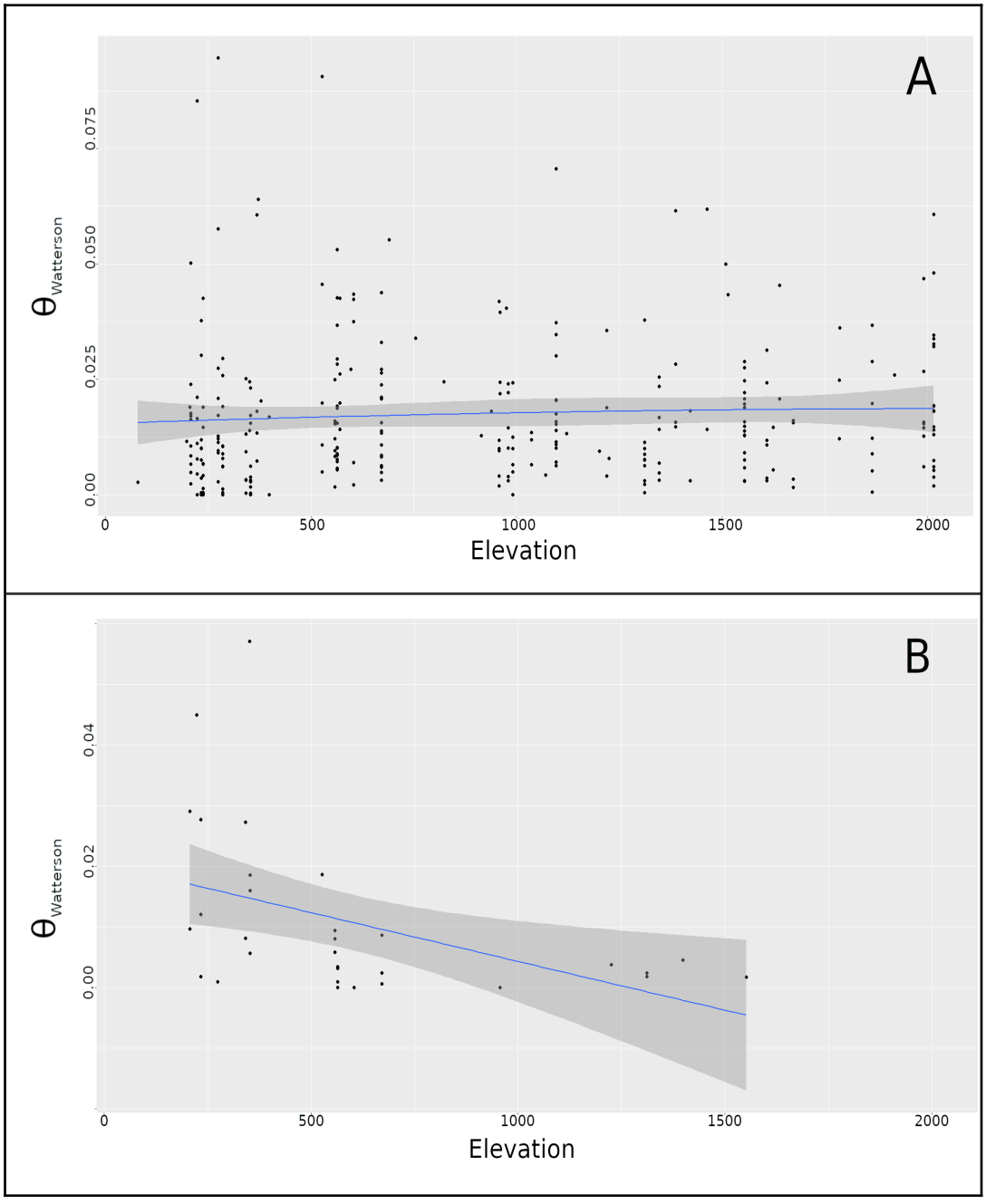


Figure 5.4. Algal and cyanobacterial photobiont diversity values plotted against substrate.

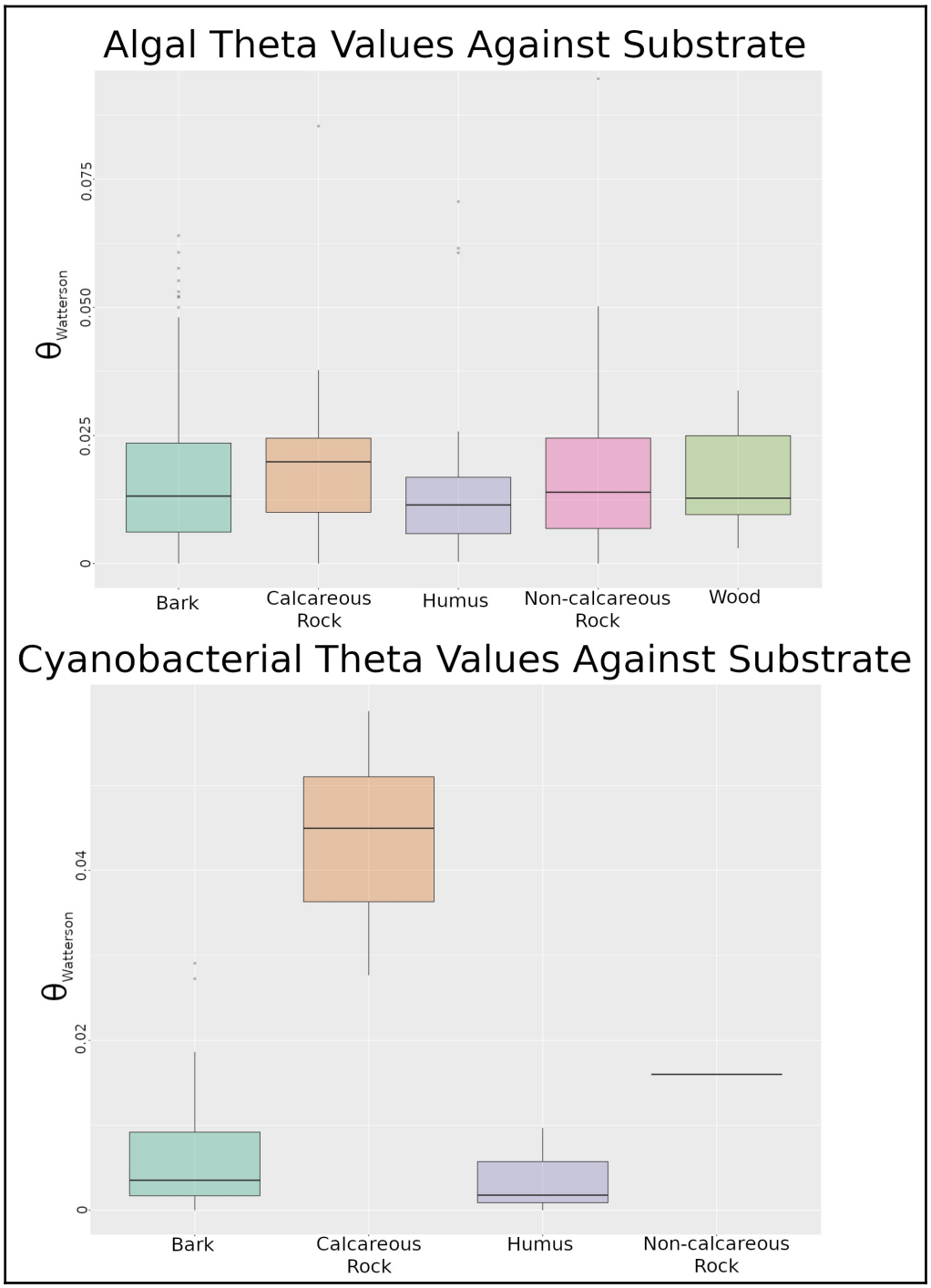


Table S5.1. Difference in the number of variants in lichens that should not have abundant cyanobacterial sequences and lichens that use exclusively cyanobacteria as their photobiont. Positive regions have a glut of variants that came from non-cyanobacterial sequences, and were thus not diagnostic for Nostoc cyanobacterial reads. Only reads that mapped to the negative regions were used to calculate cyanobacterial diversity.

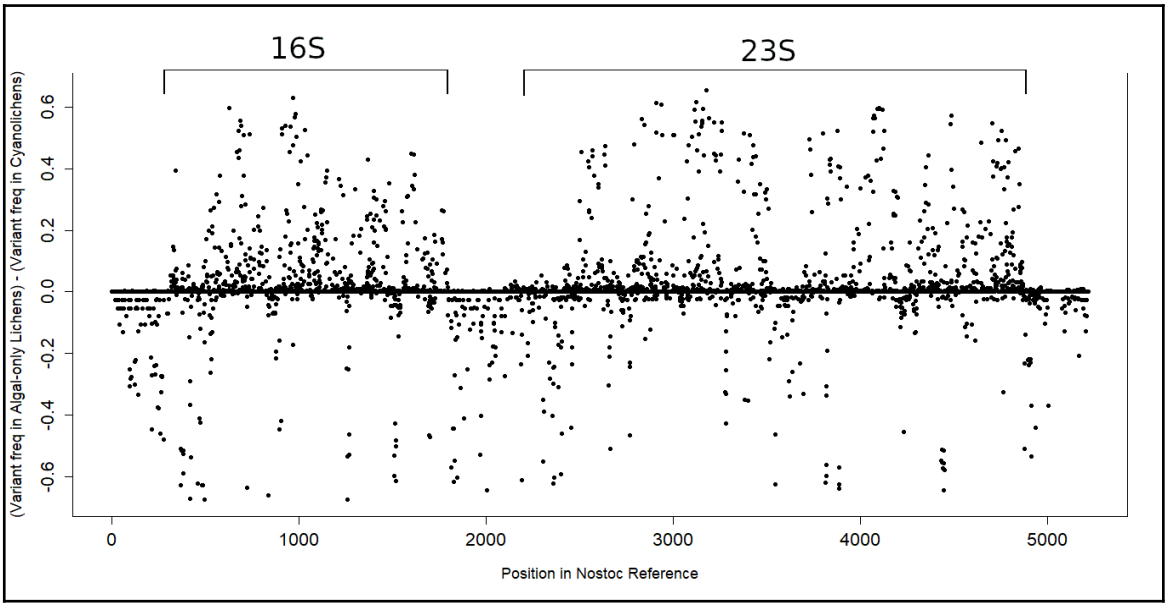


Table 5.1. Character traits of the 405 samples used in this study. Assemblies were named after the field identification of the voucher, whereas the name may differ in the Current Determination column if the voucher was initially misidentified. Umbilicate and squamulose growth forms were simplified to "Foliose" for the sake of concision. Values of "NA" for Theta and Pi columns were given to species that lacked enough reads mapping to the appropriate locus to meet the threshold of listing a value in that column. Values of "NA" for reproductive mode mean the exact mode was not known. Values in Substrate of CR=Calcareous rock and NCR=Non-calcareous rock.

Current Determination	Family	Photobiont	Reprod. Mode	Growth Form	Substrate	Elevation (m)	θ_{Algal}	θ_{Cyano}	π_{Algal}	π_{Cyano}
Absconditella delutula	Stictidaceae	Coccoid	Sexual	Crustose	Bark	1036	0.0135	NA	0.0135	NA
Acanthothecis fontana	Graphidaceae	Trentepohlioid	Sexual	Crustose	Bark	223	0.0111	NA	0.0078	NA
Acarospora	Acarosporaceae	Trentepohlioid	NA	Crustose	NA	1096	0.0139	NA	0.0179	NA
Acarospora sinopica	Acarosporaceae	Coccoid	NA	Crustose	NA	1096	0.0152	NA	0.0170	NA
Acrocordia megalospora	Monoblastiaceae	Trentepohlioid	Sexual	Crustose	Bark	558	0.0096	NA	0.0101	NA
Acrocordia megalospora	Monoblastiaceae	Trentepohlioid	Sexual	Crustose	Bark	NA	NA	NA	NA	NA
Ahtiana aurescens	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	1347	0.0255	NA	0.0253	NA
Anaptychia palmulata	Physciaceae	Coccoid	Sexual	Foliose	Bark	671	0.0048	NA	0.0049	NA
Anaptychia palmulata	Physciaceae	Coccoid	Sexual	Foliose	Bark	368	NA	NA	NA	NA
Anisomeridium sp.	Monoblastiaceae	Trentepohlioid	NA	Crustose	NA	980	NA	NA	NA	NA
Anthracothecium nanum	Unknown	Trentepohlioid	Sexual	Crustose	Bark	NA	0.0189	NA	0.0169	NA
Anzia colpodes	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	1036	0.0119	NA	0.0114	NA
Anzia ornata	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	NA	NA	NA	NA	NA
Arthonia anglica	Arthoniaceae	Trentepohlioid	Sexual	Crustose	Bark	671	0.0438	NA	0.0457	NA
Arthonia cupressina	Arthoniaceae	Coccoid	NA	Crustose	NA	1786	0.0361	NA	0.0348	NA
Arthonia kermesina	Arthoniaceae	Trentepohlioid	NA	Crustose	Bark	1609	NA	NA	NA	NA
Arthonia kermesina	Arthoniaceae	Trentepohlioid	NA	Crustose	Bark	1609	NA	NA	NA	NA
Arthonia ruana	Arthoniaceae	Trentepohlioid	Sexual	Crustose	Bark	1609	0.0242	NA	0.0247	NA
Arthonia rubella	Arthoniaceae	Trentepohlioid	Sexual	Crustose	Bark	237	0.0013	NA	0.0010	NA
Arthonia susa	Arthoniaceae	Coccoid	Sexual	Crustose	Bark	564	0.0089	NA	0.0046	NA
Arthonia vinosa	Arthoniaceae	Trentepohlioid	Sexual	Crustose	Bark	1609	NA	NA	NA	NA
Arthothelium spectabile	Arthoniaceae	Trentepohlioid	Sexual	Crustose	Bark	564	NA	NA	NA	NA
Aspicilia laevata	Megalosporaceae	Coccoid	Sexual	Crustose	NCR	237	0.0066	NA	0.0054	NA
Bacidia schweinitzii	Bacidiaceae	Coccoid	Sexual	Crustose	Bark	564	NA	NA	NA	NA
Bacidia sorediata	Bacidiaceae	Coccoid	Both	Crustose	Bark	1311	NA	NA	NA	NA
Bagliettoa baldensis	Verrucariaceae	Coccoid	Sexual	Crustose	CR	233	0.0000	NA	0.0000	NA
Bathelium carolinianum	Trypetheliaceae	Trentepohlioid	Sexual	Crustose	Bark	342	0.0032	NA	0.0021	NA
Biatora appalachensis	Ramalinaceae s. str.	Coccoid	Asexual	Crustose	Bark	1640	0.0454	NA	0.0497	NA
Biatora chrysantha	Ramalinaceae s. str.	Coccoid	Asexual	Crustose	Humus	1387	0.0615	NA	0.0618	NA
Biatora longispora	Ramalinaceae s. str.	Coccoid	Sexual	Crustose	Bark	1311	NA	NA	NA	NA
Biatora pontica	Ramalinaceae s. str.	Coccoid	Both	Crustose	Bark	1311	0.0087	NA	0.0083	NA
Botryolepraria lesdainii	Verrucariaceae	Coccoid	Asexual	Crustose	CR	1202	NA	NA	NA	NA
Botryolepraria lesdainii	Verrucariaceae	Coccoid	Asexual	Crustose	CR	274	NA	NA	NA	NA
Brianaria bauschiana	Pilocarpaceae	Coccoid	Sexual	Crustose	NCR	1387	0.0147	NA	0.0132	NA
Brigantiaea leucoxantha	Brigantaeaceae	Coccoid	Sexual	Crustose	Bark	725	NA	NA	NA	NA
Bryoria bicolor	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	2015	0.0052	NA	0.0039	NA
Bryoria nadvornikiana	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	2015	0.0038	NA	0.0010	NA
Bryoria tenuis	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	2015	0.0074	NA	0.0052	NA
Buellia mamillana	Caliciaceae	Coccoid	Sexual	Crustose	NCR	342	0.0033	NA	0.0023	NA
Buellia spuria	Caliciaceae	Coccoid	Sexual	Crustose	NCR	1096	0.0063	NA	0.0047	NA
Buellia spuria	Caliciaceae	Coccoid	Sexual	Crustose	NCR	207	0.0171	NA	0.0167	NA
Buellia spuria race stictic	Caliciaceae	Coccoid	NA	Crustose	NCR	353	0.0031	NA	0.0015	NA
Buellia stillingiana	Caliciaceae	Coccoid	Sexual	Crustose	Bark	564	0.0192	NA	0.0129	NA
Buellia vernicoma	Caliciaceae	Coccoid	Sexual	Crustose	Bark	671	0.0238	NA	0.0237	NA
Bulbothrix scortella	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	353	0.0038	NA	0.0014	NA
Byssoloma meadii	Pilocarpaceae	Coccoid	Sexual	Crustose	Bark	NA	0.0522	NA	0.0528	NA
Byssoloma subdiscordans	Pilocarpaceae	Coccoid	Sexual	Crustose	Leaf	597	0.0272	NA	0.0177	NA
Caloplaca camptidia	Teloschistaceae	Coccoid	Sexual	Crustose	Bark	671	0.0272	NA	0.0229	NA
Caloplaca chrysophthalma	Teloschistaceae	Coccoid	Asexual	Crustose	Bark	980	NA	NA	NA	NA
Caloplaca feracissima	Teloschistaceae	Coccoid	Sexual	Crustose	CR	527	0.0199	NA	0.0132	NA
Catillaria lenticularis	Catillariaceae	Coccoid	Sexual	Crustose	CR	223	0.0854	NA	0.0847	NA
Cetrelia chicitae	Parmeliaceae	Coccoid	Both	Foliose	Bark	1311	0.0113	NA	0.0107	NA
Cetrelia olivetorum	Parmeliaceae	Coccoid	Both	Foliose	Bark	957	0.0118	NA	0.0123	NA
Chaenotheca balsamconensis	Coniocybaceae	Coccoid	NA	Crustose	NA	527	0.0906	NA	0.0893	NA
Chaenotheca furfuracea	Coniocybaceae	Coccoid	Sexual	Crustose	NCR	527	NA	NA	NA	NA
Chrysothrix onokoensis	Chrysotrichaceae	Coccoid	Asexual	Crustose	NCR	274	0.0946	NA	0.0945	NA
Chrysothrix susquehannensis	Chrysotrichaceae	Coccoid	Asexual	Crustose	NCR	1096	0.0158	NA	0.0117	NA
Chrysothrix xanthina	Chrysotrichaceae	Coccoid	Asexual	Crustose	Bark	527	0.0456	NA	0.0468	NA
Chrysothrix xanthina	Chrysotrichaceae	Coccoid	Asexual	Crustose	Bark	NA	0.0245	NA	0.0243	NA
Chrysothrix xanthina	Physciaceae	Coccoid	Asexual	Crustose	Bark	237	NA	NA	NA	NA
Cladonia apodocarpa	Cladoniaceae	Coccoid	Asexual	Foliose	Humus	957	0.0041	NA	0.0026	NA
Cladonia arbuscula	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	1554	0.0158	NA	0.0108	NA
Cladonia caroliniana	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	285	0.0106	NA	0.0103	NA
Cladonia coccifera	Cladoniaceae	Coccoid	NA	Polymorphic	Humus	1096	0.0114	NA	0.0079	NA
Cladonia coniocraea	Cladoniaceae	Coccoid	NA	Polymorphic	Humus	671	0.0133	NA	0.0110	NA
Cladonia didyma	Cladoniaceae	Coccoid	NA	Polymorphic	Wood	980	0.0101	NA	0.0052	NA
Cladonia didyma	Cladoniaceae	Coccoid	NA	Polymorphic	Wood	NA	NA	NA	NA	NA
Cladonia furcata	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	233	0.0005	NA	0.0001	NA
Cladonia furcata	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	207	0.0048	NA	0.0024	NA
Cladonia grayi	Cladoniaceae	Coccoid	NA	Polymorphic	Humus	1554	0.0247	NA	0.0196	NA
Cladonia leporina	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	285	0.0078	NA	0.0078	NA

Current Determination	Family	Photobiont	Reprod, Mode	Growth Form	Substrate	Elevation (m)	θ_{Algal}	θ_{Cyano}	π_{Algal}	π_{Cyano}
Cladonia macilenta	Cladoniaceae	Coccoid	NA	Polymorphic	Wood	1554	0.0128	NA	0.0075	NA
Cladonia mateocyatha	Cladoniaceae	Coccoid	Sexual	Polymorphic	NCR	1554	0.0075	NA	0.0046	NA
Cladonia mateocyatha	Cladoniaceae	Coccoid	Sexual	Polymorphic	NCR	1096	0.0347	NA	0.0365	NA
Cladonia ochrochlora	Cladoniaceae	Coccoid	NA	Polymorphic	Humus	671	0.0211	NA	0.0190	NA
Cladonia petrophila	Cladoniaceae	Coccoid	Sexual	Foliose	NCR	274	0.0171	NA	0.0151	NA
Cladonia peziziformis	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	398	0.0169	NA	0.0172	NA
Cladonia polycarpoides	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	368	0.0606	NA	0.0608	NA
Cladonia pyxidata	Cladoniaceae	Coccoid	NA	Polymorphic	NCR	1096	0.0301	NA	0.0312	NA
Cladonia rangiferina	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	1554	0.0091	NA	0.0052	NA
Cladonia rangiferina	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	285	0.0004	NA	0.0002	NA
Cladonia ravenelii	Cladoniaceae	Coccoid	NA	Polymorphic	Wood	285	0.0295	NA	0.0295	NA
Cladonia robbinsii	Cladoniaceae	Coccoid	Asexual	Foliose	Humus	285	0.0104	NA	0.0086	NA
Cladonia squamosa	Cladoniaceae	Coccoid	NA	Polymorphic	Humus	980	0.0145	NA	0.0141	NA
Cladonia squamosa	Cladoniaceae	Coccoid	NA	Polymorphic	Humus	1737	0.0129	NA	0.0088	NA
Cladonia squamosa	Cladoniaceae	Coccoid	NA	Polymorphic	Humus	285	0.0258	NA	0.0217	NA
Cladonia stipitata	Cladoniaceae	Coccoid	Sexual	Foliose	NCR	1554	NA	NA	NA	NA
Cladonia stipitata	Cladoniaceae	Coccoid	Sexual	Foliose	NCR	1096	0.0372	NA	0.0367	NA
Cladonia strepsilis	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	1096	0.0706	NA	0.0722	NA
Cladonia subtenuis	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	285	0.0013	NA	0.0009	NA
Cladonia uncialis	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	1554	0.0058	NA	0.0038	NA
Cladonia uncialis	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	1096	0.0101	NA	0.0100	NA
Cladonia uncialis	Cladoniaceae	Coccoid	Sexual	Polymorphic	Humus	197	0.0115	NA	0.0114	NA
Coccocarpia palmicola	Coccocarpiaceae	Cyanobacterium	Asexual	Foliose	Bark	564	NA	0.0034	NA	0.0012
Collema coccophorum	Collemataceae	Cyanobacterium	NA	Foliose	CR	223	NA	0.0450	NA	0.0424
Collema furfuraceum	Collemataceae	Cyanobacterium	Asexual	Foliose	Bark	353	NA	0.0186	NA	0.0065
Collema subflaccidum	Collemataceae	Cyanobacterium	Both	Foliose	Bark	671	NA	0.0023	NA	0.0022
Conotrema urceolatum	Stictidaceae	Coccoid	Sexual	Crustose	Bark	1311	0.0100	NA	0.0063	NA
Crespoa crozalsiana	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	342	0.0093	NA	0.0085	NA
Cresponea flava	Roccellaceae	Trentepohlioid	Sexual	Crustose	Bark	207	NA	NA	NA	NA
Crocodia aurata	Lobariaceae	Coccoid	Asexual	Foliose	Bark	671	0.0208	NA	0.0196	NA
Crocodia aurata	Lobariaceae	Coccoid	Asexual	Foliose	Bark	NA	0.0339	NA	0.0329	NA
Cystocoleus ebeneus	Cystocoleaceae	Trentepohlioid	Asexual	Fruticose	NCR	1021	NA	NA	NA	NA
Cystocoleus ebeneus	Cystocoleaceae	Trentepohlioid	Asexual	Fruticose	NCR	207	0.0084	NA	0.0084	NA
Dendriscocaulon intricatulum	Lobariaceae	Cyanobacterium	Asexual	Fruticose	Bark	1311	NA	0.0018	NA	0.0003
Dermatocarpon luridum	Verrucariaceae	Coccoid	Sexual	Foliose	NCR	398	0.0000	NA	0.0000	NA
Dermatocarpon muhlenbergii	Verrucariaceae	Coccoid	Sexual	Foliose	CR	274	0.0209	NA	0.0215	NA
Dermiscellum oulocheilum	Caliciaceae	Coccoid	NA	Polymorphic	NA	79	0.0027	NA	0.0022	NA
Dibaeis absoluta	Baeomycetaceae	Coccoid	Sexual	Crustose	NCR	353	0.0000	NA	0.0000	NA
Dibaeis sorediata	Baeomycetaceae	Coccoid	Asexual	Crustose	NCR	207	0.0176	NA	0.0176	NA
Dictyocatenulata alba	Unknown	Trentepohlioid	NA	Crustose	Bark		0.0404	NA	0.0396	NA
Dictyomeridium proponens	Trypetheliaceae	Trentepohlioid	Sexual	Crustose	Bark	207	0.0023	NA	0.0009	NA
Dimelaena oreina	Caliciaceae	Coccoid	Sexual	Crustose	NCR	1554	0.0129	NA	0.0075	NA
Diploschistes scruposus	Graphidaceae	Coccoid	Sexual	Crustose	NCR	1096	NA	NA	NA	NA
Dirinaria frostii	Caliciaceae	Coccoid	Asexual	Foliose	NCR	237	0.0146	NA	0.0144	NA
Enchylium conglomeratum	Collemataceae	Cyanobacterium	NA	Foliose	NA	1347	NA	NA	NA	NA
Ephebe solida	Lichinaceae	Cyanobacterium	Sexual	Fruticose	NCR	353	NA	0.0160	NA	0.0086
Fissurina insidiosa	Graphidaceae	Trentepohlioid	Sexual	Crustose	Bark	274	0.0091	NA	0.0056	NA
Flakea papillata	Verrucariaceae	Coccoid	Asexual	Foliose	NCR	207	0.0106	NA	0.0096	NA
Flavoparmelia baltimorensis	Parmeliaceae	Coccoid	Both	Foliose	NCR	1096	0.0109	NA	0.0108	NA
Flavoparmelia baltimorensis	Parmeliaceae	Coccoid	Both	Foliose	NCR	957	0.0418	NA	0.0429	NA
Flavopunctelia flaventior	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	1673	0.0034	NA	0.0016	NA
Fuscopannaria leucosticta	Pannariaceae	Cyanobacterium	Sexual	Foliose	Bark	564	NA	0.0010	NA	0.0004
Fuscopannaria leucosticta	Pannariaceae	Cyanobacterium	Sexual	Foliose	Bark		NA	NA	NA	NA
Gomphillaceae	Gomphilaceae	Coccoid	NA	Crustose	NA	527	NA	NA	NA	NA
Gomphillus americanus	Gomphilaceae	Coccoid	NA	Crustose	Bark	564	0.0076	NA	0.0064	NA
Gomphillus calycioides	Gomphilaceae	Coccoid	Sexual	Crustose	Bark	1515	0.0433	NA	0.0458	NA
Graphis scripta	Graphidaceae	Trentepohlioid	Sexual	Crustose	Bark	564	0.0085	NA	0.0105	NA
Gyalecta farlowii	Gyalectaceae	Trentepohlioid	Sexual	Crustose	CR	237	0.0005	NA	0.0002	NA
Gyalideopsis bartramiorum	Gomphilaceae	Coccoid	NA	Crustose	Bark	285	0.0000	NA	0.0000	NA
Halecania pepegospora	Leprocaulaceae?	Coccoid	Both	Crustose	NCR	353	0.0231	NA	0.0221	NA
Herteliana schuyleriana	Squamarinaceae	Coccoid	Asexual	Crustose	NCR	1554	0.0288	NA	0.0306	NA
Heterodermia albicans	Physciaceae	Coccoid	Asexual	Foliose	Bark	223	0.0045	NA	0.0044	NA
Heterodermia appalachensis	Physciaceae	Coccoid	Asexual	Foliose	Bark	558	0.0159	NA	0.0101	NA
Heterodermia appalachensis	Physciaceae	Coccoid	Asexual	Foliose	Bark	980	0.0030	NA	0.0024	NA
Heterodermia casarettiana	Physciaceae	Coccoid	Asexual	Foliose	Bark	671	0.0031	NA	0.0028	NA
Heterodermia casarettiana	Physciaceae	Coccoid	Asexual	Foliose	NCR	351	NA	NA	NA	NA
Heterodermia crocea	Physciaceae	Coccoid	Asexual	Foliose	Bark		NA	NA	NA	NA
Heterodermia echinata	Physciaceae	Coccoid	Sexual	Foliose	Bark	233	0.0075	NA	0.0042	NA
Heterodermia granulifera	Chrysotrichaceae	Coccoid	Asexual	Foliose	Bark	237	NA	NA	NA	NA
Heterodermia hypoleuca	Physciaceae	Coccoid	Sexual	Foliose	Bark	1036	0.0065	NA	0.0041	NA
Heterodermia neglecta	Physciaceae	Coccoid	Asexual	Foliose	Bark	957	0.0099	NA	0.0092	NA
Heterodermia obscurata	Physciaceae	Coccoid	Asexual	Foliose	Bark		NA	NA	NA	NA
Heterodermia speciosa	Physciaceae	Coccoid	Asexual	Foliose	Bark	558	0.0016	NA	0.0002	NA
Heterodermia speciosa	Physciaceae	Coccoid	Asexual	Foliose	Bark	223	0.0077	NA	0.0065	NA
Heterodermia squamulosa	Physciaceae	Coccoid	Asexual	Foliose	Bark	1311	0.0030	NA	0.0015	NA
Hypocenomyce scalaris	Ophioparmaceae	Coccoid	Both	Foliose	Wood	1609	0.0030	NA	0.0028	NA
Hypogymnia incurvoides	Parmeliaceae	Coccoid	Both	Foliose	Bark	2015	0.0147	NA	0.0135	NA
Hypogymnia krogiae	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	2015	0.0193	NA	0.0193	NA
Hypogymnia vittata	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	2015	0.0327	NA	0.0326	NA
Hypotrachyna catawbiensis	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	1865	0.0122	NA	0.0102	NA
Hypotrachyna lividescens	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	1347	0.0032	NA	0.0026	NA
Hypotrachyna minarum	Parmeliaceae	Coccoid	Both	Foliose	Bark	564	0.0155	NA	0.0131	NA
Hypotrachyna minarum	Parmeliaceae	Coccoid	Both	Foliose	Bark	1311	0.0076	NA	0.0077	NA
Hypotrachyna osseoalba	Parmeliaceae	Coccoid	Both	Foliose	Bark	285	0.0060	NA	0.0051	NA
Icmadophila ericetorum	Baeomycetaceae	Coccoid	Sexual	Crustose	Humus	2015	0.0019	NA	0.0011	NA
Immersaria athroocarpa	???	Coccoid	Sexual	Crustose	NCR	1554	0.0138	NA	0.0066	NA
Imshaugia aleurites	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	1865	0.0006	NA	0.0004	NA
Ionaspis alba	Hymeneliaceae	Coccoid	Sexual	Crustose	NCR	1387	0.0283	NA	0.0222	NA
Ionaspis lacustris	Hymeneliaceae	Coccoid	Sexual	Crustose	NCR	353	0.0017	NA	0.0008	NA
Kephartia crystalligera	???	Coccoid	Sexual	Crustose	CR	233	0.0101	NA	0.0060	NA
Lasallia papulosa	Umbilicariaceae	Coccoid	Sexual	Foliose	NCR	1096	0.0070	NA	0.0084	NA

Current Determination	Family	Photobiont	Reprod, Mode	Growth Form	Substrate	Elevation (m)	θ_{Algal}	θ_{Cyano}	π_{Algal}	π_{Cyano}
Lasallia pensylvanica	Umbilicariaceae	Coccoid	Sexual	Foliose	NCR	1554	NA	NA	NA	NA
Lecania croatica	Ramalinaceae s. str.	Coccoid	Both	Crustose	Bark	604	NA	NA	NA	NA
Lecanora albella	Lecanoraceae	Coccoid	Sexual	Crustose	Bark	1554	0.0188	NA	0.0202	NA
Lecanora cinereofusca	Lecanoraceae	Coccoid	Sexual	Crustose	Bark	564	0.0054	NA	0.0045	NA
Lecanora hybocarpa	Lecanoraceae	Coccoid	Sexual	Crustose	Bark	1640	0.0207	NA	0.0206	NA
Lecanora markjohnstonii	Lecanoraceae	Coccoid	Asexual	Crustose	NCR	342	0.0132	NA	0.0129	NA
Lecanora masana	Lecanoraceae	Coccoid	Sexual	Crustose	Bark	2015	0.0193	NA	0.0121	NA
Lecanora nothocaesiella	Lecanoraceae	Coccoid	Both	Crustose	Bark	991	0.0000	NA	0.0000	NA
Lecanora oreinoides	Lecanoraceae	Coccoid	Sexual	Crustose	NCR	1096	0.0175	NA	0.0155	NA
Lecanora rugosella	Lecanoraceae	Coccoid	Sexual	Crustose	Bark	2015	0.0192	NA	0.0168	NA
Lecanora saxigena	Lecanoraceae	Coccoid	Sexual	Crustose	NCR	237	0.0002	NA	0.0002	NA
Lecanora strobilina	Lecanoraceae	Coccoid	Sexual	Crustose	Bark	570	0.0142	NA	0.0122	NA
Lecanora thysanophora	Lecanoraceae	Coccoid	Asexual	Crustose	Bark	564	NA	NA	NA	NA
Lecidea nylanderi	Lecideaceae	Coccoid	Asexual	Crustose	Bark	1609	NA	NA	NA	NA
Lecidea roseotincta	Lecideaceae	Coccoid	Sexual	Crustose	Bark	2015	0.0321	NA	0.0360	NA
Lecidea tessellata	Lecideaceae	Coccoid	Sexual	Crustose	NCR	1554	0.0148	NA	0.0146	NA
Lecidea tessellata	Lecideaceae	Coccoid	Sexual	Crustose	NCR	1096	0.0205	NA	0.0198	NA
Lecidella	Lecanoraceae	Coccoid	NA	Crustose	NA	1865	0.0289	NA	0.0272	NA
Lepra amara	Variolariaceae	Coccoid	Sexual	Crustose	Bark	564	0.0188	NA	0.0191	NA
Lepra amara	Variolariaceae	Coccoid	Sexual	Crustose	Bark	957	0.0096	NA	0.0081	NA
Lepra andersoniae	Variolariaceae	Coccoid	Both	Crustose	NCR	1122	0.0132	NA	0.0102	NA
Lepra pustulata	Variolariaceae	Coccoid	Both	Crustose	Bark	564	0.0294	NA	0.0324	NA
Lepra pustulata	Variolariaceae	Coccoid	Both	Crustose	Bark	1347	0.0068	NA	0.0072	NA
Lepra trachythallina	Variolariaceae	Coccoid	Sexual	Crustose	Bark	2015	0.0182	NA	0.0199	NA
Lepraria caesiella	Stereocaulaceae	Coccoid	Asexual	Crustose	Bark	1673	0.0047	NA	0.0034	NA
Lepraria disjuncta	Stereocaulaceae	Coccoid	Asexual	Crustose	CR	233	0.0378	NA	0.0399	NA
Lepraria finkii	Stereocaulaceae	Coccoid	Asexual	Crustose	Bark	207	0.0162	NA	0.0119	NA
Lepraria friabilis	Stereocaulaceae	Coccoid	Asexual	Crustose	Bark		0.0152	NA	0.0116	NA
Lepraria leprolomopsis	Stereocaulaceae	Coccoid	Asexual	Crustose	NCR	207	0.0502	NA	0.0502	NA
Lepraria normandinoides	Stereocaulaceae	Coccoid	Asexual	Crustose	Bark	991	0.0099	NA	0.0106	NA
Lepraria oxybapha	Stereocaulaceae	Coccoid	Asexual	Crustose	Bark	1609	0.0107	NA	0.0108	NA
Lepraria xanthonica	Leprocaulaceae?	Coccoid	Asexual	Crustose	Bark	671	NA	NA	NA	NA
Leptogium austroamericanum	Collemataceae	Cyanobacterium	Asexual	Foliose	Bark	527	NA	0.0186	NA	0.0081
Leptogium chloromelum	Collemataceae	Cyanobacterium	Sexual	Foliose	Bark	558	NA	0.0081	NA	0.0025
Leptogium corticola	Collemataceae	Cyanobacterium	Sexual	Foliose	Bark	564	NA	0.0031	NA	0.0012
Leptogium corticola	Collemataceae	Cyanobacterium	Sexual	Foliose	Bark	1311	NA	0.0024	NA	0.0015
Leptogium corticola	Collemataceae	Cyanobacterium	Sexual	Foliose	Bark	342	NA	0.0273	NA	0.0248
Leptogium cyanescens	Collemataceae	Cyanobacterium	Both	Foliose	Bark	671	NA	0.0006	NA	0.0003
Leptogium cyanescens	Collemataceae	Cyanobacterium	Both	Foliose	Bark	274	NA	NA	NA	NA
Leptogium hirsutum	Collemataceae	Cyanobacterium	Both	Foliose	Bark	558	NA	0.0094	NA	0.0050
Leptogium hirsutum	Collemataceae	Cyanobacterium	Both	Foliose	Bark	342	NA	0.0081	NA	0.0044
Leucodecton	Graphidaceae	Trentepohlioid	NA	Crustose	NA	274	0.0122	NA	0.0100	NA
Leucodecton subcompunctum	Graphidaceae	Trentepohlioid	Sexual	Crustose	Bark	NA	NA	NA	NA	NA
Lobaria pulmonaria	Lobariaceae	Polymorphic	Both	Foliose	Bark	671	0.0059	NA	0.0049	NA
Lobaria quercizans	Lobariaceae	Polymorphic	Sexual	Foliose	Bark	604	0.0423	NA	0.0433	NA
Lobaria ravanelii	Lobariaceae	Polymorphic	Sexual	Foliose	Bark		0.0249	NA	0.0231	NA
Lopadium disciforme	Physciaceae	Coccoid	Sexual	Crustose	Bark	1865	0.0367	NA	0.0369	NA
Loxospora elatina	Loxosporaceae	Coccoid	Both	Crustose	Bark	1609	0.0118	NA	0.0129	NA
Loxospora ochrophaea	Loxosporaceae	Coccoid	Sexual	Crustose	Bark	2015	0.0607	NA	0.0624	NA
Megalospora porphyritis	Megalosporaceae	Coccoid	Both	Crustose	Bark	604	0.0434	NA	0.0451	NA
Melanelia culbersonii	Parmeliaceae	Coccoid	Asexual	Foliose	NCR	1387	0.0157	NA	0.0155	NA
Melanelia stygia	Parmeliaceae	Coccoid	Sexual	Foliose	NCR	1554	0.0221	NA	0.0227	NA
Melanohalea halei	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	2015	0.0180	NA	0.0181	NA
Melanohalea halei	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	1673	NA	NA	NA	NA
Menegazzia subsimilis	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	564	NA	NA	NA	NA
Micarea neostipitata	Pilocarpaceae	Coccoid	Sexual	Crustose	Bark	570	0.0262	NA	0.0255	NA
Micarea peliocarpa	Pilocarpaceae	Coccoid	Sexual	Crustose	Bark	207	NA	NA	NA	NA
Micareopsis irriguata	???	Coccoid	Both	Crustose	NCR	351	0.0139	NA	0.0136	NA
Micareopsis irriguata	???	Coccoid	Both	Crustose	NCR	207	NA	NA	NA	NA
Mycoblastus sanguinarioides	Mycoblastaceae	Coccoid	Sexual	Crustose	Bark	2015	0.0481	NA	0.0504	NA
Mycoblimbia	Ramalinaceae s. str.	Coccoid	NA	Crustose	NA	604	0.0375	NA	0.0378	NA
Myelochroa obsessa	Parmeliaceae	Coccoid	Both	Foliose	NCR	237	0.0189	NA	0.0202	NA
Nadvornikia sorediata	Graphidaceae	Trentepohlioid	Asexual	Crustose	Bark	233	0.0302	NA	0.0320	NA
Nephroma helveticum	Nephromataceae	Cyanobacterium	Sexual	Foliose	Bark	671	NA	0.0086	NA	0.0036
Nephroma helveticum	Nephromataceae	Cyanobacterium	Sexual	Foliose	Bark	1552	NA	0.0017	NA	0.0004
Nigrovothelium tropicum	Trypetheliaceae	Trentepohlioid	Sexual	Crustose	Bark	372	0.0640	NA	0.0638	NA
Ochrolechia arborea	Ochrolechiaceae	Coccoid	Asexual	Crustose	Bark	1554	0.0208	NA	0.0141	NA
Ochrolechia trochophora	Ochrolechiaceae	Coccoid	Sexual	Crustose	Bark	991	0.0125	NA	0.0123	NA
Ochrolechia yasudae	Ochrolechiaceae	Coccoid	Both	Crustose	NCR	1387	NA	NA	NA	NA
Opegrapha moroziana	Opegraphaceae	Trentepohlioid	Both	Crustose	NCR	274	0.0126	NA	0.0060	NA
Opegrapha varia	Opegraphaceae	Trentepohlioid	Sexual	Crustose	Bark	564	NA	NA	NA	NA
Opegrapha viridis	Opegraphaceae	Trentepohlioid	Sexual	Crustose	Bark	558	0.0249	NA	0.0284	NA
Opegrapha vulgata	Opegraphaceae	Trentepohlioid	Sexual	Crustose	Bark	564	0.0427	NA	0.0431	NA
Pannaria subfusca	Parmeliaceae	Cyanobacterium	Sexual	Foliose	Bark	1067	NA	NA	NA	NA
Pannaria tavaresii	Pannariaceae	Cyanobacterium	Both	Foliose	Bark	353	NA	0.0056	NA	0.0024
Parmelia squarrosa	Parmeliaceae	Coccoid	Both	Foliose	Bark	671	0.0156	NA	0.0154	NA
Parmelia squarrosa	Parmeliaceae	Coccoid	Both	Foliose	Bark	1347	NA	NA	NA	NA
Parmelia sulcata	Parmeliaceae	Coccoid	Both	Foliose	Bark	1463	NA	NA	NA	NA
Parmotrema arnoldii	Parmeliaceae	Coccoid	Both	Foliose	Bark	233	0.0035	NA	0.0024	NA
Parmotrema austrosinense	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	205	0.0190	NA	0.0192	NA
Parmotrema cetratum	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	558	0.0121	NA	0.0107	NA
Parmotrema cetratum	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	223	0.0000	NA	0.0000	NA
Parmotrema crinitum	Parmeliaceae	Coccoid	Both	Foliose	Bark	1609	0.0036	NA	0.0011	NA
Parmotrema crinitum	Parmeliaceae	Coccoid	Both	Foliose	Bark	1311	0.0378	NA	0.0384	NA
Parmotrema diffractaicum	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	671	0.0108	NA	0.0110	NA
Parmotrema gardneri	Parmeliaceae	Coccoid	Both	Foliose	Bark	570	0.0425	NA	0.0413	NA
Parmotrema hypotropum	Parmeliaceae	Coccoid	Both	Foliose	Bark	570	0.0198	NA	0.0129	NA
Parmotrema hypotropum	Parmeliaceae	Coccoid	Both	Foliose	Bark	980	0.0241	NA	0.0229	NA
Parmotrema internexum	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	233	0.0003	NA	0.0002	NA
Parmotrema margaritatum	Parmeliaceae	Coccoid	Both	Foliose	Bark	690	0.0552	NA	0.0552	NA
Parmotrema mellissii	Parmeliaceae	Coccoid	Both	Foliose	Bark	1067	NA	NA	NA	NA

Current Determination	Family	Photobiont	Reprod, Mode	Growth Form	Substrate	Elevation (m)	θ_{Algal}	θ_{Cyano}	π_{Algal}	π_{Cyano}
Parmotrema mellissii	Parmeliaceae	Coccoid	Both	Foliose	Bark	991	0.0242	NA	0.0248	NA
Parmotrema neotropicum	Parmeliaceae	Coccoid	Both	Foliose	Bark	1071	0.0042	NA	0.0046	NA
Parmotrema neotropicum	Parmeliaceae	Coccoid	Both	Foliose	Bark	353	0.0004	NA	0.0001	NA
Parmotrema perlatum	Parmeliaceae	Coccoid	Both	Foliose	Bark	1311	0.0030	NA	0.0028	NA
Parmotrema rampoddense	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	NA	0.0072	NA	0.0074	NA
Parmotrema rampoddense	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	207	0.0066	NA	0.0054	NA
Parmotrema reticulatum	Parmeliaceae	Coccoid	Both	Foliose	Bark	1219	0.0040	NA	0.0030	NA
Parmotrema reticulatum	Parmeliaceae	Coccoid	Both	Foliose	Bark	1347	0.0141	NA	0.0140	NA
Parmotrema simulans	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	991	0.0049	NA	0.0048	NA
Parmotrema stuppeum	Parmeliaceae	Coccoid	Both	Foliose	Bark	1625	0.0145	NA	0.0115	NA
Parmotrema subisidiosum	Parmeliaceae	Coccoid	Both	Foliose	Bark	305	NA	NA	NA	NA
Parmotrema submarginale	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	274	0.0095	NA	0.0097	NA
Parmotrema subsumptum	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	980	0.0039	NA	0.0034	NA
Parmotrema tinctorum	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	237	0.0000	NA	0.0000	NA
Parmotrema ultralucens	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	353	0.0171	NA	0.0162	NA
Parmotrema xanthinum	Parmeliaceae	Coccoid	Both	Foliose	Bark	527	0.0108	NA	0.0056	NA
Peltigera	Peltigeraceae	Cyanobacterium	NA	Foliose	NA	558	NA	0.0058	NA	0.0047
Peltigera neckeri	Peltigeraceae	Cyanobacterium	Both	Foliose	Humus	207	NA	0.0097	NA	0.0085
Peltigera neopolydactyla	Peltigeraceae	Cyanobacterium	Sexual	Foliose	Humus	558	NA	NA	NA	NA
Peltigera phyllidiosa	Peltigeraceae	Cyanobacterium	Both	Foliose	Bark	1225	NA	0.0037	NA	0.0037
Peltigera phyllidiosa	Peltigeraceae	Cyanobacterium	Both	Foliose	Bark	233	NA	0.0120	NA	0.0120
Peltigera praetextata	Peltigeraceae	Cyanobacterium	Both	Foliose	Humus	564	NA	0.0000	NA	0.0000
Peltigera praetextata	Peltigeraceae	Cyanobacterium	Both	Foliose	Humus	233	NA	0.0018	NA	0.0006
Pertusaria epixantha	Pertusariaceae	Coccoid	Sexual	Crustose	Bark	378	0.0203	NA	0.0213	NA
Pertusaria epixantha	Pertusariaceae	Coccoid	Sexual	Crustose	Bark	NA	NA	NA	NA	NA
Pertusaria macounii	Pertusariaceae	Coccoid	Sexual	Crustose	Bark	564	0.0071	NA	0.0061	NA
Pertusaria obruta	Pertusariaceae	Coccoid	Sexual	Crustose	Bark	223	0.0211	NA	0.0211	NA
Pertusaria ostiolata	Pertusariaceae	Coccoid	Sexual	Crustose	Bark	558	0.0083	NA	0.0089	NA
Pertusaria paratuberculifera	Pertusariaceae	Coccoid	Sexual	Crustose	Bark	671	0.0086	NA	0.0073	NA
Pertusaria plittiana	Pertusariaceae	Coccoid	Sexual	Crustose	NCR	1423	0.0031	NA	0.0043	NA
Pertusaria plittiana	Pertusariaceae	Coccoid	Sexual	Crustose	NCR	237	0.0067	NA	0.0061	NA
Pertusaria propinqua	Pertusariaceae	Coccoid	Sexual	Crustose	Bark	957	0.0019	NA	0.0019	NA
Pertusaria rubefacta	Pertusariaceae	Coccoid	Sexual	Crustose	Bark	1423	NA	NA	NA	NA
Pertusaria subpertusa	Pertusariaceae	Coccoid	Sexual	Crustose	Bark	671	0.0138	NA	0.0133	NA
Phaeophyscia adiastola	Physciaceae	Coccoid	Both	Foliose	NCR	274	0.0274	NA	0.0256	NA
Phaeophyscia hispidula	Physciaceae	Coccoid	NA	Foliose	NA	1673	0.0156	NA	0.0155	NA
Phaeophyscia rubropulchra	Physciaceae	Coccoid	Both	Foliose	Bark	1463	0.0142	NA	0.0170	NA
Phaeophyscia rubropulchra	Physciaceae	Coccoid	Both	Foliose	Bark	342	0.0252	NA	0.0267	NA
Phaeophyscia squarrosa	Physciaceae	Coccoid	Asexual	Foliose	Bark	558	0.0154	NA	0.0151	NA
Phlyctis boliviensis	Phlyctidaceae	Coccoid	NA	Crustose	Bark	564	0.0057	NA	0.0041	NA
Phlyctis boliviensis	Phlyctidaceae	Coccoid	NA	Crustose	Bark	1463	NA	NA	NA	NA
Phlyctis boliviensis	Phlyctidaceae	Coccoid	NA	Crustose	Bark	233	0.0119	NA	0.0112	NA
Phlyctis petraea	Phlyctidaceae	Coccoid	Asexual	Crustose	NCR	233	NA	NA	NA	NA
Phlyctis speirea	Phlyctidaceae	Coccoid	Sexual	Crustose	Bark	1673	0.0160	NA	0.0126	NA
Phyllopsora corallina	Ramalinaceae s. str.	Coccoid	Both	Foliose	Bark	564	0.0531	NA	0.0527	NA
Phyllopsora corallina	Ramalinaceae s. str.	Coccoid	Both	Foliose	Bark	274	0.0576	NA	0.0593	NA
Phyllopsora parvifolia	Ramalinaceae s. str.	Coccoid	Both	Foliose	Bark	274	0.0114	NA	0.0116	NA
Physcia americana	Physciaceae	Coccoid	Both	Foliose	Bark	980	NA	NA	NA	NA
Physconia leucoleiptes	Physciaceae	Coccoid	Asexual	Foliose	Bark	690	NA	NA	NA	NA
Physconia subpallida	Physciaceae	Coccoid	NA	Foliose	NA	933	NA	NA	NA	NA
Pilophorus fibula	Cladoniaceae	Coccoid	Sexual	Polymorphic	NCR	NA	0.0290	NA	0.0291	NA
Placidium arboreum	Pyrenulaceae	Coccoid	Sexual	Foliose	Bark	980	NA	NA	NA	NA
Placidium arboreum	Verrucariaceae	Coccoid	Sexual	Foliose	Bark	223	0.0165	NA	0.0139	NA
Placynthium petersii	Placynthiaceae	Cyanobacterium	Sexual	Foliose	CR	351	NA	0.0571	NA	0.0419
Platismatia glauca	Parmeliaceae	Coccoid	Both	Foliose	Bark	2015	0.0061	NA	0.0040	NA
Platismatia tuckermanii	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	2015	0.0130	NA	0.0127	NA
Platismatia tuckermanii	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	1347	0.0168	NA	0.0175	NA
Polysporina simplex	Acarosporaceae	Coccoid	Sexual	Crustose	NCR	1991	0.0060	NA	0.0067	NA
Porina heterospora	Porinaceae	Trentepohlioid	Sexual	Crustose	Bark	527	NA	NA	NA	NA
Porina heterospora	Porinaceae	Trentepohlioid	Sexual	Crustose	Bark	274	NA	NA	NA	NA
Porina scabrida	Porinaceae	Trentepohlioid	Both	Crustose	Bark	980	NA	NA	NA	NA
Porina scabrida	Porinaceae	Trentepohlioid	Both	Crustose	Bark	237	NA	NA	NA	NA
Porpidia albocaerulescens	Lecideaceae	Coccoid	Sexual	Crustose	NCR	353	0.0062	NA	0.0062	NA
Porpidia contraponenda	Lecideaceae	Coccoid	Sexual	Crustose	NCR	1991	0.0158	NA	0.0130	NA
Porpidia crustulata	Lecideaceae	Coccoid	Sexual	Crustose	NCR	1920	0.0259	NA	0.0200	NA
Porpidia macrocarpa	Lecideaceae	Coccoid	Sexual	Crustose	NCR	960	0.0395	NA	0.0419	NA
Porpidia subsimplex	Lecideaceae	Coccoid	Sexual	Crustose	NCR	353	0.0028	NA	0.0020	NA
Protoblastenia rupestris	Psoraceae	Coccoid	Sexual	Crustose	CR	233	0.0100	NA	0.0105	NA
Pseudevernia cladonia	Parmeliaceae	Coccoid	Asexual	Foliose	Bark	2015	0.0140	NA	0.0135	NA
Pseudocyphellaria holarctica	Lobariaceae	Cyanobacterium	Asexual	Foliose	Bark	604	NA	0.0000	NA	0.0000
Pseudosagedia cestrensis	Porinaceae	Trentepohlioid	Sexual	Crustose	Bark	237	NA	NA	NA	NA
Pseudosagedia chlorotica	Porinaceae	Trentepohlioid	Sexual	Crustose	NCR	237	0.0426	NA	0.0482	NA
Pseudosagedia isidiata	Porinaceae	Trentepohlioid	Asexual	Crustose	Bark	671	0.0264	NA	0.0258	NA
Pseudosagedia rhaphidosperma	Porinaceae	Trentepohlioid	Sexual	Crustose	Bark	564	0.0367	NA	0.0407	NA
Punctelia appalachensis	Parmeliaceae	Coccoid	Both	Foliose	Bark	1625	0.0053	NA	0.0050	NA
Punctelia caseana	Parmeliaceae	Coccoid	Both	Foliose	Bark	285	0.0062	NA	0.0020	NA
Pyrenula leucostoma	Pyrenulaceae	Trentepohlioid	Sexual	Crustose	Bark	564	0.0283	NA	0.0291	NA
Pyrenula pseudobufonia	Pyrenulaceae	Trentepohlioid	Sexual	Crustose	Bark	564	0.0101	NA	0.0096	NA
Pyrenula pseudobufonia	Pyrenulaceae	Trentepohlioid	Sexual	Crustose	Bark	960	0.0219	NA	0.0221	NA
Pyrenula santensis	Pyrenulaceae	Trentepohlioid	Sexual	Crustose	Bark	NA	0.0339	NA	0.0362	NA
Pyrenula subelliptica	Pyrenulaceae	Trentepohlioid	Sexual	Crustose	Bark	960	0.0243	NA	0.0217	NA
Pyrrhospora varians	Lecanoraceae	Coccoid	Sexual	Crustose	Bark	980	0.0221	NA	0.0212	NA
Pyrrhospora varians	Lecanoraceae	Coccoid	Sexual	Crustose	Bark	368	0.0134	NA	0.0134	NA
Pyxine albovirens	Caliciaceae	Coccoid	Asexual	Foliose	Bark	237	NA	NA	NA	NA
Pyxine sorediata	Caliciaceae	Coccoid	Both	Foliose	Bark	671	0.0063	NA	0.0060	NA
Ramalina americana	Ramalinaceae s. str.	Coccoid	Sexual	Fruticose	Bark	1347	0.0016	NA	0.0020	NA
Ramalina culbersoniorum	Ramalinaceae s. str.	Coccoid	Sexual	Fruticose	Bark	1311	0.0005	NA	0.0005	NA
Ramalina intermedia	Physciaceae	Coccoid	Asexual	Fruticose	NCR	1494	0.0083	NA	0.0063	NA
Ramalina intermedia	Ramalinaceae s. str.	Coccoid	Asexual	Fruticose	NCR	274	0.0028	NA	0.0019	NA
Ramboldia blochiana	Unknown	Coccoid	Asexual	Crustose	Wood		0.0127	NA	0.0112	NA
Rhizocarpon geographicum	Rhizocarpaceae	Coccoid	Sexual	Crustose	NCR	1785	0.0121	NA	0.0103	NA

Current Determination	Family	Photobiont	Reprod, Mode	Growth Form	Substrate	Elevation (m)	θ_{Algal}	θ_{Cyano}	π_{Algal}	π_{Cyano}
Rhizocarpon subgeminatum	Rhizocarpaceae	Coccoid	Sexual	Crustose	NCR	1554	0.0275	NA	0.0266	NA
Rinodina adirondackii	Physciaceae	Coccoid	Sexual	Crustose	Bark	564	0.0101	NA	0.0103	NA
Rinodina ascociscana	Physciaceae	Coccoid	Sexual	Crustose	Bark	1509	0.0500	NA	0.0505	NA
Rinodina brauniana	Physciaceae	Coccoid	Both	Crustose	Bark	285	0.0089	NA	0.0076	NA
Rinodina buckii	Physciaceae	Coccoid	Both	Crustose	Bark	671	NA	NA	NA	NA
Rinodina chrysidiata	Physciaceae	Coccoid	Both	Crustose	Bark	1219	0.0356	NA	0.0360	NA
Rinodina dolichospora	Physciaceae	Coccoid	Sexual	Crustose	Bark	939	0.0180	NA	0.0172	NA
Rinodina tephraspis	Physciaceae	Coccoid	Sexual	Crustose	NCR	914	0.0127	NA	0.0151	NA
Rockerfellera crossophylla	Pannariaceae	Cyanobacterium	Sexual	Foliose	NCR		NA	NA	NA	NA
Ropalospora chlorantha	Fuscideaceae	Coccoid	Sexual	Crustose	Bark	2015	0.0345	NA	0.0353	NA
Schismatomma glaucescens	Roccellaceae	Trentepohlioid	Sexual	Crustose	Bark	527	NA	NA	NA	NA
Scoliciosporum umbrinum	Scoliciosporaceae	Coccoid	Sexual	Crustose	NCR	1265	0.0380	NA	0.0449	NA
Scytinium dactylinum	Collemataceae	Cyanobacterium	Sexual	Foliose	Bark	207	NA	0.0291	NA	0.0261
Scytinium lichenoides	Collemataceae	Cyanobacterium	Sexual	Foliose	CR	233	NA	0.0277	NA	0.0252
Sporodophoron americanum	Roccellaceae	Trentepohlioid	NA	Crustose	Bark	342	NA	NA	NA	NA
ssc	???	Coccoid	NA	Crustose	NA	1096	0.0571	NA	0.0569	NA
ssc	???	Coccoid	NA	Crustose	NA	991	0.0065	NA	0.0053	NA
Stereocaulon dactylophyllum	Stereocaulaceae	Polymorphic	Sexual	Fruticose	NCR	1991	0.0468	NA	0.0479	NA
Sterile sorediate crust	???	Coccoid	NA	Crustose	NA	237	0.0041	NA	0.0035	NA
Sticta	Lobariaceae	Cyanobacterium	NA	Foliose	NA	1399	NA	0.0045	NA	0.0011
Sticta beauvoisii	Lobariaceae	Cyanobacterium	Asexual	Foliose	Bark	671	NA	NA	NA	NA
Sticta carolinensis	Lobariaceae	Cyanobacterium	Asexual	Foliose	Bark	274	NA	0.0009	NA	0.0005
Sticta deyana	Lobariaceae	Cyanobacterium	NA	Foliose	NA		0.0618	NA	0.0596	NA
Sticta fragilinata	Lobariaceae	Cyanobacterium	Asexual	Foliose	Bark	957	NA	0.0000	NA	0.0000
Tephromela atra	Tephromelaceae	Coccoid	Sexual	Crustose	Bark	1554	0.0159	NA	0.0146	NA
Thelotrema defectum	Graphidaceae	Trentepohlioid	Sexual	Crustose	Bark	353	0.0155	NA	0.0084	NA
Thelotrema subtile	Graphidaceae	Trentepohlioid	Sexual	Crustose	Bark		0.0154	NA	0.0175	NA
Thelotrema subtile	Graphidaceae	Trentepohlioid	Sexual	Crustose	Bark	342	0.0003	NA	0.0001	NA
Trapelia coarctata	Trapeliaceae	Coccoid	Sexual	Crustose	NCR	671	0.0081	NA	0.0052	NA
Trapelia placodioides	Trapeliaceae	Coccoid	Both	Crustose	NCR	368	0.0072	NA	0.0045	NA
Trapeliopsis flexuosa	Trapeliaceae	Coccoid	Both	Crustose	Wood	1991	0.0267	NA	0.0283	NA
Trapeliopsis viridescens	Trapeliaceae	Coccoid	Both	Crustose	Wood	1865	0.0198	NA	0.0181	NA
Trentepohlia sp	Unknown	NA	NA	NA	NA		0.0144	NA	0.0131	NA
Tuckermanopsis ciliaris	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	1265	0.0520	NA	0.0514	NA
Tuckermanopsis ciliaris	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	1347	0.0234	NA	0.0227	NA
Tuckermanopsis ciliaris	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	285	0.0191	NA	0.0192	NA
Umbilicaria mammulata	Umbilicariaceae	Coccoid	Sexual	Foliose	NCR	1423	0.0182	NA	0.0182	NA
Umbilicaria mammulata	Umbilicariaceae	Coccoid	Sexual	Foliose	NCR	1311	0.0063	NA	0.0060	NA
Usnea angulata	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	NA	NA	NA	NA	NA
Usnea ceratina	Parmeliaceae	Coccoid	Both	Fruticose	Bark	604	0.0021	NA	0.0011	NA
Usnea ceratina	Parmeliaceae	Coccoid	Both	Fruticose	Bark	1311	0.0075	NA	0.0063	NA
Usnea cornuta	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	604	0.0069	NA	0.0055	NA
Usnea fulvoreagens	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	1347	0.0015	NA	0.0004	NA
Usnea halei	Parmeliaceae	Coccoid	Asexual	Fruticose	NCR	1554	0.0029	NA	0.0015	NA
Usnea merrillii	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	1554	0.0030	NA	0.0009	NA
Usnea mutabilis	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	368	0.0181	NA	0.0170	NA
Usnea strigosa	Parmeliaceae	Coccoid	Sexual	Fruticose	Bark	1096	0.0205	NA	0.0212	NA
Usnea subfusca	Parmeliaceae	Coccoid	Sexual	Fruticose	Bark	1609	0.0313	NA	0.0324	NA
Usnea subgracilis	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	1311	0.0023	NA	0.0025	NA
Usnea subscabrosa	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	527	0.0049	NA	0.0013	NA
Usnea subscabrosa	Parmeliaceae	Coccoid	Asexual	Fruticose	Bark	NA	0.0065	NA	0.0055	NA
Usnocetraria oakesiana	Parmeliaceae	Coccoid	Both	Foliose	Bark	1865	0.0052	NA	0.0042	NA
Viridothelium virens	Trypetheliaceae	Trentepohlioid	NA	Crustose	NA	671	0.0330	NA	0.0329	NA
Viridothelium virens	Trypetheliaceae	Trentepohlioid	Sexual	Crustose	Bark	207	0.0239	NA	0.0255	NA
Vulpicida viridis	Parmeliaceae	Coccoid	Sexual	Foliose	Bark	NA	NA	NA	NA	NA
Willeya diffractella	Verrucariaceae	Coccoid	Sexual	Crustose	CR	351	0.0245	NA	0.0221	NA
Xanthoparmelia mexicana	Parmeliaceae	Coccoid	NA	Foliose	NA	1554	0.0196	NA	0.0196	NA
Xylographa trunciseda	Xylographaceae	Coccoid	Sexual	Crustose	Wood	1865	0.0088	NA	0.0082	NA
Xylographa vitiligo	Xylographaceae	Coccoid	Asexual	Crustose	Wood	2015	0.0338	NA	0.0338	NA
Xylopsora friesii	Unknown	Coccoid	Sexual	Foliose	Wood	NA	0.0094	NA	0.0063	NA

Table 5.2. P-values of $\theta_{Watterson}$ covariate comparisons. For categorical covariates, pairwise t-tests were performed. A linear regression was performed for the elevation p-values.

Algal Photobionts				
	Sexual	Both		
Asexual	0.1355	0.5652		
Both	0.4068			
Coccoid/ Trentepohlioid	0.1638			
Elevation	0.289			
Elevation^2	0.0109			
	Humus	Wood	Calcareous Rock	Non-calcareous Rock
Bark	0.9238	0.9873	0.4800	0.6567
Humus		0.9309	0.5535	0.8857
Wood			0.4968	0.7834
Calcareous Rock				0.5547
Cyanobacterial Photobionts				
	Sexual	Both		
Asexual	0.1131	0.8096		
Both	0.06159			
	Bark	Humus	Non-calcareous Rock	
Calcareous Rock	0.04711	0.03263	0.08772	
Bark		0.3496	0.0003646	
Humus			0.01646	
Elevation	0.01093			

Table 5.3. Parameters for the the PGLS model selection. BM=PGLS under Brownian motion. OU=PGLS under Ornstein-Uhlenbeck. TIPS=Non-phylogenetic ANOVA.

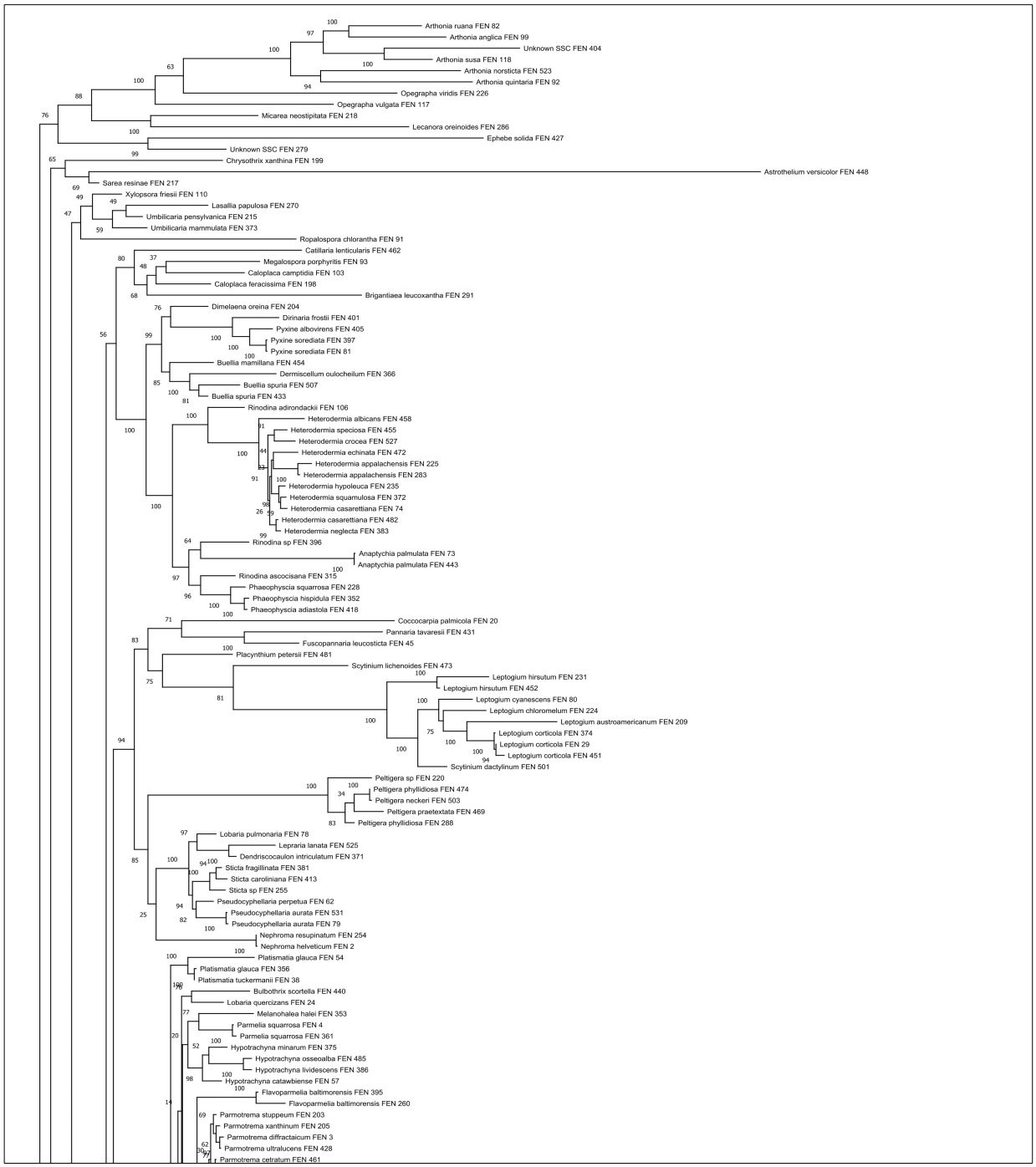
Growth Form vs Algal Diversity

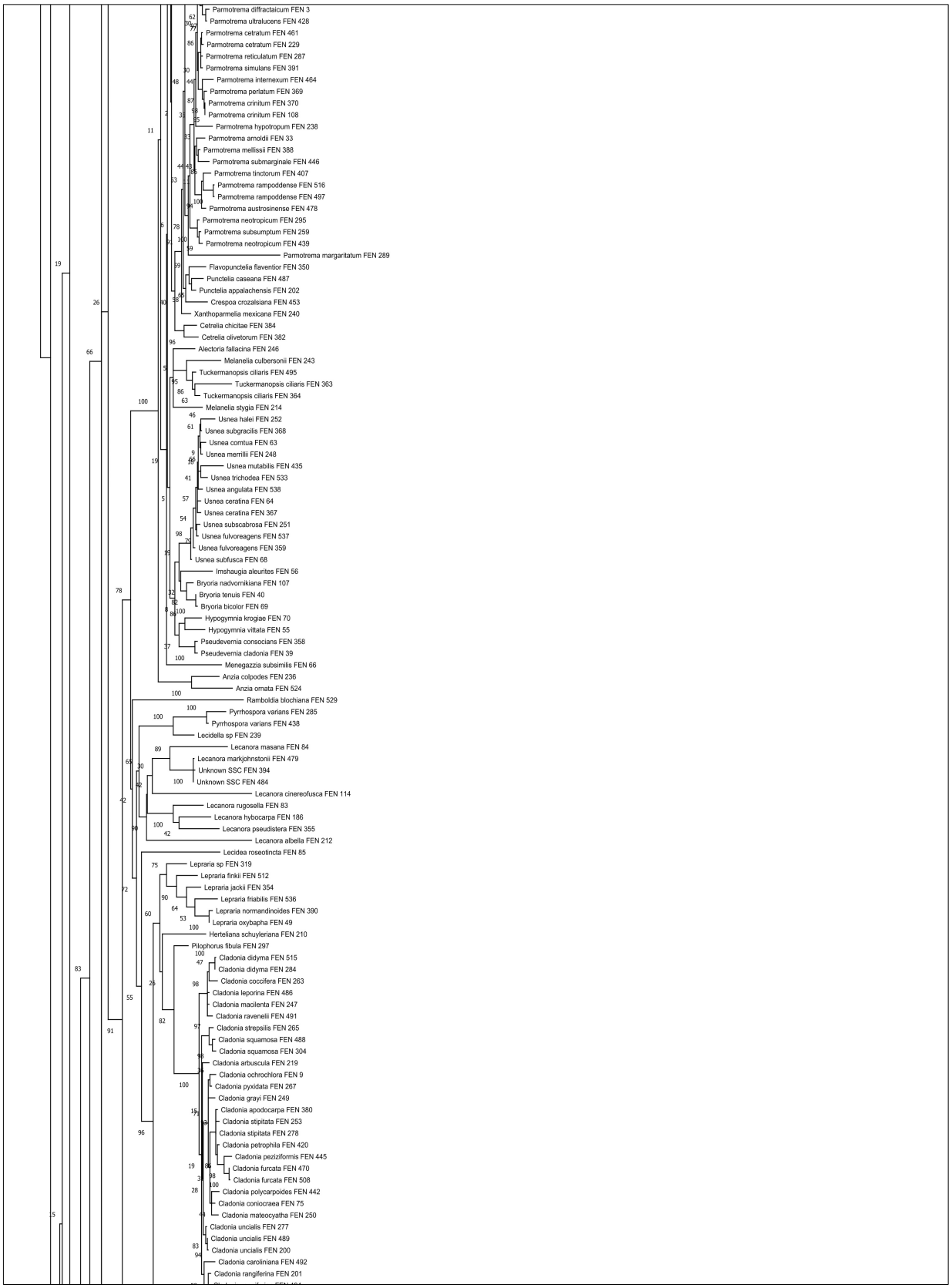
	BM	AIC = -775.2627	OU	AIC= -1144.99	TIPS	AIC= -1138.147
	Coefficient	P-Value	Coefficient	P-Value	Coefficient	P-Value
(intercept)	0.03046	0.9000	0.02027	0.0000	0.01925	0.0000
Foliose	0.00876	0.0518	-6.66E-03	0.0060	-0.00573	0.0115
Fruticose	-0.00494	0.3974	-1.33E-02	0.0035	-0.0125	0.0024
Polymorphic	-6.28E-05	0.9940	-0.0033	0.3155	-0.00234	0.4637
				α=5804.8		

Growth Form vs Cyanobacterial Diversity

	BM	AIC= -129.5674	OU	AIC= -130.5188	TIPS	AIC= -121.5585
	Coefficient	P-Value	Coefficient	P-Value	Coefficient	P-Value
(intercept)	0.01489	0.3720	0.01272	0.0925	0.0084	0.0037
Foliose	0.00895	0.0276	0.00895	0.0559	0.00758	0.5555
				α=2.4023		

Figure 5.S2. Phylogeny generated from the rDNA sequences of 297 species in this study. The tree was used to phylogenetically correct the correlations between growth form and photobiont community diversity.




Parmotrema diffractaicum FEN 3
Parmotrema ultralucens FEN 428
Parmotrema cetratum FEN 461
Parmotrema cetratum FEN 229
Parmotrema reticulatum FEN 287
Parmotrema simulans FEN 391
Parmotrema internexum FEN 464
Parmotrema perlatum FEN 369
Parmotrema crinitum FEN 370
Parmotrema crinitum FEN 108
Parmotrema hypotropum FEN 238
Parmotrema arnoldii FEN 33
Parmotrema mellissii FEN 388
Parmotrema submarginale FEN 446
Parmotrema tinctorum FEN 407
Parmotrema rampoddense FEN 516
Parmotrema rampoddense FEN 497
Parmotrema austrosinense FEN 478
Parmotrema neotropicum FEN 295
Parmotrema subsumptum FEN 259
Parmotrema neotropicum FEN 439
Parmotrema margaritatum FEN 289
Flavopunctelia flaventior FEN 350
Punctelia caseana FEN 487
Punctelia appalachensis FEN 202
Crespoa crozalsiana FEN 453
Xanthoparmelia mexicana FEN 240
Cetrelia chicitae FEN 384
Cetrelia olivetorum FEN 382
Alectoria fallacina FEN 246
Melanelia culbersonii FEN 243
Tuckermanopsis ciliaris FEN 495
Tuckermanopsis ciliaris FEN 363
Tuckermanopsis ciliaris FEN 364
Melanelia stygia FEN 214
Usnea halei FEN 252
Usnea subgracilis FEN 368
Usnea comtua FEN 63
Usnea merrillii FEN 248
Usnea mutabilis FEN 435
Usnea trichodea FEN 533
Usnea angulata FEN 538
Usnea ceratina FEN 64
Usnea ceratina FEN 367
Usnea subscabrosa FEN 251
Usnea fulvoreagens FEN 537
Usnea fulvoreagens FEN 359
Usnea subfusca FEN 68
Imshaugia aleurites FEN 56
Bryoria nadvornikiana FEN 107
Bryoria tenuis FEN 40
Bryoria bicolor FEN 69
Hypogymnia krogiae FEN 70
Hypogymnia vittata FEN 55
Pseudevernia consocians FEN 358
Pseudevernia cladonia FEN 39
Menegazzia subsimilis FEN 66
Anzia colpodes FEN 236
Anzia ornata FEN 524
Ramboldia blochiana FEN 529
Pyrrhospora varians FEN 285
Pyrrhospora varians FEN 438
Lecidella sp FEN 239
Lecanora masana FEN 84
Lecanora markjohnstonii FEN 479
Unknown SSC FEN 394
Unknown SSC FEN 484
Lecanora cinereofusca FEN 114
Lecanora rugosella FEN 83
Lecanora hybocarpa FEN 186
Lecanora pseudistera FEN 355
Lecanora albella FEN 212
Lecidea roseotincta FEN 85
Lepraria sp FEN 319
Lepraria finkii FEN 512
Lepraria jackii FEN 354
Lepraria friabilis FEN 536
Lepraria normandinoides FEN 390
Lepraria oxybapha FEN 49
Herteliana schuyleriana FEN 210
Pilophorus fibula FEN 297
Cladonia didyma FEN 515
Cladonia didyma FEN 284
Cladonia coccifera FEN 263
Cladonia leporina FEN 486
Cladonia macilenta FEN 247
Cladonia ravenelii FEN 491
Cladonia strepsilis FEN 265
Cladonia squamosa FEN 488
Cladonia squamosa FEN 304
Cladonia arbuscula FEN 219
Cladonia ochrochlora FEN 9
Cladonia pyxidata FEN 267
Cladonia grayi FEN 249
Cladonia apodocarpa FEN 380
Cladonia stipitata FEN 253
Cladonia stipitata FEN 278
Cladonia petrophila FEN 420
Cladonia peziziformis FEN 445
Cladonia furcata FEN 470
Cladonia furcata FEN 508
Cladonia polycarpoides FEN 442
Cladonia coniocraea FEN 75
Cladonia mateocyatha FEN 250
Cladonia uncialis FEN 277
Cladonia uncialis FEN 489
Cladonia uncialis FEN 200
Cladonia caroliniana FEN 492
Cladonia rangiferina FEN 201

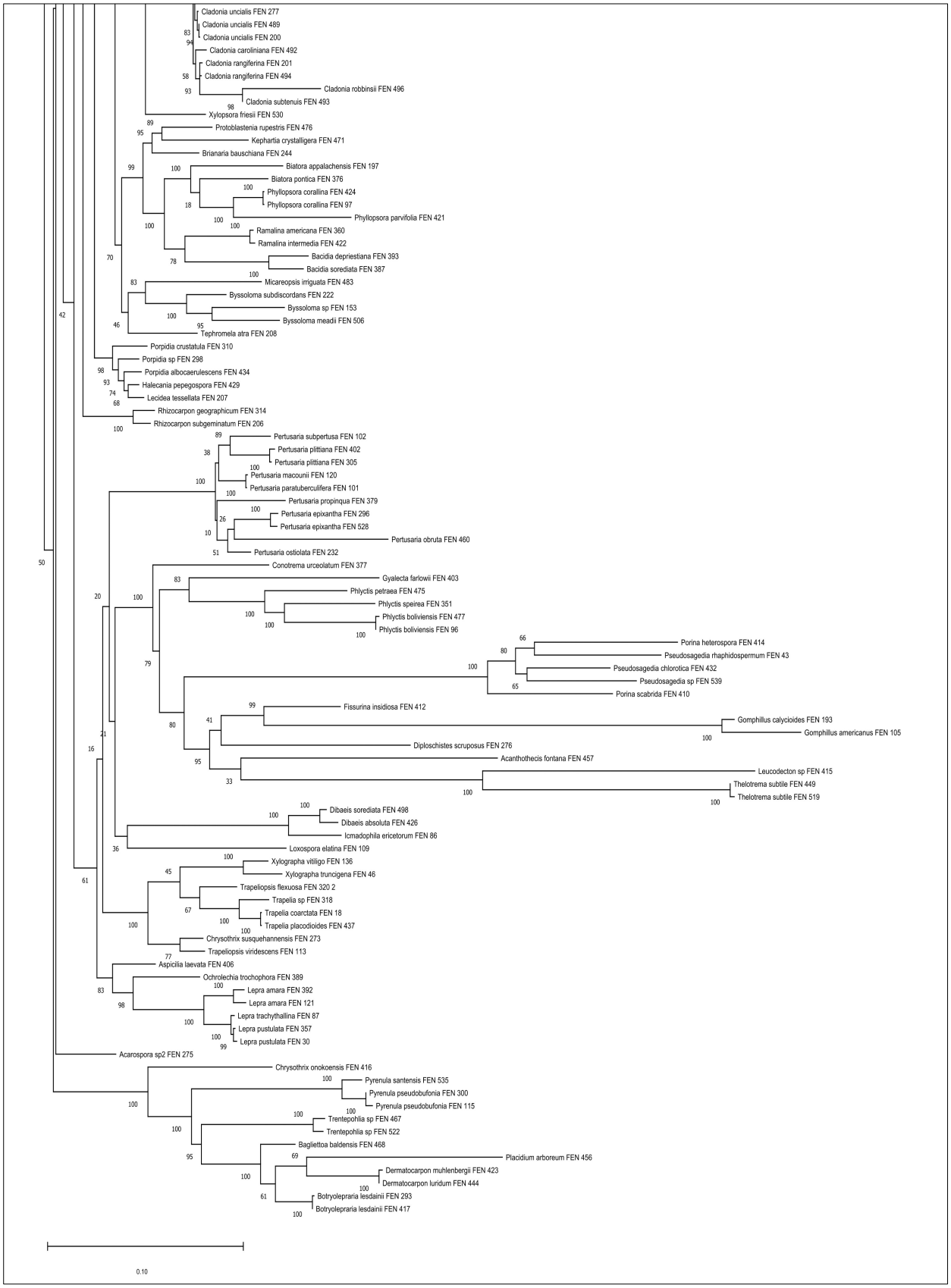
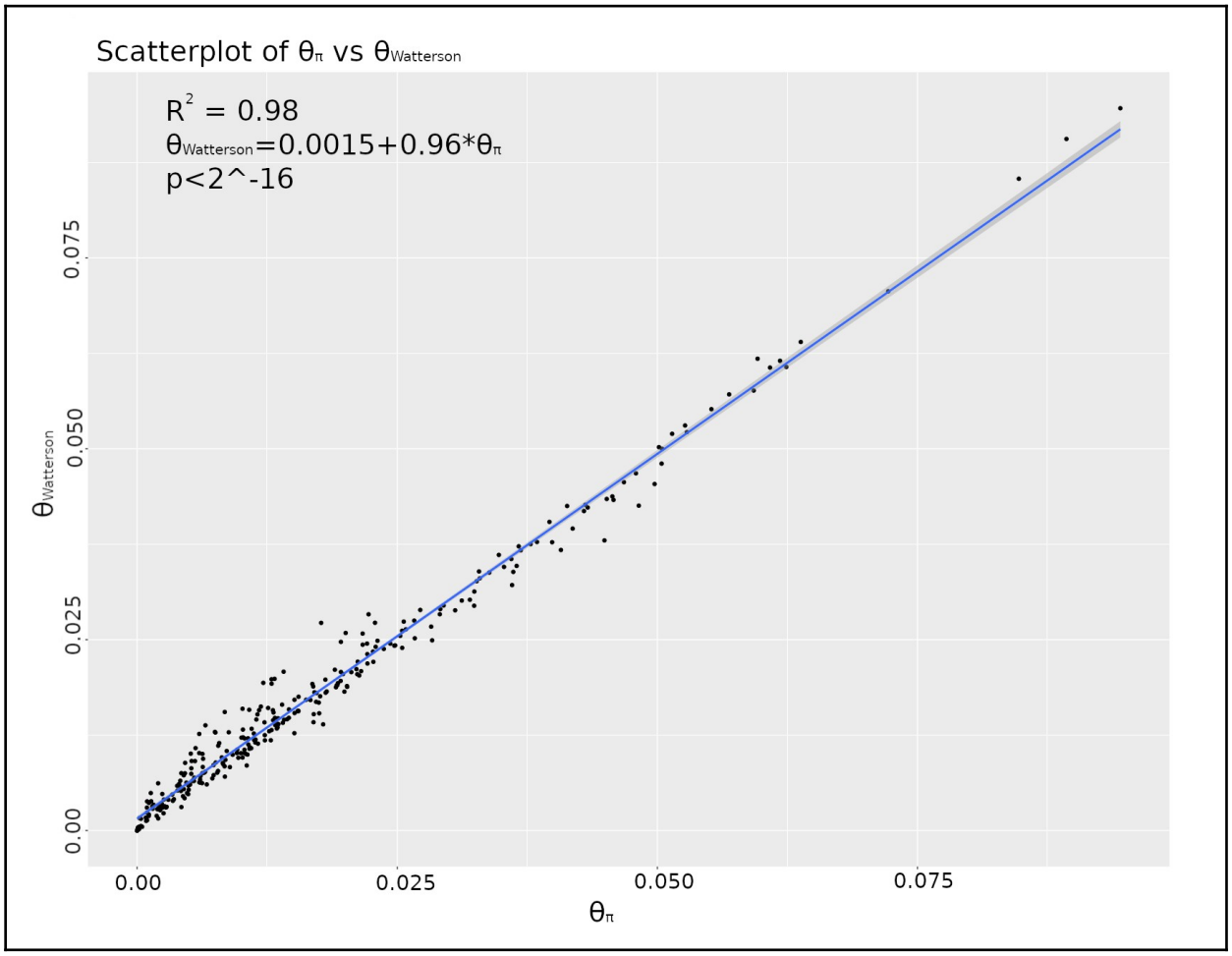

Cladonia uncialis FEN 277
Cladonia uncialis FEN 489
Cladonia uncialis FEN 200
Cladonia caroliniana FEN 492
Cladonia rangiferina FEN 201
Cladonia rangiferina FEN 494
Cladonia robbinsii FEN 496
Cladonia subtenuis FEN 493
Xylopsora friesii FEN 530
Protoblastenia rupestris FEN 476
Kephartia crystalligera FEN 471
Brianaria bauschiana FEN 244
Biatora appalachensis FEN 197
Biatora pontica FEN 376
Phyllopsora corallina FEN 424
Phyllopsora corallina FEN 97
Phyllopsora parvifolia FEN 421
Ramalina americana FEN 360
Ramalina intermedia FEN 422
Bacidia depriestiana FEN 393
Bacidia sorediata FEN 387
Micareopsis irriguata FEN 483
Byssoloma subdiscordans FEN 222
Byssoloma sp FEN 153
Byssoloma meadii FEN 506
Tephromela atra FEN 208
Porpidia crustatula FEN 310
Porpidia sp FEN 298
Porpidia albocaerulescens FEN 434
Halecania pepegospora FEN 429
Lecidea tessellata FEN 207
Rhizocarpon geographicum FEN 314
Rhizocarpon subgeminatum FEN 206
Pertusaria subpertusa FEN 102
Pertusaria plittiana FEN 402
Pertusaria plittiana FEN 305
Pertusaria macounii FEN 120
Pertusaria paratuberculifera FEN 101
Pertusaria propinqua FEN 379
Pertusaria epixantha FEN 296
Pertusaria epixantha FEN 528
Pertusaria obruta FEN 460
Pertusaria ostiolata FEN 232
Conotrema urceolatum FEN 377
Gyalecta farlowii FEN 403
Phlyctis petraea FEN 475
Phlyctis speirea FEN 351
Phlyctis boliviensis FEN 477
Phlyctis boliviensis FEN 96
Porina heterospora FEN 414
Pseudosagedia rhaphidospermum FEN 43
Pseudosagedia chlorotica FEN 432
Pseudosagedia sp FEN 539
Porina scabrida FEN 410
Fissurina insidiosa FEN 412
Gomphillus calycioides FEN 193
Gomphillus americanus FEN 105
Diploschistes scruposus FEN 276
Acanthothecis fontana FEN 457
Leucodecton sp FEN 415
Thelotrema subtile FEN 449
Thelotrema subtile FEN 519
Dibaeis sorediata FEN 498
Dibaeis absoluta FEN 426
Icmadophila ericetorum FEN 86
Loxospora elatina FEN 109
Xylographa vitiligo FEN 136
Xylographa truncigena FEN 46
Trapeliopsis flexuosa FEN 320 2
Trapelia sp FEN 318
Trapelia coarctata FEN 18
Trapelia placodioides FEN 437
Chrysothrix susquehannensis FEN 273
Trapeliopsis viridescens FEN 113
Aspicilia laevata FEN 406
Ochrolechia trochophora FEN 389
Lepra amara FEN 392
Lepra amara FEN 121
Lepra trachythallina FEN 87
Lepra pustulata FEN 357
Lepra pustulata FEN 30
Acarospora sp2 FEN 275
Chrysothrix onokoensis FEN 416
Pyrenula santensis FEN 535
Pyrenula pseudobufonia FEN 300
Pyrenula pseudobufonia FEN 115
Trentepohlia sp FEN 467
Trentepohlia sp FEN 522
Bagliettoa baldensis FEN 468
Placidium arboreum FEN 456
Dermatocarpon muhlenbergii FEN 423
Dermatocarpon luridum FEN 444
Botryolepraria lesdainii FEN 293
Botryolepraria lesdainii FEN 417
0.10

Figure 5.S3. Correlation between $\theta_{Watterson}$ and θ_{π} to check for population demographic factors that might render $\theta_{Watterson}$ inappropriate for use as an estimator for population genetic diversity.



5.5 DISCUSSION

We hypothesized that photobiont community diversity would vary according to three covariates – growth form, reproductive mode, and elevation. However, we found that algal photobiont community diversity is primarily determined by growth form. We found this pattern to be true using a model that incorporates phylogenetic relationships, as well as a non-phylogenetic model (Table 5.3). A biological mechanism for these differences could lie with how lichens acquire their photobionts. Lichens that primarily rely on vegetative propagation of their photobionts should tend to have a less diverse community within their thalli. We expect this mode of acquisition to occur in species that grow outward in a way that make it challenging to encounter free-living photobionts, such as the three-dimensional fruticose lichens. Conversely, lichens that opportunistically incorporate suitable photobionts from their environment should have more diversity in their photobiont communities. Lichens with the greatest opportunity to encounter suitable free-living photobionts are those most intimately acquainted with their substrate – the crustose lichens.

There are mixed findings regarding the diversity of cyanobacterial communities across an elevational gradient. In biological soil crusts (BSCs), cyanobacterial diversity does not decrease with elevation, and that abundance actually increases (Williams et al., 2016; Čapková et al., 2016). However, another study found that the abundance of cyanobacterial phyla in stream biofilms decreased with elevation (Wang et al., 2012) and that phylogenetic clustering of microbes in general increases as well. One possibility is that the roles of cyanobacterial communities differ among stream biofilms, BSCs and cyanolichens. These findings add another facet to our understanding of how cyanobacterial communities exist across an elevational gradient, and further research will be needed to understand the exact nature of the physiological

interactions between cyanobacteria and their lichen hosts that leads to a decline in diversity at high elevations.

We found that there was a very slight but significant inverse-quadratic pattern in the algal photobiont communities. Previous research has found that microbes do not follow the diversity pattern of plants and animals, which are highest in diversity at intermediate elevations (Fierer et al., 2011). However, that study was focused on bacterial microbes, whereas our study is an example of a pattern of diversity within microbial eukaryotes that behave more similarly to animals and plants than with bacteria.

Surprisingly, reproductive mode does not play a role in photobiont diversity, leading to the conclusion that, although asexual species carry a cadre of photobionts with them in their lichenized propagules to help them establish in their new home, asexual species still opportunistically incorporate suitable free-living photobionts just as sexually reproducing species do. From an evolutionary perspective, this makes sense, as lineages of asexually propagating lichens that limited its pool of suitable photobionts would be poorly adapted to weathering changing environmental conditions or pathogens, and would be more likely to go extinct, thus precluding their inclusion in this study. This finding has important implications for conservation. Namely, that asexual species may be more resilient in the face of changing environmental conditions than sexual species. Not only do asexual species incorporate environmental photobionts as their thalli grow, they have the additional benefit of being able to establish in a new area as soon as their propagule lands, as opposed to having to find suitable photobionts in a new location, as sexual propagules must. Moreover, one oft-mentioned trade-off of asexual reproduction is that a lack of genetic diversity through sexual recombination exposes vulnerabilities to parasitism (Hamilton et al., 1990; Lively and Morran 2014). While that trade-

off certainly applies to the genome of the mycobiont in these species, it does not apply to their photobiont communities.

We found that lichens living on calcareous rock and non-calcareous rock contain more diverse cyanobiont communities (Table 5.2). However, although that association is statistically significant, it is possibly attributable to sampling bias due to very small sample size, with just 5 samples taken from calcareous rock, and just one sample in non-calcareous rock. We thus withhold positing any biological significance to this pattern. Algal photobiont community diversity did not vary according to substrate (Table 5.2), tempering any fear of bias in our findings of patterns within growth form as being attributable to substrate.

Our sampling of photobiont communities relied on a whole genome shotgun approach, as opposed to a traditional amplicon-based approach that relies on amplifying the barcoding region with PCR. We have previously demonstrated that a WGS approach detects more species in an environmental metagenomic survey of fungi (Keepers et al., 2019). Whole genome shotgun metagenomics avoids the potential species abundance biases that are introduced via PCR (Hajibabaei et al, 2011; Piñol et al., 2015). Moreover, data quality scores on Illumina platforms decrease as a function of sequence similarity in amplicon sequencing (Kreuger et al., 2011), which introduces an additional bias in surveys, such as this one, that assess diversity among communities with varying levels of diversity.

This study utilized a bioinformatic pipeline that was developed to analyze community diversity in a novel way. The small metagenome size of lichens makes them ideal candidates for the development of whole rDNA databases for both the mycobiont and photobiont, comprised of representatives from many diverse species. One challenge we encountered was the mapping of non-algal reads present in the metagenomic libraries to highly conserved regions in the algal

database, which required careful masking of the database in all regions that were causing introgression of non-algal into the diversity estimates.

Use of $\theta_{Watterson}$ – Demographic factors within the populations being examined, such as recent bottlenecks or rapid expansion, can bias $\theta_{Watterson}$ as an estimator for genetic diversity within it. We performed a linear regression between our estimates of $\theta_{Watterson}$ (also referred to as the expectation of θ_{π}) and θ_{π} to detect any problematic deviations from the expectation of θ_{π} (Figure 5.S3). We found that the two estimates were highly correlated ($p<2^{-16}$, $R^2 = 0.98$). These two estimators are expected to be equal when population sizes are large, panmictic, and not changing in size, and their close correspondence in this system suggest either estimator would be appropriate for describing the diversity of photobiont communities.

Caveats – There are some considerations that may affect these findings. Many of the species analyzed in this study are crustose (191 out of 404), and for some vouchers, it is possible that thalli were limited in size and a sample of tissue was not possible from a single thallus present in the voucher, leading to the collection of tissue from more than one thallus in the voucher. Instances of this shortcoming are likely very rare, as care was taken to avoid this circumstance. Moreover, vouchers were selected, in part, for their quality as museum specimens, thus the majority of these vouchers were charismatic and large representatives of their species, being large enough to collect all tissue from a single thallus.

The statistical analysis that incorporated phylogenetic structure utilized a phylogeny generated from the ribosomal DNA complex of the mycobiont of each lichen. There are well-documented issues that are worth mentioning. First, our use of just a single locus to infer the species relationships is inferior to the use of a large dataset of many nuclear single-copy genes

popular among sophisticated phylogenetic studies (Smith and Kriebel 2018). Second, rDNA evolves concertedly – that is, there are several copies of the locus located within each genome that evolve in tandem. However, despite these concerns, the use of rDNA for inferring relatedness has long-been a widely-used and important locus for use in phylogenetics (Olsen and Woese 1993; Hwang and Kim 1999). The small ribosomal subunit, 18S, has been shown to be a reliable locus for inferring relationships specifically within fungi (Yarza et al., 2017). Moreover, the species relationships on the tree used in this study are in line with expectations based on expert taxonomic designations. Species within genera cluster together as expected. The few instances of species not clustering with congeners are on branches with low bootstrap support, or of uncertain taxonomic designation and may represent the true taxonomic placement of the seemingly errant taxon.

An additional caveat to be considered is that the estimates we present in this study represent the diversity of photobionts contained within the sample taken from the museum vouchers, not a broad-scale diversity of the entire lichen thallus. We were as consistent as possible with sampling a 1cm x 1cm piece of tissue taken from a localized piece of thallus, but more accurate estimates of community diversity could be obtained by homogenizing entire lichen thalli of similar area.

In summary, we have shown that the method of aligning short-reads from metagenomic community data to the commonly shared rDNA locus is an effective method of interrogating relationships contributing to patterns of diversity within those communities. This method allows for the assessment of algal diversity within a lichen thallus, which we have shown to vary in ways that have important ways based on the fungal growth form and the habitat. Our findings will have implications for our understanding of fungal biology, and could affect the way decisions are made regarding the conservation of vulnerable, endemic lichen species.

Specifically, because asexual species, as well as sexual species, appear to acquire photobionts opportunistically from their environment, as well as inheriting them from their lichenized propagules, it provides lichens with the potential to be more robust in the face of changing environmental conditions that may limit the functioning of any one algal genotype within a thallus.

Data availability – All genomic libraries used in this study are available on NCBIs SRA database, at Bioproject https://www.ncbi.nlm.nih.gov/bioproject/PRJNA731936.

CHAPTER 6

An Annotated Bibliography of My Collaborative Works Generated During My Tenure at the University of Colorado, Boulder

During my tenure as a graduate student in the Ecology and Evolutionary Biology department at the University of Colorado, Boulder, I derived much satisfaction and fulfillment from my collaborations, many of which resulted in publications in scientific or pedagogical journals. Owing to its nature as one of the more purely bioinformatic labs in the EBIO department, the Kane Lab saw many graduate students and researchers from other labs, and even other departments, seeking the expertise of someone in the lab to elevate their research by adding a genomic or bioinformatic element to it. Herein I detail my contributions to the collaborations I was privileged to take part in, grouped according to the study system.

Lichens

Although a large component of this dissertation focuses on two bioinformatic pipelines I developed for the Dimensions in Biodiversity Grant to analyze large lichen datasets, there were several other publications that resulted from the genomics-side of the project.

Pogoda, C. S., Keepers, K. G., Nadiadi, A. Y., Bailey, D. W., Lendemer, J. C., Tripp, E. A., & Kane, N. C. (2019). Genome streamlining via complete loss of introns has occurred multiple times in lichenized fungal mitochondria. *Ecology and evolution*, *9*(7), 4245-4263.

This project was an analysis of the intron compositions within the mitochondria of 58 diverse species of lichen mycobionts that had been assembled in Nolan Kane's genomics class by undergraduate students. Much of the analysis of the project was performed by two talented undergraduate researchers in the Kane Lab, Arif Nadiadi and Dustin Bailey, under the direction of Dr. Cloe Pogoda and myself. I performed the phylogenetic analyses, helped with the gene clustering analysis, and the pairwise similarity matrix. I also helped write and edit the manuscript.

Pogoda, C. S., Keepers, K. G., Lendemer, J. C., Kane, N. C., & Tripp, E. A. (2018). Reductions in complexity of mitochondrial genomes in lichen-forming fungi shed light on genome architecture of obligate symbioses. *Molecular Ecology*, *27*(5), 1155-1169.

Forming one of the dissertation chapters of Dr. Pogoda,we found in this paper that multiple lineages of lichen mycobionts had lost the gene *atp9* from their mitochondria. We also characterized patterns of synteny, intron content, and gene duplications. I was involved in the assembly and annotation of many of the genomes, as well as helped with the downstream analyses such as the phylogenetics and synteny analysis. I helped write and edit the manuscript, as well.

Funk, E. R., Adams, A. N., Spotten, S. M., Van Hove, R. A., Whittington, K. T., Keepers, K. G., ... & Kane, N. C. (2018). The complete mitochondrial genomes of five lichenized fungi in the genus Usnea (Ascomycota: Parmeliaceae). *Mitochondrial DNA Part B*, *3*(1), 305-308.

and

Brigham, L. M., Allende, L. M., Shipley, B. R., Boyd, K. C., Higgins, T. J., Kelly, N., ... & Kane, N. C. (2018). Genomic insights into the mitochondria of 11 eastern North American species of Cladonia. *Mitochondrial DNA Part B, 3*(2), 508-512.

These two papers were the result of the student-led assemblies and annotations of lichen mycobiont mitochondria in Nolan Kane's genomics class. The papers provided the mitogenomes as resources to the broader scientific community, as well as contextualized the phylogenetic placement of the taxa in the studies. Each student was in charge of their own mitochondrial genome, and as the teaching assistant of the class in the semester these papers were written, I was responsible for vetting the quality of both their assemblies and annotations. I also guided the graduate students in charge of these two manuscripts, Laurel Brigham, Luis Allende, Eric Funk and Alex Adams, with the phylogenetics and genome content analyses, as well as edited the manuscripts for submission to the journal.

Stewart, C. R. A., Lendemer, J. C., Keepers, K. G., Pogoda, C. S., Kane, N. C., McCain, C. M., & Tripp, E. A. (2018). Lecanora markjohnstonii (Lecanoraceae, lichenized Ascomycetes), a new sorediate crustose lichen from the southeastern United States. *The Bryologist, 121*(4), 498-512.

and

Tripp, E. A., Morse, C. A., Keepers, K. G., Stewart, C. A., Pogoda, C. S., White, K. H., ... & McCain, C. M. (2019). Evidence of substrate endemism of lichens on Fox Hills

Sandstone: Discovery and description of Lecanora lendemeri as new to science. *The Bryologist, 122*(2), 246-259.

These are two novel species descriptions. In the former, I had the pleasure to collaborate with my colleague Carly Anderson-Stewart on the genomics and phylogenetics that were included in the description of *Lecanora markjohnstonii*, a sorediate crustose lichen collected as part of the Dimensions in Biodiversity Grant described in Chapters 4 and 5. It was named after Mark Johnston, in honor of his lifelong contributions to environmental education and conservation of old-growth forests and wetlands of Alabama. In the latter paper, we describe a species endemic to the Fox Hill Sandstone outcrop located on the Front Range of Colorado. I again contributed to the genomics and phylogenetics aspects of the manuscript. We found that the species is nested within the enigmatic *L. dispersa* group, which do not appear to form thalli. It is named after Dr. James Lendemer, who is the curator of lichenology at the New York Botanical Gardens, in honor of his contributions to the field.

Lendemer, J. C., Keepers, K. G., Tripp, E. A., Pogoda, C. S., McCain, C. M., & Kane, N. C. (2019). A taxonomically broad metagenomic survey of 339 species spanning 57 families suggests cystobasidiomycete yeasts are not ubiquitous across all lichens. *American journal of botany*, *106*(8), 1090-1095.

A landmark 2016 study in *Science* (Spribille et al., 2016) found basidiomycete yeasts in the cortexes of lichens belonging to 52 different genera within Parmeliaceae, and that these lichens fail to grow in culture unless the basidiomycete yeasts are also present. Their findings hinted at a

possible third obligate member of the lichen symbiosis. We wished to assess the taxonomic breadth of this pattern. Along with my co-author James Lendemer, I looked for basidiomycete sequences in 413 of our metagenomic lichen assemblies, which constituted 339 unique species, spanning 57 families. We found basidiomycete sequences in 2.7% of them, mostly in Parmeliaceae species in which they were found previously. These results suggest that basidiomycete yeasts are not ubiquitous, and are not likely to be an obligate member of the lichen symbiosis.

Cannabis

When the recreational drug, *Cannabis sativa*, was legalized in the state of Colorado, the genetics and genomics of a cash crop worth billions annually (Caulkins et al., 2016) became legal to study. These papers represent my fruitful collaboration with Dr. Daniela Vergara, who conceptualizes and writes most of these papers, and who serves as a deft liaison between science and industry.

Vergara, D., White, K. H., Keepers, K. G., & Kane, N. C. (2016). The complete chloroplast genomes of Cannabis sativa and Humulus lupulus. *Mitochondrial DNA Part A*, *27*(5), 3793-3794.

and

White, K. H., Vergara, D., Keepers, K. G., & Kane, N. C. (2016). The complete mitochondrial genome for Cannabis sativa. *Mitochondrial DNA Part B*, *1*(1), 715-716.

In the same sense that most metazoans contain mitochondrial genomes, as visited a few times in the lichens section of this chapter, plants like Cannabis and Humulus (hops) contain genomes in their chloroplasts as well. The chloroplast genomes of most tracheophytes are highly structured, with two single-copy sections separated by two long inverted-repeats, which are identical in sequence to one another. For these two papers, I helped with assembly and annotation of the organellar genomes, including with helping to solve the puzzle of fitting the structural components of the chloroplast genome in the correct order.

Vergara, D., Baker, H., Clancy, K., Keepers, K. G., Mendieta, J. P., Pauli, C. S., ... & Kane, N. C. (2016). Genetic and genomic tools for Cannabis sativa. *Critical Reviews in Plant Sciences*, *35*(5-6), 364-377.

and

Gray, D. J., Baker, H., Clancy, K., Clarke, R. C., deCesare, K., Fike, J., ... & Trigiano, R. N. (2016). Current and future needs and applications for cannabis. *Critical Reviews in Plant Sciences*, *35*(5-6), 425-426.

Cannabis, for better or worse, ranks among the world's most valuable crops. As such, these two papers assessed the state of the research industry around the plant. My contribution was to compare the qualities of the best genomic assemblies available at the time.

Vergara, D., Huscher, E. L., Keepers, K. G., Givens, R. M., Cizek, C. G., Torres, A., ... & Kane, N. C. (2019). Gene copy number is associated with phytochemistry in Cannabis sativa. *AoB Plants*, *11*(6), plz074.

In addition to its industrial uses, Cannabis is consumed recreationally for its psychoactive effects. The cannabinoid synthesis pathway produces the molecules responsible for those effects, THCA and CBDA, or delta-9 tetrahydrocannabinolic acid and cannabidiolic acid, respectively. The recent discovery that the gene THCA-synthase, once thought to be a single-copy gene, actually exists in multiple copies in close proximity in the Cannabis genome (McKernan et al., 2015; Weiblen et al., 2015). We investigated how gene copy number associates with the phytochemistry of the leaf trichomes in drug-type plants. In this paper, I identified the THCA-synthase gene copies in two genomic assemblies we had available. I also performed the phylogenetic analysis comparing all of the different gene copies to one another.

Vergara, D., Huscher, E. L., Keepers, K. G., Pisupati, R., Schwabe, A. L., McGlaughlin, M. E., & Kane, N. C. (2021). Genomic evidence that governmentally produced Cannabis sativa poorly represents genetic variation available in state markets. *bioRxiv*.

Prior to the legalization of Cannabis in several states, the sole provider of Cannabis for conducting medical research was the National Institute on Drug Abuse (NIDA). In this preprint, we show that the cultivars provided by NIDA are genetically distinct from the majority of plants that are consumed for recreation. Accordingly, medical studies that use NIDA plants should be interpreted with caution. I contributed to the assembly and genomic analysis for this study.

Diatoms

Diatoms are ubiquitous single-celled algae responsible for the production of 25% of the oxygen in the atmosphere (Field et al., 1998). There are estimated to be between 12,000 and 30,000 species of diatoms (Guiry 2012; Mann and Vanormelingen 2013), yet genomic resources derived from them remain sparse. I collaborated with Drs. Sarah Hamsher, Joshua Stepanek and Patrick Kociolek to sequence and assemble the mitochondrial and chloroplast genomes of several species of diatoms, including one, *Halamphora calidilacuna*, that was new to science.

Pogoda, C. S., Keepers, K. G., Hamsher, S. E., Stepanek, J. G., Kane, N. C., & Kociolek, J. P. (2019). Comparative analysis of the mitochondrial genomes of six newly sequenced diatoms reveals group II introns in the barcoding region of cox1. *Mitochondrial DNA Part A*, *30*(1), 43-51.

In this paper, we not only present the fully assembled and annotated mitochondrial genomes of six diatom species, our analysis found group II introns, or parasitic retrotransposons, had inserted themselves into the gene encoding cytochrome c oxidase subunit I, or COI. This gene was previously put forth as a potential universal barcoding marker for diatoms (Evans et al., 2007; Trobajo et al., 2010; Hamsher et al., 2011), but was found difficult to consistently amplify, even among closely related species (Trobajo et al., 2010; Hamsher et al., 2011). Our analysis presents a potential reason why -- a high variability in the number and locations of group II introns in the gene. Thus, in this paper, we ruled out COI as a universal barcoding marker for diatoms and proposed that researchers seek alternative barcodes in the chloroplast or nuclear genomes.

Hamsher, S. E., Keepers, K. G., Pogoda, C. S., Stepanek, J. G., Kane, N. C., & Kociolek, J. P. (2019). Extensive chloroplast genome rearrangement amongst three closely related Halamphora spp. (Bacillariophyceae), and evidence for rapid evolution as compared to land plants. *PloS one*, *14*(7), e0217824.

In this paper, we compared the chloroplast genomes of three congener diatoms, *Halamphora americana*, *H. calidilacuna*, and *H. coffeaeformis*. We found extensive chloroplast genome rearrangement and a rate of nucleotide evolution of between 4 and 7 times faster than the chloroplasts of land plants. This was the first study comparing diatoms chloroplast genomes within a single genus. My contributions to both papers involved assembly and annotation of the genomes, as well as consultation on the comparative analyses.

Pedagogy

One of the courses that I had the fortune of assisting in teaching three times during graduate school was EBIO 4640/5640: Computational Genomics. My colleague Dr. Cloe Pogoda and I wanted to make the adoption and teaching of genomics in other colleges and universities more accessible. We realized that the format of the class developed by Professor Kane could be adapted to serve as a teaching module by other universities. The class is taught as a CURE, or course-based undergraduate research experience, in which the students take charge of the assembly and annotation of their own, uniquely-assigned mitochondrial genome, typically with data derived from projects being conducted by researchers within the EBIO department. We wrote a comprehensive, simple guide to teaching genomics that emphasizes the importance of

students taking ownership of their projects, as well as the value of being able to claim published genomic resources on their academic CVs.

Pogoda, C. S., Keepers, K. G., Stanley, J. T., & Kane, N. C. (2019). A CURE-based approach to teaching genomics using mitochondrial genomes. CourseSource.

Conclusion

Science has long moved past the tired trope of the ‘lone genius’ producing brilliant science from thin air. Collaborations are the true catalyst that move the science zeitgeist forward. I contend that developing a unique and sought-after skill set is an excellent way of fostering productive collaborations with colleagues seeking to elevate their own research projects with analyses that utilize said skill set. I am very fortunate to have a modest talent with genomics and bioinformatics that has enabled me to collaborate with such uniquely knowledgeable scientists in their fields. I have provided in this chapter a small glimpse into my history of productive collaborations, and hope to continue such fruitful endeavors long into the future.

CHAPTER 7

7.1 KEY RESULTS

In the grand scheme of humanity's study of biology, bioinformatics is a relatively nascent toolset for conservation and ecology whose applications are still being discovered. The advent of "big data" in the 1990s has enabled the field to explode in popularity. I have presented four chapters that all use genomics or bioinformatic data in different applications, each of which have implications for the conservation of species made vulnerable by habitat destruction, invasive species, and climate change.

In my second chapter, I follow the fate of an introgression of healthy pupfish into the South Scruggs Spring population that was suffering a demographic collapse. My research established an expectation of the magnitude of the effect of a genetic rescue, finding that the genetically admixed offspring of the introgression enjoyed a 20% higher survivorship than South Scruggs residents prior to the introgression. I also contributed the first fully assembled and annotated mitochondrial genome of the Warm Springs pupfish, which is a useful 'ultra-barcode' (Kane et al., 2012) for population genetics studies of this endangered fish.

In addition to their charismatic and enigmatic presence, lichens are critical bellwethers for understanding the impacts of pollution on other species in an area. Interestingly, lichens are often the first species to establish on lands that were previously glaciated (Nascimbene et al, 2017; Sancho et al, 2019), serving as a stark reminder of how rapidly the climate landscape is changing. As such, a better understanding of the conditions that allow lichens to thrive in their environment will enable researchers to understand why they don't live in some environments, which may be due to a lack of suitable conditions for photobionts, and thus the lichen as a whole, to grow in the environment. I developed the bioinformatics pipeline and the database I described

in Chapter 4 which can be used to detect the hidden diversity of lichen propagules in any environment that has representative ribosomal DNA sequences in the database. Moreover, my analysis of how photobiont diversity varies among lichens of different lineages, dominant reproductive modes, growth forms, and primary photobiont type showed that the primary predictor of photobiont diversity in lichens using algal photobionts is the growth form. In cyanolichens, the best predictor is elevation. Critically, diversity does not depend on reproductive mode, suggesting that asexually reproducing species may be more robust to changing availability of photobionts than previously thought.

7.2 DATA LIMITATIONS

The fungal metagenomic survey pipeline used in chapter 4 is dependent upon the availability of ribosomal DNA of the species the researcher is trying to detect. If the propagule of a species of lichen is present in a metagenomic sample but a corresponding ribosomal DNA sequence is unavailable in the database, the species will remain undetected. It is also limited to some degree by how variable the sequences of related species are. Species that have highly similar rDNA will fail to be detected due to the fact that reads may align to both sequences, which decreases the SAM CIGAR score below the threshold for detection.

In Chapter 5, the reference sequence for cyanobacterial photobionts, *Nostoc sphaeroides* rDNA, was mapping many bacterial sequences that did not belong to cyanobacteria. We circumvented the problem by discovering regions of the sequence that are diagnostic for cyanobacteria, but those regions only summed to 1,269 bp out of the total of 5,219 bp in the reference. It was long enough to get estimates of the community diversity, but a barcode with a greater sum of usable sequence would probably yield better estimates.

7.3 FUTURE DIRECTIONS

Several hundred more lichens, from the broader survey under which Chapters 4 and 5 fell, have been sequenced and assembled. The rDNAs of many new species will be added to the fungal rDNA database and will be used to measure the disparity between observed lichen diversity and the potential diversity as measured by the pipeline in Chapter 4.

The difficulties of restricting metagenomic reads in the cyanobacterial analysis in Chapter 5 revealed the ubiquitous presence of diverse bacterial communities present on or within the lichen thallus that remains to be studied in such a broad taxonomic dataset. One approach of analyzing the community assembly of the 'hologenome' (Zilber-Rosenberg and Rosenberg 2008; Moran and Sloan 2015; Tripp et al., 2017) is to bin genomic reads with MetaBin (Sharma et al., 2012), which is highly accurate and sensitive taxonomic assignments by aligning the six open reading frames of each read to a database using Blat (WJ Kent, 2002). Genomic binning of assembled contigs may be performed with either metaBAT (Kang et al., 2015) or tetramerFreqs (Dick et al., 2009), both of which use deeply conserved evolutionary patterns of tetramer frequencies to bin genomic contigs. Each taxonomic bin may be identified using BLAST. To visualize the patterns in community assembly, tetramerFreqs incorporates an Emergent Self-Organizing Map (ESOM) visualization (Ultsch and Moerchen, 2005) similar to an ordination plot.

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