Lichen Conservation in Eastern North America: Population Genomics, Climate Change, and Translocations

Jessica Allen
The Graduate Center, City University of New York

How does access to this work benefit you? Let us know!
Follow this and additional works at: https://academicworks.cuny.edu/gc_etds

Part of the Biodiversity Commons, Bioinformatics Commons, Botany Commons, Climate Commons, Genomics Commons, Natural Resources and Conservation Commons, and the Population Biology Commons

Recommended Citation
https://academicworks.cuny.edu/gc_etds/2059

This Dissertation is brought to you by CUNY Academic Works. It has been accepted for inclusion in All Dissertations, Theses, and Capstone Projects by an authorized administrator of CUNY Academic Works. For more information, please contact deposit@gc.cuny.edu.
LICHEN CONSERVATION IN EASTERN NORTH AMERICA:
POPULATION GENOMICS, CLIMATE CHANGE, AND TRANSLOCATIONS

By

JESSICA L. ALLEN

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2017
LICHEN CONSERVATION IN EASTERN NORTH AMERICA: POPULATION GENOMICS, CLIMATE CHANGE, AND TRANSLOCATIONS

by

Jessica L. Allen

This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

__________________________
Date Dr. William Buck

Chair of Examining Committee

__________________________
Date Dr. Laurel Eckhardt

Executive Officer

Supervisory Committee:

Dr. Elizabeth Alter

Dr. Joseph Rachlin

Dr. James Lendemer

Dr. Richard Harris

Dr. Bernard Goffinet

THE CITY UNIVERSITY OF NEW YORK
ABSTRACT

LICHEN CONSERVATION IN EASTERN NORTH AMERICA:
POUPLATION GENOMICS, CLIMATE CHANGE, AND TRANSLOCATIONS

by

Jessica L. Allen

Advisor: William Buck

Conservation biology is a scientific discipline that draws on methods from diverse fields to address specific conservation concerns and inform conservation actions. This field is overwhelmingly focused on charismatic animals and vascular plants, often ignoring other diverse and ecologically important groups. This trend is slowly changing in some ways; for example, increasing number of fungal species are being added to the IUCN Red-List. However, a strong taxonomic bias still exists. Here I contribute four research chapters to further the conservation of lichens, one group of frequently overlooked organisms. I address specific conservation concerns in eastern North America using modern methods. The results of these studies provide insight into lichen conservation in each situation, implications for the broader ecosystems within the study regions, and advancement of methods for the study of lichen conservation and biology.

The first research chapter (Chapter 2) is a population genomics study based on whole genome shotgun sequencing of *Cetradonia linearis*, an endangered, lichenized fungus. These data were used to 1) assemble and annotate a reference genome, 2) characterize the mating system, 3) test for isolation by distance (IBD) and isolation by environment (IBE), and 4) investigate the biogeographic history of the species. Approximately 70% of the genome (19.5 Mb) was assembled. Using this assembly, only a single mating type was located, suggesting the
species could be unisexual. There was strong evidence for both low rates of recombination and for Isolation by Distance, but no evidence for Isolation by Environment. The hypothesis that *C. linearis* had a larger range during the last glacial maximum, especially in the southern portion of its current extent, was supported by Hindcast species distribution models and the spatial distribution of genetic diversity. Given the findings here, it is recommended that *C. linearis* remain protected by the U.S. Endangered Species Act and listed as Vulnerable on the International Union for the Conservation of Nature Red-List.

The third chapter is an estimation of the impacts of climate change on high-elevation, endemic lichens in the southern Appalachians, a global diversity hotspot for many groups, including lichens. Extensive field surveys in the high elevations of the region were carried out to accurately document the current distributions of eight narrowly endemic species. These data were compared with herbarium records, and species distribution modeling was used to predict how much climatically suitable area will remain within, and north of, the current range of the target species at multiple time points and climate change scenarios. Fieldwork showed that target species ranged from extremely rare to locally abundant and models predicted average losses of suitable area within the current distribution of species ranging from 93.8 to 99.7%. The results indicate that climate change poses a significant threat to high-elevation lichens, and illustrates the application of current modeling techniques for rare, montane species.

In the fourth chapter, a dataset of >13,000 occurrence records for lichens in the Mid-Atlantic Coastal Plain (MACP) of eastern North America was used to model distributions of 193 species. The resulting models were used to quantify the amount of each species’ distribution that is occupied by unsuitable land use types, along with the potential area that will be lost to sea-level rise (SLR). These analyses showed that species have likely already lost an average of 32%
of their distributional area to development and agriculture, and are predicted to lose an average of 12.4 and 33.7% of their distributional area with one foot (~0.3 m) and six feet (~1.8 m) of SLR, respectively. Functional and taxonomic groups were compared to identify specific effects of SLR. Species reproducing with symbiotic propagules were found to have significantly larger distributions than species that reproduce sexually with fungal spores alone, and the sexually reproducing species were predicted to lose greater distributional area to SLR. *Cladonia* species occupy significantly less area in the MACP than *Parmotrema* species and were predicted to lose more of their distributions to SLR. Patterns of total species diversity showed that the area with the highest diversity is the Dare Peninsula in North Carolina, which was also predicted to lose the most land area to SLR. The workflow established here is flexible and applicable to estimating SLR impacts worldwide and can provide essential insights for local conservation planning.

The fifth chapter describes the results of three experiments conducted to test new and established methods for lichen transplantation. First, small fragments of *Graphis sterlingiana*, *Hypotrachyna virginica*, and *Lepraria lanata* were placed on medical gauze attached to each of the species’ most common substrate to test the feasibility of transplanting narrowly endemic species. Second, burlap, cheesecloth, medical gauze, and a plastic air filter were directly compared for their use as artificial transplant substrates with *Lepraria finkii* as the test lichen. Third, transplants of *Usnea angulata* were established to test its amenability to transplantation via hanging fragments on monofilament. The first two experiments were established on Roan Mountain, North Carolina and the third experiment at Highlands Biological Station, North Carolina. In the first two experiments medical gauze did not withstand local weather conditions and nearly all pieces fell from the trees within 6 months. The plastic air filter and burlap performed best as artificial substrates for transplants, with a 100% and 80% success rate,
respectively. Cheesecloth remained attached to the trees, but only 20% of lichen fragments remained attached to the substrate after one year. In the third experiment *U. angulata* grew 3.5 ± 1.4 cm in 5 months, exceeding previously reported growth rates for this species. These results advance methods for conservation-focused lichen transplants, and expand established methods to a new region and new species.
ACKNOWLEDGEMENTS

I would like to sincerely thank the following people for their help with this research: my committee, Dr. James Lendemer, Dr. William Buck, Dr. Richard Harris, Dr. Elizabeth Alter, and Dr. Joseph Rachlin for their guidance in designing, implementing, and presenting these projects, and for Dr. Bernard Goffinet serving as an outside reviewer; Sean McKenzie for his unfailing support, countless hot meals, and contagious enthusiasm for science; Jenna Dorey, Nelson Salinas, Annie Virnig, Troy McMullin, Natalie Howe, Grace McKenzie, and André de Carvalho for their friendship and support throughout the past five years: my family for grounding and humor: my mother Teri Seidl, father Greg Allen, and their spouses Ron Seidl and Jill Allen, and my siblings Annie Allen and Daniel Aguirre; Dr. Robin O’Quinn for her mentorship, guidance, and an ever -welcoming home away from home, along with Tom Groessbeck and Freya; for their field assistance I would like to thank Alex Cecil, who diligently helped with most of the rock gnome sampling, Jenna Dorey, Sean McKenzie, and Charlie Zimmerman; NYBG graduate students past and present, especially Julián Aguirre, Kate Armstrong, Fernando Bittencourt-Matos, Dario Cavaliere, Naveed Davoodian, Jenna Dorey, Stephen Gottschalk, Lance Jones, Jordan Hoffman, Ricardo Kreibel, Elizabeth McCarthy, Donald McClelland, William Perez, Carlo Rodrigues, Marcelo Reginato, Nelson Salinas, Robin Sleith, Alejandra Vasco, and Annie Virnig; many of the curators and staff at NYBG whom I have had the pleasure to interact with, including Dr. Barbara Ambrose, Daniel Atha, Dr. Brian Boom, Dr. Doug Daly, Dr. Lisa Campbell, Dr. Roy Halling, Dr. Jackie Kalunki, Dr. Ken Karrol, Dr. Larry Kelly, Dr. Damon Little, Dr. Fabián Michelangeli, Dr. Robbin Morrall, Dr. Rob Naczi, Matt Sewell, Dr. Dennis Stevenson; Everyone who keeps the physical and digital herbarium running, especially Dr. Barbara Thiers, Nicole Tarnowsky, Melissa Tulig, Kim Watson, Elizabeth Keirnan, Elen Bloch,
Ana Maria Ruiz, Lin Li, and Joel Ramirez; Dr. Christoph Scheidegger for many helpful discussions regarding lichen transplants and population genetics; all of the Tuckerman workshop participants and network of eastern North American lichenologists, professionals and enthusiasts alike; the many professional scientist and botanists in the southern Appalachians who supported this research through discussions and permitting, including Gary Kauffman, Paul Super, Mara Alexander, Chris Ulrey, Susan Sachs, and Ed Corey; the staff at Highlands Biological Station, Jim Costa, Karen Kandl, Alyssa Fuller, Michelle Ruigrok, Cynthia Soderstrom, Sonya Carpenter, Russell Funderburk, and Katie Lapish; and Dolly Parton for her endless inspiration.

Chapter 2, “Climate change impacts on endemic, high-elevation lichens in a biodiversity hotspot,” was originally published in Biodiversity and Conservation, DOI:10.1007/s10531-016-1071-4. Chapter 3, “Quantifying the impacts of sea-level rise on coastal biodiversity: A case study on lichens in the mid-Atlantic Coast of eastern North America,” was originally published in Biological Conservation, DOI:10.1016/j.biocon.2016.08.031.

Funding for these projects came from the National Science Foundation Graduate Research Fellowship, NSF DEB#1145511, Highlands Biological Station, Southern Appalachian Botanical Society, City University of New York Doctoral Research Grant, and The American Bryological and Lichenological Society.
TABLE OF CONTENTS

Abstract iv
Acknowledgments viii
Table of Contents x
List of Figures xii

Chapter 1. Introduction 1

Chapter 2. Population Genomics of an Endangered, Lichenized Fungus Characterized by Low Rates of Recombination and Strong Isolation by Distance 14
  2.1 Abstract 14
  2.2 Introduction 14
  2.3 Methods 18
  2.4 Results 24
  2.5 Discussion 28
  2.6 Acknowledgements 33
  2.7 Figures and Tables 35

Chapter 3. Climate change impacts on endemic, high-elevation lichens in a biodiversity hotspot 41
  3.1 Abstract 41
  3.2 Introduction 41
  3.3 Methods 44
  3.4 Results 48
Chapter 4. Quantifying the impacts of sea-level rise on coastal biodiversity: A case study on lichens in the mid-Atlantic Coast of eastern North America

4.1 Abstract
4.2 Introduction
4.3 Methods
4.4 Results
4.5 Discussion
4.6 Acknowledgements
4.7 Figures and Tables

Chapter 5. Testing Lichen Transplant Methods for Conservation Applications in the Southern Appalachians

5.1 Abstract
5.2 Introduction
5.3 Methods
5.4 Results
5.5 Discussion
5.6 Acknowledgements
5.7 Figures and Tables

Supplement 1

Bibliography
FIGURES AND TABLES

Figure 1.1. Map of dissertation study regions. 11

Table 1.1. Table citing the total number of species in each taxonomic group on the IUCN Red-List and protected by the U.S. Endangered Species Act. 12

Figure 2.1. Morphology, habit, and habitat of Cetradonia linearis. 35

Figure 2.2. Scatterplot showing results of Blobology pipeline used to identify contaminating reads. 36

Figure 2.3. Population genetic structure of Cetradonia linearis. 37

Figure 2.4. Scatterplot of genetic vs. geographic distance and outcome of statically significant partial Mantel test. 38

Figure 2.5. Species distribution model of Cetradonia linearis. 38

Table 2.1. Site names, mountain range, individuals sampled, average percent SNPs covered for each site/mountain range (% Coverage), and average nucleotide diversity for all sampled sites and mountain ranges. 39

Table 2.2. Pairwise Fst between all sampled sites. 40

Table 2.3. Results of partial Mantel test showing a significant relationship between genetic distance (Fst) and a geographic distance (km), but not between genetic distance and any environmental variables. 40

Table 2.4. Results of BEDASSLE showing that more genetic differentiation among populations is due to their geographic rather than environmental distance. 40

Figure 3.1. Targeted high-elevation, endemic lichen species. A) Arthonia kermesina, B) Arthopyrenia betulicola, C) Buellia sharpiana, D) Cladonia appalachensis, E) Graphis sterlingiana, F) Lecanora masana, G) Lepraria lanata, H) Hypotrachyna virginica. 55
Figure 3.2. Sites sampled for high-elevation, endemic lichen search. 55

Figure 3.3. Examples of distribution models for *Graphis sterlingiana*. 56

Figure 3.4. Larger geographic area highlighting suitable areas for multiple species, the disappearance of suitable habitat in the southern Appalachians, and the agricultural and developed areas that will likely act as dispersal barriers among forested areas. 57

Table 3.1. Records and land area estimates by species. 58

Figure 4.1. Distribution and heatmaps of sampling sites from regional biodiversity survey and collections held at NYBG combined with survey sites. 76

Figure 4.2. Distribution size and predicted loss of distribution for the most speciose genera in the MACP. 77

Figure 4.3. Distribution size and predicted area loss of functional groups. 78

Figure 4.4. Total species diversity in the MACP, spatial distribution of unsuitable land use types, and two levels of SLR, 1 foot and 6 feet. 79

Figure 4.5. Distribution patterns for most diverse genera in the MACP. 79

Figure 5.1. Diversity of substrates and species used in transplant studies. 94

Figure 5.2. Total annual precipitation at Highlands Biological Station 1961-2016. 95

Table 5.1. Results of *Graphis sterlingiana, Hypotrachyna virginica, Lepraria lanata,* and *Lepraria finkii* transplants. 95
Chapter 1. Introduction

The term biodiversity encompasses the immense variety of ecosystems, taxa, and genes on our planet (CBD 1992, Mace et al. 2011). In essence, all living diversity is included in this definition, species from the smallest virus to the largest whale, and everything in between, along with their interactions and genetic diversity. Beyond viewing biodiversity with awe, humans value it for many reasons. We are completely dependent on other species for our food, and maintaining crop diversity is one key element of ensuring a sustainable future for human civilization (Jacobson et al. 2013). Most of our medicines originate from natural sources, and many species produce compounds that have the potential to become important drugs (Cordell 2000, Aly et al. 2011, David et al. 2015). Somewhat less directly, ecosystem services provide clean air and water, protection from severe storms, and crop pollination (Potts et al. 2010, Mace et al. 2011, Tilman et al. 2014). Biodiverse, functioning ecosystems support human life in myriad, well-documented ways (Millennium Ecosystem Assessment 2005).

Because humans are completely dependent on biodiversity, the current loss of species and reduction in species’ abundances and distributions is causing alarm (Dirzo and Raven 2003, Butchart 2010, Barnosky et al. 2011, Ceballos et al. 2015). There are 860 documented extinctions that have been caused by humans and recognized by the International Union for the Conservation or Nature (IUCN) (IUCN 2016). There are likely additional extinctions that have not been documented, especially in groups like insects, where there are 70 documented extinctions but thousands estimated (Dunn 2005). Not only are numbers of species declining, but there is a steady trend of higher extinction risk, and lower habitat extent, habitat quality, and other metrics that measure the health and safety of biodiversity (Butchart et al. 2010).
The major drivers of biodiversity loss are well-documented (Chivian and Bernstein 2008). Land use conversion and habitat destruction, especially for resource extraction purposes, are the biggest drivers of biodiversity loss (De Baan 2013), with over half of the terrestrial area of the Earth already altered to some degree by human activity (Chivian and Bernstein 2008). Overharvesting is another major driver, especially for species that are already rare and endangered (Barron 2011, Wittemyer et al. 2014, Baricevic et al. 2015). Invasive species are marginalizing and crowding out diverse natives and altering how ecosystems function (Carruthers 2003, Loo 2009, Mainka and Howard 2010). Pollution is degrading all habitats, causing mass die-offs in many cases, along with serious health impacts to humans (Newport et al. 2014, John and Shaïke 2015, Rai 2016). Taken together, these clear, co-occurring forces synergize and lead to compounding impacts on biodiversity.

Climate change is predicted to exacerbate current conservation challenges and accelerate extinction rates (Brook et al. 2008, Mainka and Howard 2010, Bellard et al. 2012). It is well established that greenhouse gas emissions from humans, including carbon dioxide and methane, are the major driver of climate change (IPCC 2014). Relatively little action has been taken to slow the process, and given the current socio-political situation, continued increases in Earth surface temperatures are very likely (IPCC 2014). The rapid changes in temperature, precipitation, and weather patterns for most of the globe have serious implications for biodiversity. Species distributions are already shifting (Colwell et al. 2008, Kelly and Goulden 2008, Harsch et al. 2009). Migration will likely be limited by habitat fragmentations and human erected barriers to dispersal (Ackerly et al. 2010), or by a lack of suitable nearby conditions (e.g., mountain-top extinction) (Dullinger et al. 2012). Migration rates among species and groups of organisms are uneven, and may result in loss of essential biological interactions for some species.
The climate projections, migrations and adaptation restrictions, and all other threats taken together are pointing to a rapid acceleration in extinction rates in the coming century (Brook et al. 2008).

One major focus of conservation biology is addressing the biodiversity extinction crisis. Conservation biologists use research techniques from diverse empirical scientific fields to address conservation concerns for biodiversity, ecosystems, and human well-being (Kareiva and Marvier 2012, Wiederholt et al. 2015). For instance, studies of societal perspectives of, and interactions with, wildlife improve understanding and implementation of conservation actions and education (Pooley et al. 2014). Population genetics and genomics aid in planning and prioritizing land areas for protection (Luikart et al. 2003, Manel et al. 2003). Spatial analyses and remote sensing data provide insight into habitat quality and extent (Cabello et al. 2012). DNA barcoding is frequently used to identify plants and animals that are illegally harvested and sold (Kim et al. 2014). The breadth and depth of this field is rapidly expanding as techniques and interest in conservation biology increase (Wiederholt et al. 2015).

The taxonomic scope of major conservation efforts and attention beyond charismatic animals and vascular plants is one major advancement that is much needed in the field of conservation biology (Dunn 2004, Griffith and Dos Santos 2012, Allen and Lendemer 2015). Taxonomic bias in conservation is sometimes supported by the claim or assumption that conserving large, charismatic groups will inherently result in the conservation of other groups of organisms (Caro 2010). However, the relationship among diversity patterns in multiple groups of organisms is rarely empirically evaluated, and it is often not supported when it is evaluated (Lindenmayer and Likens 2011). This bias is clearly observed throughout the field of conservation biology. A thorough review of conservation literature found that vertebrates were
the most frequently published on groups of organisms, with 58% of articles published in 2001 and 54% in 2011 focusing on them (Velasco et al. 2015). A total of 25% of the papers in 2001 and 20% in 2011 were specifically on mammals, and all non-animal organisms were grouped under the ambiguous terms “microorganisms” and “vegetation” (Velasco et al. 2015). The IUCN Red List is another publication that clearly shows taxonomic bias (IUCN 2016). There are 102,780 animals on the list representing 71% of the listed diversity, and 76,226 of the species are vertebrates representing 53% of all listed species. This is in stark contrast to the 40,946 plants (28% of listed species), and 64 fungi (0.0004% of listed species) (Table 1.1; IUCN 2016). In the United States the pattern is equally biased, with 1,372 listed animals (59% of species), 941 listed plants (41%), and 2 fungi (0.0009%) protected by the Endangered Species Act (Table 1.1; https://www.fws.gov/endangered/ [accessed Dec. 2016]). These metrics are simple indicators of the broader trend of groups like insects and fungi being consistently under-represented in conservation research and policies (Griffith and Dos Santos 2012).

Lichens, fungi that form obligate symbioses with algae and/or cyanobacteria, are one group of ecologically important fungi that are under-represented in conservation biology studies. Lichens grow in terrestrial ecosystems worldwide, dominating ~8% of the Earth’s surface (Purvis 2000). There are ~20,000 described species of lichens, representing 20% of fungal diversity (Lutzoni et al. 2001, Lücking et al. 2016). They function as primary producers, nitrogen fixers, soil stabilizers, and soil formers (Brodo et al. 2001). Many animals eat lichens, from tardigrades to elk and nematodes to flying squirrels (Henderson and Hackett 1986; Cumming 1992; Petterson et al. 1995). Caribou (Rangifer spp.) are dependent on lichens, and their winter diets in some areas consist of >85% lichens (Thompson et al. 2015). Within the thallus, diverse communities of bacteria and fungi rely on lichens as an important habitat (Arnold et al., 2009,
Hodkinson & Lutzoni, 2009). Because they are very sensitive to environmental quality and change, lichens have been used worldwide as indicators of air pollution (Conti and Cecchetti 2001, Will-Wolf et al. 2006). Lichens produce a wide diversity of chemical compounds, some of which are regularly used by humans as antibiotics, and many of which have shown potential to kill cancerous cells (Shrestha and St. Clair 2013). Thus, while each individual lichen is usually small, taken together they form an essential component of functioning, terrestrial ecosystems, and provide a number of services to human societies.

Four major threats to lichen biodiversity have been clearly identified. First, lichens are highly sensitive to air pollution (Conti and Cecchetti 2001, Giordani et al. 2002). The impacts of air pollution on lichens are readily observable in many cities where lichens are generally infrequent (Nash and Gries 1999). Habitat destruction is another major threat (Belinchon et al. 2009, Boch et al. 2016). For epiphytic species, clear-cutting forests is particularly damaging (Johansson 2008). Because primary forests consistently have the highest species diversity (Lesica et al. 1991), recovery from clear-cutting is slow or impossible. For soil crusts, any mechanical disturbance, such as grazing or driving, destroys the crust, which either cannot regenerate or remains significantly negatively impacted (Root et al. 2011). In the past, overharvesting lichens for dyes was a serious issue in some parts of Europe (Casselman 2011), but harvesting for this particular use is no longer a threat. Now, climate change poses a serious threat to lichens, with clearly documented effects like shrub encroachment in Arctic regions leading to lichen declines (Moffat et al. 2015), and shifts in species distributions documented worldwide (Ellis 2013) (See Chapter 3 for greater detail). While these general trends in lichen conservation issues are established, significant research is still needed to address region-specific conservation concerns and identify any new, emerging threats.
Despite the clear threats to lichens and their ecological importance, in much of the world lichens are not recognized as warranting protection or granted any conservation status. Fortunately, the exceptions to this rule are slowly growing. In 2003 two species were added to the IUCN Red-List, two species were added in 2014, and in 2016 four species were added (IUCN 2016). Now, there are efforts underway to continue building this list. In the United States two lichens are protected by the Endangered Species Act, and many species are protected by the Survey and Manage program in the Pacific Northwest (Molina et al. 2006). While 33 states have no lichens on their rare species lists, a few do, with Oregon (205 species), Washington (133 species), and Alaska (64 species) tracking the most species (Allen and Lendemer 2015). Some countries are far ahead of the United States in their protection of lichens, such as Finland, Serbia, and the United Kingdom, which have explicit frameworks to specifically conserve fungi (Minter 2014). Continued efforts are required to ensure that lichens are properly and equally considered by conservation scientists and policy-makers worldwide.

Here I present the results of four studies that aim both to advance knowledge of lichens to address specific conservation concerns in the eastern United States, and also to expand the methods used for investigating the basic biology of lichens. All these studies were conducted in the southeastern United States, a region that hosts an incredible diversity of lichens, with high species-level diversity reported from Florida (Harris 1995) to the Ozarks (Harris and Ladd 2005), including numerous endemics. Not only is this region notable for its lichen diversity, but there are a number of specific conservation concerns that need to be addressed (Holzmueller et al. 2010, Nagy et al. 2011, Gutierrez et al. 2016).

Most of the studies in this dissertation focus on one subregion, the southern Appalachians (Fig. 1.1). The core of this region is in western North Carolina, and its edges span to Alabama,
Georgia, Tennessee, South Carolina, and Virginia (Fig. 1.1, Manos and Meireles 2015). It hosts a great diversity of plant and animal species, including endemics that span many groups of organisms, from trees like *Abies fraseri*, to spiders like *Microhexura montivaga* (Harper 1948, Manos and Meireles 2015, Seaborn and Catley 2016). Specific threats to biodiversity in this region include invasive pests that have repeatedly swept through forests and caused mass die-offs of tree species, beginning with the American Chestnut (Milgroom and Cortesi 2004), followed by the Elm (Evans and Finkral 2010), then *Abies fraseri* (Hollingsworth and Hain 1992, Pauley and Clebsch 1990, White et al. 2012), and now *Tsuga caroliniana* (Jetton et al. 2008). Air pollution is a serious issue in the Blue Ridge Province, where some of the highest rates of acid deposition have been reported in the eastern United States (Elwood et al. 1991). Much of the region was clear-cut by the early 1900’s, leaving very little primary forest (White et al. 2012). Now, climate change poses a threat with predictions of warmer temperatures and changes in seasonality (Ingram et al. 2013).

Lichens are particularly diverse in the southern Appalachians, and have long been studied by botanists visiting the region (summarized by DePriest (1984)). The first botanists to explore the area could not help but collect lichens along with plants. André Michaux collected 14 species of lichens during his exploration in the late 18th century. In the 19th century Henry William Ravenel and Moses Ashley Curtis were the most prominent regional collectors, and both corresponded regularly with Edward Tuckerman, arguably the most prominent lichenologist of the time. Lichenological research in the region has increased rapidly in the 20th and 21st centuries, with publications from many prominent collectors (Degelius 1942; Yoshimura and Sharp 1968; Dey 1978, 1979; DePriest 1983, 1984; Tønsberg 2005; Keller et al. 2007; Lendemer et al. 2013). All these studies have culminated in the knowledge that this portion of the
Appalachian Mountains has some of the highest species richness of lichens in North America. There are over 800 species documented from Great Smoky Mountains National Park (Lendemer et al. 2013), and well over 1,000 lichen species occurring in the region as a whole (Lendemer, pers. comm.).

The first two research chapters of this dissertation focus on rare lichens endemic to the southern Appalachians. The first study (Chapter 2), is on the population genomics of the most well-known southern Appalachian endemic lichen, the rock gnome (*Cetradonia linearis*), one of two fungi on the endangered species list (Allen and Lendemer 2015). The results of this study provide evidence for low rates of recombination, a self-fertile mating system, strong signal of isolation-by-distance, and signatures of refugial populations in the southern portion of its range. Chapter 3 (published as Allen and Lendemer 2016a) reports a study of eight species that are narrowly endemic to high-elevations (>4,500 ft.) in the region. Included in that chapter are the results of a thorough and successful search for previously undocumented populations all of the target endemics, along with estimations of how their distributions will shift with the projected warming trend for the southern Appalachians (Ingram et al. 2013).

The Mid-Atlantic Coastal Plain (MACP) is the other focal subregion. This vast, low-lying area spans along the United States coast from southern New Jersey through South Carolina (EPA 2013). It encompasses diverse ecosystems, including dunes, pocosins, hardwood- and conifer-dominated swamps, pine savannahs and maritime forests (EPA 2013). These ecosystems provide important habitat for migratory birds and mammals, including endangered red wolves, amphibians, and reptiles (USFWS 2008). The MACP has seen some of the most turnover in land use over the past century compared to other regions in United States, and now only ~12.4% of the land area remains in a natural state (Auch 2000). Ongoing threats of resource extraction and
invasive pests are further compounded by climate change, with sea-level rise being a particularly

While there have been some studies of the lichens in parts of the MACP (Torrey 1937,
Crichton 1994, Culberson et al. 1982), a large-scale survey of the region was only recently
undertaken. This survey was conducted from 2012 to 2015 and was led by J.C. Lendemer and
R.C. Harris. During the survey 215 sites were visited and a voucher specimen of every species
found at each site was collected. Because I spent weeks conducting field work as part of the team
of researchers on this project, and was thus intimately familiar with the dataset, I used the
generated lichen diversity data to test a method for quantifying the estimated impacts of sea-level
rise. This study is detailed in Chapter 4 (published as Allen and Lendemer 2016b), and involved
using species distribution modeling to identify diversity hotspots, and taxonomic and functional
groups that are more threatened. It also quantified actual land area likely to be lost to sea-level
rise. The results of this study are examined in light of possible conservation solutions, including
translocations and construction of biodiversity corridors (Keddy 2009).

In the final chapter of my dissertation I detail three lichen transplant studies that aim to
advance our knowledge and methods for successful lichen translocations. While there have been
a number of lichen translocation studies for conservation purposes in the past (Smith 2015),
many challenges still remain. The biggest challenge is successfully transplanting crustose
lichens, a feat that has only been successfully completed twice (Smith 2015). More generally,
transplant attempts conducted with a greater diversity of species and regions are required. In
chapter five I report on transplants of *Graphis sterlingiana*, *Hypotrachyna virginica*, *Lepraria
finkii*, *Lepraria lanata*, and *Usnea angulata*, three crustose species, one foliose species, and one
fruticose species. These studies include tests of established and new methods for lichen transplants.

Advances in the field of conservation biology, and specifically in the conservation of lichens, are encouraging. More and better scientific research is available to guide conservation actions and policies to increase their efficiency and efficacy (Wiederholt et al. 2015). Broader perspectives and approaches seek to involve local communities in conservation, benefitting both biodiversity and people (UNDP 2016). Agricultural practices that incorporate and support greater species and genetic diversity are increasingly recognized and developed (Bengtsson et al. 2005). Most encouragingly, previously overlooked diversity is gaining recognition in the conservation community for its contribution to functioning ecosystems and human health (Rosenfeld 2002, Vanbergen et al. 2013, Heilmann-Clausen et al. 2014). Continued focus and effort on conducting sound scientific research to advance our understanding and conservation of understudied biodiversity is essential to ensure adequate protection for these diverse groups of organisms.
1.1 Figures and Tables

**Figure 1.1.** Study regions included in this dissertation. Dark purple shading highlights the Southern Appalachians. Pink shading highlights the Mid-Atlantic Coastal Plain

**Table 1.1.** (Following two pages). Table citing the total number of species in each taxonomic group on the IUCN Red-List and protected by the U.S. Endangered Species Act.
<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Phylum</th>
<th>Endangered</th>
<th>Threatened</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animalia</td>
<td>Annelida</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animalia</td>
<td>Arthropoda</td>
<td>112</td>
<td>15</td>
<td>127</td>
</tr>
<tr>
<td>Animalia</td>
<td>Chordata</td>
<td>851</td>
<td>228</td>
<td>1079</td>
</tr>
<tr>
<td>Animalia</td>
<td>Cnidaria</td>
<td>0</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Animalia</td>
<td>Echinodermata</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animalia</td>
<td>Mollusca</td>
<td>118</td>
<td>26</td>
<td>144</td>
</tr>
<tr>
<td>Animalia</td>
<td>Nemertina</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animalia</td>
<td>Onychophora</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animalia</td>
<td>Platyhelminthes</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animalia Total</td>
<td></td>
<td>1081</td>
<td>291</td>
<td>1372</td>
</tr>
<tr>
<td>Chromista</td>
<td>Ochrophyta</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fungi</td>
<td>Ascomycota</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Fungi</td>
<td>Basidiomycota</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fungi Total</td>
<td></td>
<td>0</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Plantae</td>
<td>Anthoceratophyta</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plantae</td>
<td>Bryophyta</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plantae</td>
<td>Charophyta</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plantae</td>
<td>Marchantiophyta</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plantae</td>
<td>Rhodophyta</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plantae</td>
<td>Tracheophyta</td>
<td>772</td>
<td>169</td>
<td>941</td>
</tr>
<tr>
<td>Plantae Total</td>
<td></td>
<td>772</td>
<td>169</td>
<td>941</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1853</td>
<td>460</td>
<td>2315</td>
</tr>
<tr>
<td>Kingdom</td>
<td>Phylum</td>
<td>Extinct</td>
<td>Extinct in Wild</td>
<td>Critically Endangered</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------</td>
<td>---------</td>
<td>-----------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Animalia</td>
<td>Annelida</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Animalia</td>
<td>Arthropoda</td>
<td>81</td>
<td>2</td>
<td>425</td>
</tr>
<tr>
<td>Animalia</td>
<td>Chordata</td>
<td>363</td>
<td>17</td>
<td>1673</td>
</tr>
<tr>
<td>Animalia</td>
<td>Cnidaria</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Animalia</td>
<td>Echinodermata</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animalia</td>
<td>Mollusca</td>
<td>297</td>
<td>14</td>
<td>586</td>
</tr>
<tr>
<td>Animalia</td>
<td>Nemertina</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Animalia</td>
<td>Onychophora</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Animalia</td>
<td>Platyhelminthes</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Animalia Total</td>
<td></td>
<td>744</td>
<td>33</td>
<td>2706</td>
</tr>
<tr>
<td>Chromista</td>
<td>Ochrophyta</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Fungi</td>
<td>Ascomycota</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Fungi</td>
<td>Basidiomycota</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Fungi Total</td>
<td></td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Plantae</td>
<td>Anthoceratophyta</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plantae</td>
<td>Bryophyta</td>
<td>2</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>Plantae</td>
<td>Charophyta</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Plantae</td>
<td>Marchantiophyta</td>
<td>1</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Plantae</td>
<td>Rhodophyta</td>
<td>1</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Plantae</td>
<td>Tracheophyta</td>
<td>112</td>
<td>35</td>
<td>2477</td>
</tr>
<tr>
<td>Plantae Total</td>
<td></td>
<td>116</td>
<td>35</td>
<td>2506</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>860</td>
<td>68</td>
<td>5220</td>
</tr>
</tbody>
</table>
Chapter 2. Population Genomics of an Endangered, Lichenized Fungus Characterized by Low Rates of Recombination and Strong Isolation by Distance

2.1 Abstract

Obligate symbioses (e.g., corals) are some of the most threatened organisms globally. Population genetics in obligate symbiotic organisms is challenging, often requiring axenic isolates to develop species-specific markers. The burgeoning field of population genomics provides tools to circumvent these traditional demands by allowing detailed investigation of symbiont population structure without isolating symbionts and developing species-specific markers. Here the results of a population genomics study based on whole genome shotgun sequencing of *Cetradonia linearis*, an endangered lichen, are presented. These data were used to 1) assemble and annotate a reference genome, 2) characterize the mating system, 3) test for isolation by distance (IBD) and isolation by environment (IBE), and 4) investigate the biogeographic history of the species. 19.5 Mb of the genome (approximately 70%) was assembled, and only the MAT 1-2-1 idiomorph was located, suggesting the species could be unisexual. There was strong evidence for both low rates of recombination and for IBD, but no evidence for IBE. The hypothesis that *C. linearis* had a larger range during the last glacial maximum, especially in the southern portion of its current extent, was supported by Hindcast species distribution models and the spatial distribution of genetic diversity. Given the findings here, it is recommended that *C. linearis* continue to be protected by the U.S. Endangered Species Act and listed as Vulnerable on the International Union for the Conservation of Nature Red-List.

2.2 Introduction

Genomes from both model and non-model organisms are being sequenced and analyzed at an increasingly fast pace due to lower sequencing costs and advancing computational power
(Ellegren 2014). These extensive amounts of data have been used to study diverse topics from human immunity and adaptation (Lachance and Tishkoff 2013) to the evolution of human pathogens (Croucher et al. 2013, Billmyre et al. 2014, Comas et al. 2015), and crop species evolution (Meyer and Purugganan 2013) to the impacts of urbanization on animals (Munshi-South et al. 2016). Population genomics methods have recently begun to be applied to biodiversity conservation issues (Garner et al. 2016). Because both neutral and adaptive markers are sequenced, there is the possibility to disentangle the influences of genetic drift, gene flow, and adaptation on populations, providing more complete information to designate conservation units (Funk et al. 2012). Examples of the application of population genomics to conservation of diverse organismal groups have dramatically increased over the past few years, and addressed diverse topics including species delimitation (Picq et al. 2016), hybridization (Combosch and Kolmer 2015), and species invasions (Trumbo 2016). Results of population genomics studies are not yet necessarily translated into direct conservation action due to methodological developments and the lack of a policy framework to incorporate genomic level data into decision-making (Shafer et al. 2015). However, the ever increasing use of population genomics to study rare and endangered species will eventually make it a standard approach (Garner et al. 2016).

Of the domains of eukaryotic organisms, fungi are one of the most amenable to genomics studies due to their generally small, compact genomes (Gladieux et al. 2014). Population genomic studies have already added substantial depth and breadth to the knowledge of basic fungal biology, allowing researchers to address questions that were once intractable. For instance, fungi that have only been observed reproducing asexually show genomic evidence for sexual reproduction (Tsai et al. 2008, Stefanini et al. 2016), speciation through homoploid hybridization has been shown to occur rapidly, at least in yeast (Leducq et al. 2016), and
Glomerales, arbuscular mycorrhizal fungi, have highly flexible levels of ploidy in the heterokaryotic cells within species (Wyss et al. 2016). Studies have also focused on applied issues of virulence, fungicide resistance, and hybridization with wild relatives in plant and human pathogens (Grünwald et al. 2016). Understanding of basic and applied mycology is being transformed by genomic analyses, and these advances will likely continue as techniques are applied across even more diverse groups of fungi.

Lichens, one major group of fungi comprising >20% of all ascomycetes (Lücking et al. 2016) that form obligate symbioses with algae and/or cyanobacteria, have never before been studied using population genomics. In fact, despite their conspicuous abundance in many terrestrial ecosystems, relatively few taxa have been studied with traditional population genetics methods. To date, most population genetics studies of lichens have been conducted on Lobaria pulmonaria and its photobiont Dictyochloropsis reticulata using microsatellite markers (Widmer et al. 2010, Dal Grande et al. 2010, Nadyeina et al. 2014). These studies have shown that L. pulmonaria frequently disperses short distances via lichenized propagules (bundles of algae and fungi), and infrequently disperses long distances via sexually produced fungal spores (Werth et al. 2006). Furthermore, there is evidence of adaptation and population isolation on small spatial scales (Nadyeina et al. 2014). Population genetic patterns in Xanthoria parietina based on RAPD-PCR markers contrast starkly with the findings for L. pulmonaria, where high genetic diversity and very few clones were found within small areas, even among adjacent individuals (Itten and Honneger 2010). The pattern recovered in X. parietina is similar to a study of Parmelina carporrhizans based on microsatellite loci, where high rates of migration were recovered among populations, except for isolated island populations (Alors et al. 2017). These three highly detailed studies of lichen population genetics are only the beginning to
understanding this diverse group of fungi that have evolved at least seven times independently throughout the fungal tree of life (Schoch et al. 2009), and occupy every terrestrial ecosystem from the poles to the tropics (Brodo et al. 2001). Population genomics is a promising approach to rapidly advance our knowledge of population biology in lichens as it circumvents difficulties associated with developing species-specific markers, especially since lichens are notoriously difficult and slow to culture (Crittendon et al. 1995).

The rock gnome lichen (*Cetradoina linearis*) is an ideal study organism to both shift the current methodological paradigm and advance the knowledge of population biology in lichenized fungi, while simultaneously contributing to the growing literature on population genomics for conservation applications (Garner et al. 2016). *Cetradoina linearis* is one of two fungal species protected by the Endangered Species Act in the United States (USFWS 2013), and one of eight lichens on the IUCN Red-List (Allen et al. 2015). It is narrowly endemic to the Southern Appalachians of eastern North America, where it is known from ~100 populations, most of which are located in western North Carolina (USFWS 2013). It grows on rocks either on exposed cliffs at high-elevations or on large boulders in mid- to high-elevation streams. *Cetradoina* is a unispecific genus, whose position as the earliest diverging member of the widespread and ecologically important Cladoniaceae makes its study essential for addressing hypotheses of evolution in this family (Wei and Ahti 2002, Zhou et al. 2006). It forms colonies of simple to branched squamules with black apothecia and/or pycnidia, reproductive structures, frequently produced at the tips (Fig.1). Despite having been protected by the Endangered Species Act for over 20 years, little is known about *C. linearis* beyond its distribution, including its growth rate, age to maturity, and life span (USFWS 2013). This study aims to address a major knowledge gap for this species: its population genetic structure.
In this study, three hypotheses were tested concerning the population dynamics of *Cetradonia linearis*: 1) most reproduction and dispersal occurs through clonal processes, 2) isolation by distance is the major force shaping the genetic differentiation, while ecological adaptation plays a minor role, and 3) the southern portion of its current extent was an important refugium during the Pleistocene glaciation. To test these hypotheses low-coverage, whole genome shotgun sequencing was used to generate large-quantities of genomic data from samples throughout the species’ range. The resulting genome-wide single-nucleotide polymorphisms (SNPs) were used to measure genetic diversity, recombination, and clonality. Population genetic structure, connectivity, and evidence for isolation by environment were also investigated. This study is the first assessment of population genomics in a lichen, providing a baseline for comparison in this group of organisms, along with valuable information for the continued conservation of the endangered rock gnome lichen.

### 2.3 Methods

*Study System, Sampling, and Sequencing*

Samples were collected from populations throughout the geographic and ecological range of *Cetradonia linearis* (Fig. 2.1). At each site two to three squamules were taken from up to ten distinct colonies using surface sterilized forceps. Squamules were placed into 1.5 mL eppindorf tubes, set out to air dry for 24 hours, then stored in a -40° C freezer. Samples were washed with acetone and DNA was extracted using the Qiagen DNeasy Plant Mini Kit with the cell lysis stage extended for 4-6 hours. Thirty-two samples were chosen for sequencing based on DNA quality and yield, while maintaining the geographic and ecological breadth of samples. Sequencing was conducted at the Rockefeller University Genomics Resource Center. Libraries were prepared with the Nextera XT kit and Illumina Next Seq platform in Mid Output, 150 bp paired end read
mode was used for sequencing. All samples were sequenced at roughly equal coverage, except one sample from the Balsam Mountains, B224, which was sequenced at 5X higher coverage in order to assemble a reference genome.

**Quality Filtering, Genome Assembly, and Annotation**

A reference genome was assembled and annotated after strictly filtering contaminating reads. B224 reads were trimmed, adapters were removed, and overlapping read-pairs combined using cutadapt and FLASH (Magoc and Salzberg 2011, Martin 2011). Read pools for all other samples were trimmed, adapters were removed, and overlapping read-pairs combined using FLASH and Trimmomatic v 0.36 (Bolger et al. 2014). An initial assembly of B224 was built using Minia with a kmer size of 75 and an abundance minimum of 3 (Chikhi and Rizk 2013). To filter out contaminants the Blobology work flow and perl scripts were used (Kumar et al. 2013). Specifically, a random subset of 15,000 contigs longer that 250 bp were subjected to homology search using megablast against the whole nucleotide database and the e-value cutoff was set to 1e-5. Contigs with GC content > 0.6 and coverage < 5 were pooled to form a set of contaminant contigs (Fig. 2.2). Then, all B224 reads were aligned to the contaminant contigs using bowtie2, and all reads that did not align to the contaminants were retained for reassembly. The final assembly was built using Abyss with the paired-end read setting and a kmer size of 41 (Simpson et al. 2009). All resulting contigs shorter than 500 bp were removed from the dataset before further analyses. Genome annotation was conducted using the MAKER pipeline (Cantarel et al. 2008). SNAP was used for the ab-initio gene predictor, and protein homology evidence was drawn from *Aspergillus niger* ATCC 1015 v4.0, *Cladonia grayi* Cgr/DA2myc/ss v2.0, and *Cochliobolus heterostrophus* C5 v2.0 (Andersen et al. 2011, Ohm et al. 2012, Condon et al. 2013, McDonald et al. 2013, Leskovec and Sosic 2016). All genes were blasted against the A.
niger, C. grayi, and C. heterostrophus gene sets. Contigs were kept for downstream analysis if the gene with the highest-scoring blast hit matched most closely with a C. grayi gene.

SNP Calling and descriptive statistics

Single-nucleotide polymorphisms (SNPs) were called for all sequenced samples using the annotated contigs as a reference genome for the fungal component. For the algal component, two Chlorophyta genomes were used as reference to determine if unique SNPs from the photobiont were recovered, or if the reads were simply aligning to conserved or repetitive sequences found throughout Chlorophyta genomes. The two genomes used as reference were Trebouxia gelatinosa isolate LA000220 (GCA_000818905.1, Carniel et al. 2016), and Chlamydomonas reinhardtii strain CC-503 (Merchant et al. 2007). First, to align the reads to the contigs, the short read aligner bwa was used (Li and Durbin 2009). Then, to call the SNPs from this alignment FreeBayes was used with the ploidy set to 2, minimum alternate fraction set to 0.9, and coverage set to 5 for the mycobiont and 2 for the photobiont (Garrison and Marth 2012). The ploidy was set to two because all samples were fertile, thus there were potentially two genetic individuals present. Furthermore, analyses were conducted on SNP sets called with the ploidy set to one and the same results were found regardless of ploidy designation. Pi (π) was calculated using VCFtools (Danecek et al. 2011). Linkage disequilibrium was corrected for using the R package poppr with a threshold of 0.2 and a 1 Kb sliding window (Kamvar et al. 2014). Then, pairwise Fst was calculated among all populations with the linkage disequilibrium corrected dataset using BEDASSLE (Bradburd et al. 2013).

Statistical Analyses

First, the relationships among populations were explored to determine if there were phylogenetic signals for each distinct sampling site and mountain range, and at what level of
grouping the relationships were clearest. Discriminant Analysis of Principle Components (DAPC), a multivariate approach to identifying genetically distinct clusters of individuals, was used with the clustering algorithm implemented to define groups resulting in 10 genetic clusters chosen based on the Bayesian Information Criterion (BIC) (Jombart et al. 2010). This method was specifically designed to cope with the large quantity of next generation sequencing data and implemented in R through the package adegenet 2.0 (Jombart 2008). An un-rooted neighbor joining tree was also built to infer the relationships among individuals based on bitwise distances using the R package ape (Paradis et al. 2004).

The influence of geographic and ecological distance on genetic distance was investigated using two approaches. First, a partial Mantel test with 10,000 permutations was used to test for correlation between genetic distance measured as pairwise Fst, and geographic distance measured as Euclidean distance in kilometers, and a set of five environmental variables. The five environmental variables were habitat (boulder in stream vs. exposed rock outcrop), slope and three noncolinear variables from the worldclim dataset: mean temperature of wettest quarter (BIO8), mean temperature of warmest quarter (BIO10), and annual precipitation (BIO12) (Hijmans et al. 2005). Second, a Bayesian approach was taken to estimate the contributions of geographic and ecological distance to genetic distance in the fungal dataset (Bradburd et al. 2013). The same set of ecological and geographic distance variables were used as input data, along with allele sample sizes and frequencies in all samples. An initial Bayesian analysis, run for 1 million generations found that the effect size of BIO8 and BIO12 were essentially zero, and these were removed from the dataset. A second analysis was run for 5 million generations with a sample frequency of 10. Trace plots were examined for convergence, and mean marginal
densities and 95% confidence intervals calculated for $\alpha_E:\alpha_D$ for each environmental variable with the first 40% of generations treated as burn-in and removed.

**Determination of Fungal Mating System**

Recognition of suitable reproductive individuals in ascomycetes is largely controlled by the mating-type locus (Dyer 2008). Most species are either homothallic, meaning all genes required for sexual reproduction are on the same chromosome and individuals are able to self-fertilize, or heterothallic, meaning the two mating types are in different individuals that must come together to recombine and produce sexual spores (Wilson et al. 2015). The two mating types are MAT 1-1-1 or the alpha domain, often referred to as MAT-1, and MAT 1-2-1 or HMG-box, MAT-2 for short (Scherrer et al. 2007). Mating-type loci evolve quickly and protein sequences are significantly divergent among species, making them difficult to locate and study (Lee et al. 2010). However, mating type genes have been sequenced in a few species of lichens (Scherrer et al. 2007). Here we used previously published amino acid sequences to locate mating-type genes in *Cetradonia linearis*. First, a homology search was conducted between the published fragment of the MAT-2 locus from *Cordyceps militaris* (BAC66500.1) and all amino acid sequences from the MAKER annotation using blastp (Altschul et al. 1990). We used the *C. militaris* amino acid sequence because it closely matched the *Cladonia galindezii* (AY634274.1) nucleotide sequence. Two putative MAT-2 genes were found among the amino acids sequences annotated in the *C. linearis* genome. These prospective genes were aligned using COBALT through the NCBI webserver with all available MAT-2 amino acid sequences for ascomycetes and relationships among sequences were examined through a neighbor-joining tree (Papadopoulus and Agarwala 2007). The amino acid sequence that formed a monophyletic clade with the *Cladonia galindezii* was treated as the *C. linearis* MAT 1-2-1 gene and used for all
subsequent analyses. A homology search was also conducted between MAT-1 amino acid sequences from _Xanthoria_ sp. (Scherrer et al. 2007) and the amino acid sequences form the MAKER output using blastp (Altschul et al. 1990). No matches were found between the two datasets. Next, I determined if either MAT locus was found in the remaining 31 read pools. To search for the MAT-2 locus, reads were aligned to the contig that included the most likely putative MAT 1-2-1 gene using bowtie2 with the sensitive, local setting. The presence and depth of reads was examined at the MAT-2 sequence site in the resulting alignments. The same process was used to search for MAT-1 loci using nucleotide sequences from _Xanthoria_ sp., _Rhynchosporium secalis_, _Pyrenopeziza brassicae_, _Mycosphaerella graminicola_, and _Aspergillus nidulans_ (Singh and Asby 1998, Waalwijk et al. 2002, Linde et al. 2003, Paoletti et al. 2007, Scherrer et al. 2007). No putative MAT-1 gene was found in any of the samples. Fertile samples were then dissected to search microscopically for trichogynes, specialized hyphae that receive spermatia (conidia) to begin sexual reproduction, and fertile apothecia, structures that produce fungal spores that result from recombination. Thin sections were cut by hand with a razor blade through apothecia and mounted on slides. They were stained with phloxine and cleared with potassium hydroxide before examination under a compound microscope. No trichogynes were observed, but multiple ascospores were identified, and the presence of 8 spores/ascus was confirmed.

**Species Distribution Modeling**

Species distribution modeling was used to investigate if the populations with the highest genetic diversity were located in an area that was likely a refugium during the Last Glacial Maximum (LGM). Species distribution models (SDMs) that predict the probability of a species’ presence across the landscape must first be built for the present, then projected to past climates.
Species distribution modeling was conducted using Maxent v. 3 (Phillips et al. 2006, Phillips and Dudik 2008) after steps were taken to reduce sampling bias and calibrate the model. First, localities were thinned by a 5 km radius to reduce sampling bias by randomly excluding one of two localities when they fell within that radius, as implemented in the R package SpThin (Aiello-Lammens et al. 2015). There were 101 known localities originally, and after thinning 42 localities were retained and used for all further analyses. The worldclim dataset of 19 bioclimatic variables were used for the environmental data at 30 arc second resolution for the present and 2.5 arc minutes for the last glacial maximum, and all autocorrelated variables were first removed, leaving mean temperature of wettest quarter (BIO8), mean temperature of warmest quarter (BIO10), and annual precipitation (BIO12) (Hijmans et al. 2005). These three variables were clipped to the extent of the species known range with a small buffer for layers from the present and LGM. Two modeling parameters were tuned to identify the best level of complexity: feature classes define the allowed shape of the environmental variable response curves, and the regularization multiplier controls for complexity, with higher values increasingly penalizing complexity (Scheglovitova and Anderson 2013). The best modeling parameters were chosen based on the Akaike Information Criterion corrected for sample size (AICc) (Warren and Seifert 2011). Model tuning was implemented using the R package ENMEval with the ‘blocks’ setting (Muscarella et al. 2014). The final model was built and projected using all thinned localities with the regularization multiplier set to 3.5 and linear, quadratic, and hinge response curves allowed.

2.4 Results

High-coverage, whole-genome shotgun sequencing of one individuals of *Cetradonia linearis* was used to assemble a reference genome. Then, whole-genome shotgun sequencing of 31 additional individuals were mapped to this genome, and the resulting SNPs were used to infer
the population structure, biogeographic history, and mating system of the taxon. An unsuccessful attempt was made to assemble a similar dataset for the symbiotic alga.

*Cetradonia linearis Reference Genome*

Multiple steps of stringent quality and contaminant filtering resulted in the production of a high-quality, partial reference genome. The original read pool from the sample used to create the reference genome, sample B224, contained 55 million reads, for a total of 16 Gb. The mean PHRED quality score was 33. After trimming, filtering for low quality base calls, and merging paired ends there were 44 million merged reads (where two paired-end reads overlapped and merged into a single sequence) with a total of 5.6 Gb of sequence, and 8.3 million read pairs that did not overlap with a total of 2.1 Gb of sequence. The initial assembly using Minia built 32,669 contigs. When contigs under 500 bp were excluded, the total assembly length was 105.5 Mb and the N50 was 3,814. After filtering contaminants from the contigs using the Blobology workflow (Kumar et al. 2013), 41.9 million merged reads with 5.4 Gb of sequence remained, as well as 7.6 million paired-end read with 2.0 Gb of sequence. These filtered reads were then assembled using Abyss (Simpson et al. 2009), which built 17,199 contigs with a total length of 40.0 Mb and an N50 of 6,093. This assembly was then annotated using the MAKER pipeline with protein homology data from *Aspergillus niger* ATCC 1015 v4.0, *Cladonia grayi* Cgr/DA2myc/ss v2.0, and *Cochliobolus heterostrophus* C5 v2.0 and ab-initio prediction using SNAP. Then, only contigs where the annotated gene with the best blastp score against *C. grayi* and *A. niger* proteins most closely matched *Cladonia grayi* were retained for the final reference genome to be used in all downstream analyses. This reference genome comprised 2,703 contigs with a total length of 19.5 Mb and had an N50 of 10,095. A total of 6,295 genes were annotated on these contigs. CEGMA (Parra et al. 2007) analysis of conserved gene content showed that 74% of universally
conserved genes are present in our assembly, suggesting that our assembly is approximately 74% complete. Consistent with this, our assembly was 53-70% as large as the three genomes available for other species in the Cladoniaceae (28 Mb—37 Mb; Armeleo and May 2009, Park et al. 2013).

*Cetradonia linearis Population Structure*

To call SNPs all read pools were aligned to the reference genome. A total of 126,662 SNPs were discovered. After correcting for linkage disequilibrium 10,026 SNPs remained. This large reduction in SNPs after correcting for linkage disequilibrium suggests a low rate of recombination. Most sampled individuals had data for >80% of the SNPs used for subsequent analyses, however, the total range spanned from 8.3—99.1% (Table 2.1). Nucleotide diversity (π) within populations ranged from 0.084 for one site in the Great Smoky Mountains, to 0.18 for one site in the Black Mountains (Table 2.1). When the samples were grouped by mountain range, π = 0.079—0.338 (Table 2.1). Pairwise Fst values between sites ranged from 0.312 to 0.731 (Table 2.2).

Population structure was first explored through simple relational analyses. The unrooted NJ tree recovered distinct, monophyletic clades that corresponded to distinct mountain ranges (Fig. 2.3). Sampling sites also largely formed monophyletic clades, except PV which is paraphyletic. The one paraphyletic PV sample formed a clade with SH, a site that was only 1.5 km downstream. Ten clusters were found as the most likely grouping of the samples using DAPC. Most clusters were comprised of all individuals from single sampling sites. Group four was the only one that included samples from multiple sites, for a total of 15 individuals from nine sites that included the Great Smoky Mountains, Balsam Mountains, Nantahala Mountains,
and Roan Mountain (Fig. 2.3). Each of the three sites sampled from the Black Mountains formed their own distinct group, despite their close proximity to each other.

The influence of geographic versus environmental distance on genetic distance was tested using two methods. First, a partial Mantel Test was used to test for correlations. There was a significant correlation between genetic distance, measured as pairwise Fst, and geographic distance, measured as pairwise Euclidean distance in km, where $r = 0.489$, and $p = 0.001$ (Fig. 2.4). There were no correlations between genetic distance and any of the environmental distances (Table 2.3). The second analysis was a Bayesian approach implemented in the program BEDASSLE (Bradburd et al. 2013). Here, the relevant value is the ratio of effect size of each environmental variable versus the effect size of the geographic distance ($\alpha_E:\alpha_D$). The results were similar to the partial Mantel test, and geographic distance far outweighed the effect of environmental distance. Specifically, the effect of 10°C mean temperature of the warmest quarter was equal to the effect of 0.026 km of geographic distance ($\alpha_E:\alpha_D = 0.026$), and the effect of occurrence in different habitats was equal to 0.71 km of geographic distance ($\alpha_E:\alpha_D = 0.712$; Table 2.4). Hindcasting the SDM of Cetradonia linearis supported the hypothesis that its refugial range was located predominantly in the southern edge of its current range during the LGM (Fig. 2.5). The quality of the SDM was high, with an AUC of 0.919.

**Mating System**

The mating system of Cetradonia linearis was provisionally determined to be unisexual. Unisexuality is a particular type of homothallism where only one of the two mating type genes are present in a species (Wilson et al. 2015). Unisexual fungi are able to form ascospores through self-fertilization or outcrossing, usually resulting in the production of 8 spores/ascus, as was observed in fertile apothecia of C. linearis (Wilson et al. 2015). A MAT 1-2-1 locus was
identified in the reference genome, and in total the MAT 1-2-1 was located in 14 of 32 total samples. The absence of the MAT 1-2-1 gene from some individuals may be due to the quantity of sequence data generated for those individuals. There was no evidence that a MAT 1-1-1 gene was present in any of the individuals, nor detected in any sample by aligning reads to other fungal MAT 1-1-1 loci. Examination of selected specimens did not result in the location of any trichogynes, but ascospores were observed. The current data are thus consistent with a unisexual mating system. However, further studies using single spore isolates will be required to fully assess the mating system of this species and confirm unisexuality (Scherrer et al. 2007).

**Photobiont Detection**

Very few SNPs were recovered when either algal genome was used as a reference. There were 2,412 SNPs detected when *Trebouxia gelatinosa* was used as a reference genome, and only 57 of those remained after loci with lower than 75% coverage were removed from the dataset. Three-hundred forty-eight SNPs were found when reads were aligned to the *Chlamydomonas reinhardtii* genome, and this number was reduced to 22 after equivalent coverage filtering. No further analyses were conducted with the photobiont SNP set because so few loci were recovered. Furthermore, because the SNP set was so small any contaminating reads or sequencing errors could strongly influence the outcome of analyses.

2.5 Discussion

This study is the first to report the results of a genomic approach for investigating the population genetics of a lichen. Low-coverage whole-genome sequencing of lichen fragments produced large quantities of SNP data (>122,000 SNPs) among individuals within a species, even after contaminants were removed by stringent filtering. This demonstrates that culturing is not required for lichen population genomics. The original hypothesis that the main reproductive
strategy of *Cetradonia linearis* is through clonal propagation was not supported as no clones were identified. However, there is evidence that the species only infrequently undergoes sexual recombination based on the high rates of linkage disequilibrium (~122K SNPs reduced to ~10K), and the putative unisexual mating system. The hypothesis that there are low rates of gene flow among populations was supported by high Fst values (0.312—0.731), significant correlation between genetic and geographic distance (Mantel Test, $r = 0.489$, $p = 0.001$), and proportionally higher influence of geographic distance on genetic distance when compared to environmental distance ($\alpha_E:\alpha_D < 1$). There was no evidence for isolation by environment based on the partial Mantel test and BEDASSLE results. The populations with the highest genetic diversity were concentrated in the southern portion of the range of the taxon, suggesting that these may have been refugial areas during the LGM. This hypothesis was supported by hindcasting an SDM for the species. Attempts at simultaneously analyzing SNPs from the photobiont failed, likely because 1) the photobiont comprises a smaller portion of the biomass of *C. linearis* than the mycobiont, and thus there was far less algal DNA in the extraction, 2) sequencing depth was too low to generate enough reads for the algal genome that is likely ~100 Mb (Armeleo and May 2009), and 3) most species in Cladoniaceae associate with algae from the genus *Asterochloris*, and while the closely related *Trebouxia gelatinosa* genome was used as a reference, it is unlikely conspecific with the photobiont of *Cetradonia linearis*. To capture simultaneous photobiont-mycobiont data steps must be taken to concentrate or isolate the photobiont data separately from the mycobiont, and ideally obtain a reference genome specifically for the photobiont species.

**Influence of Reproductive Strategy on Population Genetic Structure**

Three species of lichenized fungi have been subjected to detailed population genetic studies with particular attention paid to the mating-system (Itten and Honegger 2010, Singh et al.
Xanthoria parietina is unisexual, having only the MAT 1-2-1 gene present in all individuals investigated, and no observed instances of trichogynes, though it is almost always fertile (Scherrer et al. 2005). The population genetic structure of X. parietina based on RAPD-PCR fingerprinting revealed high rates of genotypic diversity within populations, even on a microsite scale, and much lower genetic diversity between populations than within them (Itten and Honegger 2010). A study of Parmelina carporrhizans found a similar pattern of very high gene flow among most populations sampled, though it is a heterothallic species (Alors et al. 2017). This pattern starkly contrasts with that of Lobaria pulmonaria, a heterothallic species that is often observed without sexual reproductive structures, and apothecia usually are not produced until individuals are 15—25 years old (Denison 2003, Hoistad and Gjerde 2011, Singh et al. 2012). Lobaria pulmonaria consistently displays high rates of clonality within populations and sampling sites (Werth et al. 2006, Sing et al. 2012). One way to explain the difference between the population genetic patterns of the two heterothallic species is the ratio of the two alternate MAT idiomorphs: L. pulmonaria ratios are often skewed in populations while P. carporrhizans populations have equal ratios (Singh et al. 2012, Alors et al. 2017). Population genetic structure and biology of Cetradonia linearis is more similar to X. parietina and P. carporrhizans because 1) it is almost always fertile, 2) no clones have been identified, even from closely collected colonies, and 3) there is a high level of polymorphism within each population ($\pi = 0.079–0.18$; Table 2.1). However, C. linearis populations seem to have low rates of gene flow among them, which contrasts with the pattern of low genetic distance found in both X. parietina and P. carporrhizans. These results, along with the high rate of linkage disequilibrium, suggest that while C. linearis does not seem to frequently reproduce
clonally, there must be some rate of self-fertilization and dispersal restriction that leads to the genetic isolation of populations.

Historically, two major hypotheses have shaped the perspective of fungal population structure and basic biology. First, because most fungi produce very small spores their dispersal is limited only by ecological suitability, and not by geographic distance (O’Malley 2007). Second, species in which no sexual reproductive structures have been observed are assumed to reproduce only asexually (Taylor et al. 2015). Phylogenetic and population genetic studies have already challenged these hypotheses in fungi that do not form lichens, and now population genomic studies are set to overturn them. For instance, in the common and widespread fungus *Suillus brevipes* there is evidence for IBD and adaptation of coastal populations to saline environmental (Branco et al. 2015). Species in *Saccharomyces* show varying levels of geographic structure in their genetic differentiation, with *S. paradoxus* showing clear evidence of IBD and *S. cerevisiae* showing much less geographic structure (Liti et al. 2009). Taylor et al. (2015) reviewed the literature on clonal reproduction in fungi, concluding that there evidence for recombination regardless of observed reproductive structures. For detailed reviews of population genetics and genomics in other groups of fungi see Grünwald et al. (2016) and Peter and Schacherer (2016). The results presented here further support that fungal populations do not necessarily have unlimited dispersal ability, despite the frequent production of small propagules. They also show that recombination can be low despite the frequent presence of sexual spore producing structures. This finding highlights the phenomenon that observed reproductive mode does not necessarily translate directly to the frequency of recombination.

*Biogeographic History*
The southern Appalachian Mountains are one of the oldest continuously exposed land masses on earth, and have served as a refugium at multiple points in geological history (Braun 1950). Thus, though it is a relatively small area, the long and complex geological history of the region has shaped similarly strong, complex population genetics patterns in endemic species across multiple domains of life (Manos and Meireles 2015). The population genetics of *Cetradonia linearis* are no exception. The southern portion of the current extent of *C. linearis* was likely a refugium during Pleistocene glaciation. The evidence to support this hypothesis is the higher genetic diversity in southern populations and location of suitable areas predicted by the hindcast SDM (Fig. 2.5). Interestingly, the model also suggested an expansion of the range to lower elevation areas (Fig. 2.5). This finding is consistent with hypotheses that ranges of present-day high-elevation endemics expanded downslope during Pleistocene glaciation (Crespi et al. 2003, Bruhl 1997, Premoli et al. 2007, Desamore et al. 2010). While this downslope expansion could have connected populations and diminished the signal of IBD, the data generated for this study still show a strong signal of IBD. A population genetic study of *Desmognathus wrighti*, a salamander sympatric with *C. linearis*, also showed a strong signal of IBD among all sampled populations (Crespi et al. 2003). Furthermore, populations of *D. wrighti* and *C. linearis* from Roan Mountain did not group with populations from the Black Mountains, despite their close geographic proximity (Crespi et al. 2003). A group of spiders in the genus *Hypochilus* sympatric with *C. linearis* also showed high levels of IBD (Keith and Hedin 2012). One species in particular, *H. pococki*, whose distribution is nearly the same as *C. linearis*, had such high levels of divergence among populations that the authors suggested it may actually be comprised of multiple cryptic species (Keith and Hedin 2012).

*Conservation Implications*
Low rates of recombination and significant IBD support the continued endangered status of *Cetradonia linearis* under the Endangered Species Act, and Vulnerable status on the IUCN Red-List (USFWS 2013, Allen et al. 2016). Though a putatively homothallic mating system would allow this species to both self and outcross, there is a low rate of effective gene flow among populations. Most dispersal seems to be over a short distance, either through fragmentation, selfing, or asexual fungal spores (conidia). While some populations are very large, composed of colonies that are multiple meters in diameter, if these colonies are clonal, or very closely related, then a rapid and drastic change in environmental conditions, or introduction of a pathogen could result in mass decreases in population size or whole population die-off (Spielman et al. 2004). In fact, after multi-year observations of populations at the southern edge of the range conducted during this project, it is clear that some colonies are experiencing mortality, and others have become overgrown with cyanobacteria, a likely indication that they are no longer healthy. Continued monitoring of this species is required to determine long term population dynamics, and detect any emerging threats.

2.6 Acknowledgements

I would like to thank the following people for aiding with this research: Dr. Elizabeth Alter for help designing and carrying-out the project, Sean McKenzie and Robin Sleith for help analyzing the data, Alex Cecil and Jenna Dorey for their field assistance, Dr. Richard Harris for searching for trichogynes and spores, Gary Kauffman for help locating populations throughout the National Forest and many helpful discussions about *Gymnoderma*, Chris Ulrey for help locating the species on Blue Ridge National Park land, and especially for repelling expertise. Library preparations and sequencing was conducted at The Rockefeller Genome Resource Center.
Funding for this research came from Highlands Biological Station, NSF GRFP, and NSF DEB#1145511.
2.7 Figures and Tables
Figure 2.1. (Preceding Page) Morphology, habit, and habitat of *Cetradonia linearis*. A) Large granite dome where species occurs at base of large rock faces, inset shows seeping rock faces where the species occurs; B) Stream habitat where species occurs frequently on scattered rocks and boulders throughout; C) Large boulder face covered in the species illustrating sampling protocol using sterile forceps; D) Fertile colony on mossy boulder in stream; E) Large rock outcrop hosting colonies of the species, inset shows view from *Cetradonia linearis* perspective; F) Waterfall populations are very abundant, one colony outlined by black box; G) Colony displaying apothecium and potential zoochory event.

Figure 2.2. Scatterplot showing results of Blobology pipeline used to identify contaminating reads.
Figure 2.3. Population genetic structure of *Cetradonia linearis*. A) DAPC plot showing first two principle components with samples clustered into 10 distinct gene pools as identified by clustering algorithm; B) Neighbor-joining tree based on bitwise distance; C) Spatial distribution of genetic clusters as recovered by DAPC.
Figure 2.4. Scatterplot of genetic vs. geographic distance and outcome of statically significant partial Mantel test.

Figure 2.5. Species distribution model of *Cetradonia linearis* A) in the present; B) at the last Glacial Maximum. Probability of *C. linearis* grades from blue (low) to red (high). Inset gives larger spatial orientation of study area.
Table 2.1. Site names, mountain range, individuals sampled, average percent SNPs covered for each site/mountain range (% Cov), and average nucleotide diversity for all sampled sites and mountain ranges.

<table>
<thead>
<tr>
<th>Site</th>
<th>Mtn. Range</th>
<th># Ind.</th>
<th>% Cov</th>
<th>( \pi )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>Blacks</td>
<td>2</td>
<td>98.9</td>
<td>0.180</td>
</tr>
<tr>
<td>B</td>
<td>Balsams</td>
<td>2</td>
<td>98.9</td>
<td>0.125</td>
</tr>
<tr>
<td>CD</td>
<td>Smokies</td>
<td>2</td>
<td>29.9</td>
<td>0.131</td>
</tr>
<tr>
<td>FC</td>
<td>Smokies</td>
<td>2</td>
<td>98.3</td>
<td>0.079</td>
</tr>
<tr>
<td>LG</td>
<td>Balsams</td>
<td>2</td>
<td>45.0</td>
<td>0.128</td>
</tr>
<tr>
<td>MG</td>
<td>Blacks</td>
<td>2</td>
<td>99.1</td>
<td>0.134</td>
</tr>
<tr>
<td>PK</td>
<td>Blacks</td>
<td>2</td>
<td>97.8</td>
<td>0.133</td>
</tr>
<tr>
<td>PV</td>
<td>Nantahalas</td>
<td>3</td>
<td>83.2</td>
<td>0.173</td>
</tr>
<tr>
<td>R</td>
<td>Roan</td>
<td>3</td>
<td>64.4</td>
<td>0.148</td>
</tr>
<tr>
<td>RP</td>
<td>Smokies</td>
<td>1</td>
<td>26.4</td>
<td>-</td>
</tr>
<tr>
<td>SC</td>
<td>Smokies</td>
<td>2</td>
<td>62.0</td>
<td>0.084</td>
</tr>
<tr>
<td>SH</td>
<td>Nantahalas</td>
<td>2</td>
<td>8.3</td>
<td>0.165</td>
</tr>
<tr>
<td>SI</td>
<td>Nantahalas</td>
<td>2</td>
<td>88.3</td>
<td>0.094</td>
</tr>
<tr>
<td>T</td>
<td>Nantahalas</td>
<td>3</td>
<td>52.6</td>
<td>0.164</td>
</tr>
<tr>
<td>WS</td>
<td>Nantahalas</td>
<td>2</td>
<td>15.1</td>
<td>0.178</td>
</tr>
<tr>
<td>-</td>
<td>Smokies</td>
<td>4</td>
<td>58.1</td>
<td>0.338</td>
</tr>
<tr>
<td>-</td>
<td>Nantahalas</td>
<td>6</td>
<td>52.6</td>
<td>0.329</td>
</tr>
<tr>
<td>-</td>
<td>Blacks</td>
<td>12</td>
<td>98.6</td>
<td>0.268</td>
</tr>
<tr>
<td>-</td>
<td>Balsams</td>
<td>3</td>
<td>71.9</td>
<td>0.208</td>
</tr>
<tr>
<td>-</td>
<td>Roan</td>
<td>7</td>
<td>64.4</td>
<td>0.148</td>
</tr>
<tr>
<td>All</td>
<td>All</td>
<td>32</td>
<td>66.0</td>
<td>0.328</td>
</tr>
</tbody>
</table>
Table 2.2. Pairwise Fst between all sampled sites.

<table>
<thead>
<tr>
<th></th>
<th>AC</th>
<th>B</th>
<th>CD</th>
<th>FC</th>
<th>LG</th>
<th>MG</th>
<th>PK</th>
<th>PV</th>
<th>R</th>
<th>RP</th>
<th>SC</th>
<th>SH</th>
<th>SI</th>
<th>T</th>
<th>WS</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC</td>
<td>0.568</td>
<td>0</td>
<td>0.515</td>
<td>0.621</td>
<td>0.523</td>
<td>0.507</td>
<td>0.447</td>
<td>0.510</td>
<td>0.578</td>
<td>0.457</td>
<td>0.601</td>
<td>0.481</td>
<td>0.572</td>
<td>0.565</td>
<td>0.497</td>
</tr>
<tr>
<td>B</td>
<td>0</td>
<td>0</td>
<td>0.568</td>
<td>0.662</td>
<td>0.528</td>
<td>0.629</td>
<td>0.621</td>
<td>0.487</td>
<td>0.554</td>
<td>0.524</td>
<td>0.657</td>
<td>0.546</td>
<td>0.595</td>
<td>0.553</td>
<td>0.581</td>
</tr>
<tr>
<td>CD</td>
<td>0.515</td>
<td>0</td>
<td>0.621</td>
<td>0.662</td>
<td>0.528</td>
<td>0.629</td>
<td>0.621</td>
<td>0.487</td>
<td>0.554</td>
<td>0.524</td>
<td>0.657</td>
<td>0.546</td>
<td>0.595</td>
<td>0.553</td>
<td>0.581</td>
</tr>
<tr>
<td>FC</td>
<td>0.621</td>
<td>0.568</td>
<td>0.621</td>
<td>0.662</td>
<td>0.528</td>
<td>0.629</td>
<td>0.621</td>
<td>0.487</td>
<td>0.554</td>
<td>0.524</td>
<td>0.657</td>
<td>0.546</td>
<td>0.595</td>
<td>0.553</td>
<td>0.581</td>
</tr>
<tr>
<td>LG</td>
<td>0.523</td>
<td>0.409</td>
<td>0.579</td>
<td>0.699</td>
<td>0.646</td>
<td>0.611</td>
<td>0.582</td>
<td>0.410</td>
<td>0.571</td>
<td>0.505</td>
<td>0.582</td>
<td>0.410</td>
<td>0.571</td>
<td>0.505</td>
<td>0.582</td>
</tr>
<tr>
<td>MG</td>
<td>0.507</td>
<td>0.634</td>
<td>0.662</td>
<td>0.699</td>
<td>0.646</td>
<td>0.611</td>
<td>0.582</td>
<td>0.410</td>
<td>0.571</td>
<td>0.505</td>
<td>0.582</td>
<td>0.410</td>
<td>0.571</td>
<td>0.505</td>
<td>0.582</td>
</tr>
<tr>
<td>PK</td>
<td>0.447</td>
<td>0.618</td>
<td>0.602</td>
<td>0.679</td>
<td>0.582</td>
<td>0.505</td>
<td>0.505</td>
<td>0.410</td>
<td>0.571</td>
<td>0.505</td>
<td>0.505</td>
<td>0.410</td>
<td>0.571</td>
<td>0.505</td>
<td>0.505</td>
</tr>
<tr>
<td>PV</td>
<td>0.510</td>
<td>0.478</td>
<td>0.487</td>
<td>0.579</td>
<td>0.699</td>
<td>0.611</td>
<td>0.582</td>
<td>0.410</td>
<td>0.571</td>
<td>0.505</td>
<td>0.582</td>
<td>0.410</td>
<td>0.571</td>
<td>0.505</td>
<td>0.582</td>
</tr>
<tr>
<td>R</td>
<td>0.578</td>
<td>0.554</td>
<td>0.651</td>
<td>0.699</td>
<td>0.558</td>
<td>0.655</td>
<td>0.638</td>
<td>0.527</td>
<td>0</td>
<td>0.442</td>
<td>0.607</td>
<td>0.546</td>
<td>0.595</td>
<td>0.505</td>
<td>0.505</td>
</tr>
<tr>
<td>RP</td>
<td>0.457</td>
<td>0.524</td>
<td>0.496</td>
<td>0.513</td>
<td>0.464</td>
<td>0.544</td>
<td>0.595</td>
<td>0.442</td>
<td>0.607</td>
<td>0</td>
<td>0.442</td>
<td>0.607</td>
<td>0</td>
<td>0.442</td>
<td>0.607</td>
</tr>
<tr>
<td>SC</td>
<td>0.601</td>
<td>0.657</td>
<td>0.654</td>
<td>0.631</td>
<td>0.707</td>
<td>0.688</td>
<td>0.676</td>
<td>0.563</td>
<td>0.611</td>
<td>0.546</td>
<td>0.657</td>
<td>0.546</td>
<td>0.595</td>
<td>0.505</td>
<td>0.505</td>
</tr>
<tr>
<td>SH</td>
<td>0.481</td>
<td>0.436</td>
<td>0.542</td>
<td>0.581</td>
<td>0.458</td>
<td>0.547</td>
<td>0.502</td>
<td>0.312</td>
<td>0.536</td>
<td>0.479</td>
<td>0.624</td>
<td>0</td>
<td>0.479</td>
<td>0.624</td>
<td>0</td>
</tr>
<tr>
<td>SI</td>
<td>0.572</td>
<td>0.592</td>
<td>0.610</td>
<td>0.662</td>
<td>0.607</td>
<td>0.648</td>
<td>0.633</td>
<td>0.462</td>
<td>0.664</td>
<td>0.539</td>
<td>0.696</td>
<td>0.495</td>
<td>0</td>
<td>0.495</td>
<td>0</td>
</tr>
<tr>
<td>T</td>
<td>0.565</td>
<td>0.553</td>
<td>0.628</td>
<td>0.667</td>
<td>0.573</td>
<td>0.641</td>
<td>0.624</td>
<td>0.344</td>
<td>0.644</td>
<td>0.569</td>
<td>0.717</td>
<td>0.381</td>
<td>0.558</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WS</td>
<td>0.497</td>
<td>0.431</td>
<td>0.581</td>
<td>0.601</td>
<td>0.484</td>
<td>0.571</td>
<td>0.551</td>
<td>0.346</td>
<td>0.543</td>
<td>0.513</td>
<td>0.655</td>
<td>0.409</td>
<td>0.503</td>
<td>0.425</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2.3. Results of partial Mantel test showing there is a significant relationship between genetic distance (Fst) and a geographic distance (km), but not between genetic distance and any environmental variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographic distance</td>
<td>0.489</td>
<td>0.001</td>
</tr>
<tr>
<td>Habitat (cliff vs. stream)</td>
<td>0.046</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Mean temp. of wettest quarter</td>
<td>0.196</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Mean temp. of warmest quarter</td>
<td>0.07</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Annual precipitation</td>
<td>0.196</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

Table 2.4. Results of BEDASSLE showing that more genetic differentiation among populations is due to their geographic rather than environmental distance.

<table>
<thead>
<tr>
<th>Env. Variable</th>
<th>αE:αD (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat (cliff vs. stream)</td>
<td>0.712 (0.544, 0.978)</td>
</tr>
<tr>
<td>Mean temp. of warmest quarter</td>
<td>0.026 (0.020, 0.036)</td>
</tr>
</tbody>
</table>
Chapter 3. Climate change impacts on endemic, high-elevation lichens in a biodiversity hotspot

3.1 Abstract

Previous studies of the impacts of climate change on lichens and fungi have focused largely on alpine and subalpine habitats, and have not investigated the potential impact on narrowly endemic species. Here, I estimate the impacts of climate change on high-elevation, endemic lichens in the southern Appalachians, a global diversity hotspot for many groups of organisms, including lichens. I conducted extensive field surveys in the high elevations of the region to accurately document the current distributions of eight narrowly endemic lichen species. Species distribution modeling was used to predict how much climatically suitable area will remain within, and north of, the current range of the target species under multiple climate change scenarios at two time points in the future. My field work showed that target species ranged from extreme rarity to locally abundant. Models predicted over 93% distributional loss for all species investigated and very little potentially suitable area north of their current distribution in the coming century. My results indicate that climate change poses a significant threat to high-elevation lichens, and provide a case study in the application of current modeling techniques for rare, montane species. This chapter was originally published in Biodiversity and Conservation (Allen and Lendemer 2016a).

3.2 Introduction

High-elevation mountain ecosystems have been, and will continue to be, severely impacted by climate change. Distributional shifts of high-elevation species have already been documented, with species transitioning to higher elevations and becoming extirpated at the lower elevational limits of their ranges (Parmesan 2006, Colwell et al. 2008, Kelly and Goulden 2008,
Harsch et al. 2009). As the climate continues to change, rates of distributional shifts, habitat loss, and extinction are predicted to increase in mountain ranges worldwide (Colwell et al. 2008, Raxworthy et al. 2008, Dirnböck et al. 2011, Dullinger et al. 2012). Although estimates of extinction vary widely among studies and mountain ranges, they have consistently concluded that extinction rates for high-elevation endemics will be disproportionately high compared to species with other distributions (Raxworthy et al. 2008, Dirnböck et al. 2011, Dullinger et al. 2012). Even models that assume an optimistic scenario of unlimited dispersal ability predict that high-elevation endemics will face significant habitat loss and threat of extinction (Dullinger et al. 2012). It is thus well established that high-elevation endemics are threatened by climate change, and estimates of climate change impacts for specific mountain ranges and taxonomic groups are essential to understand the degree of threat and the potential for conservation actions.

Species distribution modeling is one important approach that has frequently been used to predict how climate change will impact biodiversity (Thuiller et al. 2008, Elith and Leathwick 2009, Merow et al. 2013). Species distribution modeling methods are widely used because of their applicability to presence-only data, which facilitates use of the immense amount of data in biological collections, and evidence for robust performance across diverse groups of organisms and geographic regions (Merow et al. 2013). However, a number of difficulties remain in modeling distributions of rare and montane species. Rare species will always have a limited number of documented localities, which is not ideal for building models, but they are the species for which models are most needed (Lomba et al. 2010). High climatic and geological heterogeneity in montane ecosystems make capturing the full complexity of factors influencing species distributions in such species problematic (Rull 2009, Dobrowski 2011, Spasojevic et al. 2013). Despite these challenges, investigation and estimation of the impacts of climate change on
rare, montane species, is essential as these organisms will likely be disproportionately negatively impacted by climate change.

The Appalachian Mountains are a prominent mountain system that spans much of the latitudinal gradient of eastern North America, from Alabama in the United States to Newfoundland in Canada. This range is among the oldest continuously exposed land masses on Earth and is a well-documented center of both diversity and endemism for many groups of organisms (Braun 1950, Pickering et al. 2003). The phenomena of biodiversity and endemism are especially pronounced in the southern portions of the Appalachian Mountains (ATBI 2014, Pickering et al. 2003), which also host multiple globally threatened ecosystems (Noss et al. 1995, Rollins et al. 2010, McManamay et al. 2011, White et al. 2012). Long-term studies in the region have found evidence for warming, drying, and an increased height of the clouds that engulf mountain peaks on a daily basis (Richardson et al. 2003, Wear and Greis 2011, Laseter et al. 2012). In addition to climate change, these ecosystems have been, and continue to be, significantly impacted by invasive pests, logging, and acid rain and fog (Kenis et al. 2009, White et al. 2012, Culatta and Horton 2014). The highest elevations of the southern Appalachians, areas above 5,000 ft (~1,500 m), in particular are inhabited by many rare and endemic species, such as the iconic Fraser fir (*Abies fraseri*), a conifer now widely cultivated for sale as Christmas trees, and the lesser known spruce-fir moss spider (*Microhexura montivaga*) (USFWS 1998).

The southern Appalachians are a hotspot for lichen diversity in North America (Lendemer et al. 2013), and the high elevation habitats in particular host abundant and unique communities, including many narrow endemics (Dey 1978, DePriest 1984, Lendemer et al. 2013). Understanding how lichens will be impacted by climate change provides a valuable perspective on the broader ecosystem because they occupy a key place in the web of biotic
interactions. They host diverse and unique communities of microbes and invertebrates within their thalli, provide food and nesting material for vertebrates, and contribute significant carbon and nitrogen to nutrient cycles (Henderson and Hacket 1986, Brodo et al. 2001, Arnold et al. 2009, Hodkinson and Lutzoni 2009). While there has been limited research on climate change impacts on lichens (Aptroot and Herk 2007, Bjerke 2011, Ellis et al. 2014, Lendemer and Allen 2014), much of which is restricted to alpine and subalpine habitats (Klanderud and Totland 2005, Klanderud 2008, Crabtree and Ellis 2010), there has yet to be an assessment of how severely high-elevation endemics will be affected.

Here I present the first assessment of how severely rare, endemic high-elevation lichens will be impacted by climate change. I performed extensive field studies to accurately map the current distribution of eight target species throughout the region, and used these data, along with all existing herbarium records, to predict how abiotically suitable areas will shift for these species over the next 55 years. Many of the target species were known exclusively or nearly exclusively from the Great Smoky Mountains National Park (GSMNP) before I began this project. I expected to find populations of most of these species in high elevations outside of GSMNP, though less frequently since GSMNP includes the largest and healthiest stands of old growth forests remaining in eastern North America (White et al. 2003). I also expected that the models would predict a significant loss of climatically suitable area for all species within their current distributions, and aimed to quantify loss within this century (2050 and 2070). The results of this study further illustrate the utility of a suite of modeling tools that can be applied to rare, montane species, and provide insight for conservation of this unique ecosystem.

3.3 Methods

Field Work and Locality Data
Six weeks spanning June, September, and October 2014 were spent searching for eight high-elevation, southern Appalachian endemic lichen species. The eight species (Fig. 1) were selected based on a combination of 1) having distinctive morphologies that could easily be detected and confirmed in the field, 2) evidence of rarity based on the number of previous collections and documented range previous to beginning this work, 3) representing the diverse and unique growth forms and life histories of regional lichens, and 4) robust published evidence from large scale biodiversity inventories, floristic treatments and taxonomic revisions that the species were endemic to the study area (e.g., Lendemer and Allen 2015). Three are corticolous species narrowly restricted to a single phorophyte species on which they are only found when the trees are very mature: *Arthonia kermesina* on *Picea rubens*, and two species that grow on *Betula alleghaniensis, Arthopyrenia betulicola* and *Graphis sterlingiana*; two corticolous species that are not specific to a single phorophyte: *Hypotrachyna virginica*, which grows most abundantly on *Abies fraseri*, but can also be found on hardwoods and ericaceous shrubs, and *Lecanora masana* which grows most frequently on *Abies fraseri* and ericaceous shrubs. The remaining three species are saxicolous. *Buellia sharpiana* and *Cladonia appalachensis* grow on Anakeesta rock, which is a metal rich rock that outcrops primarily at high-elevations only in a narrow ridge of GSMNP. The final species is *Lepraria lanata*, which is not restricted to a single rock type, but is only found in the cool, humid high-elevations.

To survey for these species, nearly all the highest elevation ridges and mountains of North Carolina, including the Great Smoky Mountains, Balsam Mountains, Black Mountains, and Roan Mountain were visited during six weeks of field work in 2014. Approximately 170 miles were covered on foot, both on and off trail, and 99 sites were inventoried (Fig. 3.2). A small voucher of each target species was collected at each site and deposited in herbarium of The
New York Botanical Garden (NY). Thin layer chromatography was performed to confirm the identifications of *C. appalachensis*, *H. virginica* and *L. lanata* following standard protocols of Culberson and Kristinsson (1970) with modifications according to Lendemer (2011). All populations discovered during 2014 field work were included in modeling analyses. Additionally, all specimens of the target species held by NY were examined and included in analyses, along with records from the Consortium of North American Lichen Herbaria (CNALH) online database.

**Modeling Methods**

Two target species, *Buellia sharpiana* and *Cladonia appalachensis*, remained known from fewer than five localities after our field work, most of which were closely clustered. Thus, there were only effectively two or three locations each and the species were excluded from our study because of the small number of known locations. For the remaining six target species the following modeling protocols were applied. Models were built using Maxent with all 19 variables from the worldclim dataset at the highest available resolution, which is 10 arc seconds (~1 km) (Hijmans et al. 2005, Phillips et al. 2006, Phillips and Dudik 2008). I chose to use Maxent because it has been shown to work well for modeling species with very few known localities (Elith et al. 2006, Wisz et al. 2008, Elith and Graham 2009). The worldclim variables are all derived from weather station measurements of temperature and precipitation, which works well in eastern United States as there is a high density of weather stations. Locality data were thinned with a 5 km radius rule to minimize spatial autocorrelation using the R package spThin, which randomly removes points that are within the given radius and iterates over this process the number of times specified by the user (in this case 1,000) and returns the top five results that retain the most localities (Kramer-Schadt et al. 2013, Aiello-Lammens et al. 2014). The
modeling extent used was the minimum convex hull with a ~50 km buffer around the localities for each species, thus eliminating potential areas that are climatically similar but beyond our current knowledge of the dispersal ability of species (Anderson and Raza 2010). When modeling rare species distributions in Maxent the default settings often result in overfit models (Shcheglovitova and Anderson 2013). Model tuning to find the best level of complexity for each species followed the work of Shcheglovitova and Anderson (2013), in which the feature class (allowed shape of response curve) and regularization multiplier (a value that influences the level of complexity) are varied to produce a suite of models, which are then evaluated to choose the best parameters. To find the correct level of model complexity the feature class (linear (L), linear and quadratic (LQ), linear, quadratic and hinge (LQH), and hinge (H)) and the regularization multiplier (0.5, 1, 1.5, 2, 2.5, 3, 3.5, and 4) were varied, and k-1 crossvalidation, also known as leave-one-out crossvalidation, was performed, as implemented in the R package ENMEval (Muscarella et al. 2014). In this method of crossvalidation only one locality is used to test the model, which is built using all other localities. This process is performed iteratively until each locality is used to test the model once, and this process is repeated for each different combination of feature classes and regularization multipliers. The resulting models were evaluated using AICc (Warren and Seifert 2011), and the model parameters that resulted in the lowest AICc value were chosen for each species.

Once the best model complexity was chosen, models were rerun for all species using all known localities, and projected to 2050 and 2070 with a spatial extent including most of the southeastern United States. In order to model the greatest range of possibilities two scenarios that predict very different conditions for the future of the southern Appalachians were chosen, CCMS44 and HadGEM2-AO (Mitchell et al 2014). The lowest and highest representative
concentration pathway (rcp 2.6 and 8.5) for each model at each time point were used. Climate change data were from Hijmans et al. (2005).

Modeling resulted in a total of nine models for each species: the present, 2050 CCMS4 2.6, 2050 CCMS4 8.5, 2070 CCMS4 2.6, 2070 CCMS4 8.5, 2050 HadGEM2-AO 2.6, 2050 HadGEM2-AO 8.5, 2070 HadGEM2-AO 2.6, and 2070 HadGEM2-AO 8.5. To calculate the area included in the species distribution, all models were converted to binary suitable/unsuitable based on the equal training sensitivity and specificity threshold, both before and after clipping to the extent of the current distribution of each species (Liu et al. 2013). Climatically suitable areas were calculated using R for the present, future within the current distribution of the species, and future throughout the southeastern United States (R Development Core Team 2008). Binary rasters for the southeastern United States were summed for each time period in R. Maps were created in R and QGIS (QGIS Development Team 2014), and tables were created in Microsoft Excel. Light micrographs were taken with a Nikon DS-Ri1 digital camera attached to a Nikon SMZ1500 dissecting microscope and using NIS-Elements 4.3 software. Figures were formatted using Inkscape (www.inkscape.com) and gimp (gimp.org). All R codes were deposited at GitHub and can be accessed at the following URL:

https://github.com/jallen73/Rare_spp_distr_modeling.git.

3.4 Results

Field Work, Model Complexity and Quality

At least one previously unknown population was discovered for all targeted endemic species (Table 3.1). After pooling all known records, past and present, for each species, and performing spatial filtering, the following numbers of localities were used for modeling each species distribution: nine Arthonia kermesina, seven Arthopyrenia betulicola, eight Graphis
sterlingiana, 11 Hypotrachyna virginica, 24 Lecanora masana, and eight Lepraria lanata. Models for the present had AUCs >0.97 for all species after tuning. The chosen model complexity and resulting AUC values of the model for each species were LQ1.5 and 0.978 for A. kermesina, LQ1.5 and 0.983 for A. betulicola, LQ2.5 and 0.988 for G. sterlingiana, L0.5 and 0.998 for H. virginica, L1 and 0.99 for L. masana, and L1 and 0.976 for L. lanata. There was evidence that the models overpredicted the currently suitable habitat for these species, indicating they are conservative estimates and that any subsequent climate change models would also overpredict suitable area (Supplement 1; Martinez-Meyer 2005).

**Climate Change Projections**

The projected models predicted significant losses of climatically suitable area within the current distributions of all studied species, regardless of the time frame and climate change model used (Table 3.1; Fig. 3.3 — 3.4). This is particularly striking given the aforementioned evidence that the models overpredict the species distributions. *Arthonia kermesina* is projected to lose between 75% and 100% of suitable area within its current distribution, with an average of 93.7% (± 9.8) loss. *Arthopyrenia betulicola* is projected to lose all currently occupied area for all models except CCMS4 rcp 2.6 in 2070, which predicted a 97% loss. Losses for *Arthopyrenia betulicola* average to 99.7% (± 0.9). Between 92% and 100% of currently occupied area is predicted to be lost for *Graphis sterlingiana*, and the average predicted loss is 97.6% (± 3.3) (Fig. 3.3). *Hypotrachyna virginica* is predicted to lose an average of 94.3% (± 5.7) of its current distribution, with values ranging from 83% to 100%. Losses for *Lecanora masana* range from 81% to 100%, with an average of 95.9% (± 6.6). Lastly, *Lepraria lanata* is predicted to lose between 79% and 100% of its currently occupied area, with an average of 95.4% (± 8.0). All of these predicted losses are striking, regardless of the climate model used, as the average loss for
all species is above 90%, and at least one scenario results in loss of all currently occupied area for every species.

Models were projected throughout the southeastern United States to identify areas outside of the current ranges of species that will likely be suitable in the future (Fig. 3.4). The predicted amount of suitable area outside of the current distribution varied widely among species, with *Hypotrachyna virginica* predicted to have the least suitable area (0 — 102 km²) and *Arthonia kermesina* predicted to have the most (315 — 175,875 km²). Because the studied species are almost completely sympatric, and form an important portion of high-elevation lichen communities, they can be considered together to identify areas north of the southern Appalachians which are predicted to be suitable by the majority models in the future. When all the binary models were summed they predicted that eastern West Virginia and Pennsylvania will have the most climatically suitable area in the future (Fig. 3.4).

### 3.5 Discussion

Modeling the distributions of rare, montane species and predicting how their distributions will shift with climate change are challenging but important tasks (Lomba et al. 2010). Here I applied a suite of recent methods developed to improve the predictive abilities of Maxent (Scheglovitova and Anderson 2013, Aiello-Lammens et al. 2014, Muscarella et al. 2014) to a set of narrowly endemic, high-elevation lichen species in the southern Appalachians. The workflow performed well (all AUCs > 0.97, Supplement 1) and predicted drastic losses in abiotically suitable area for all species in the coming century, with estimates ranging from 75 — 100%. These findings contribute to ongoing discussion on modeling rare species distributions, environmental variable selection, and the scale of distribution determining processes. The estimates of suitable area loss serve as a starting point for future research on climate change
impacts to this ecosystem, and further our understanding of climate change impacts to lichens more broadly.

Modeling rare species distributions is challenging due to them unavoidably having few localities, and thus limited information on their ecological tolerances. Yet, these are the very species where greater understanding of their distribution and ecology is essential for their conservation (Lomba et al. 2010). Because of this “rare species paradox,” significant advancements have been made specifically to improve models of rare species distributions. Here I chose to use Maxent because it has been shown to perform well when modeling species with few localities (Elith et al. 2006, Elith and Graham 2009), and specific methods have been developed to increase the quality of the models and address common problems (Lomba et al. 2010, Anderson and Gonzalez 2011, Scheglovitova and Anderson 2013, Muscarella et al. 2014). I found evidence that the models were performing well despite the small sample size. Models built in a smaller area and projected in geographic space with only the localities known before we began this study predicted the occurrence of populations with zero percent false negative rate, though the false positive rate varied (Supplement 1). This, along with high AUC values, suggests that models performed well, were transferable, and in fact likely serve as conservative estimates of suitable area loss since there is evidence that they overpredict the species distributions. Future studies using a variety of modeling techniques as methods continue to advance for rare species applications will further inform these predictions.

Climate, land use history, pollution, and biotic interactions all shape species distributions (Peterson et al. 2011). Previous studies on factors that shape lichen distributions in this region found that temperature and rainfall were important variables, and that pollution, urbanization, and forest stand age also contributed to diversity patterns (McCune et al. 1997). A recent study
showed the importance of the presence of lichen species sharing symbionts in shaping distributions at a fine spatial scale (Belinchón et al. 2015). Here we modeled species distributions using only climatic variables for a number of reasons. First, we opted to use only direct variables, rather than including variables such as altitude that indirectly influence the climate (Austin 2002). Second, adding additional variables to models for species with few known localities often leads to overfit models (Radosavljevic and Anderson 2014). Third, we were attempting to produce a conservative estimate of the impact of climate change. Including non-climatic variables, such as developed areas or phorophyte, would provide complementary insight into the ecology of these species. Such a study will prove useful for comparing variable selection methods when the required spatial data are available in the future.

High-elevation forests in the southern Appalachians are thought to be islands of Pleistocene refugia, where climatic conditions and historical contingency have fostered the evolution of endemic species and continued existence of disjunct populations from the northern boreal forests (Rollins et al. 2010, Lendemer and Harris 2013). In the Anthropocene they may once again serve as refugia, and specifically microrefugia (Rull 2009, Dobrowski 2011). Previous studies in montane ecosystems have investigated how habitat heterogeneity influences species responses to climate change, many of which have concluded that the heterogeneity created by the complex landscape is not fully captured in species distribution models (Dobrowski 2011, Spasojevic et al. 2013). For instance, a 20-year study of alpine plant communities in the Alps found that communities did not move directionally despite macroclimatic changes in the region, but rather appeared to track smaller scale processes (Spasojevic et al. 2013). Here we found evidence for extreme reduction in suitable area for species in the future on a macroclimatic scale, using a method that likely does not detect areas where small microclimatic refugia may remain.
Assuming that species will persist in microrefugia, it is unclear whether or not viable populations would remain, or how long the species will persist in such microrefugia. Regardless of the potential for persistence in microrefugia, our results predict that there will be significant losses to climatically suitable areas for all species modeled here, resulting in increased rarity and extinction risk.

Previous research on the impacts of climate change on lichens have found or predicted significant impact to species and communities, many of which are explored in a recent review (Ellis 2013). For instance, in the Netherlands lichens associated with Trentepohlia as the photobiont have increased, and soil-inhabiting species have decreased (Aptroot and van Herk 2005). There is evidence that Flavoparmelia caperata has established populations further north in Denmark as the climate has warmed over the past century (Søchting 2004). Other studies have focused on lichens as a group, rather than individual species, and have found evidence for future decline of lichens in montane ecosystems with climatic warming. For instance, an artificial warming experiment in the Alps found that lichen cover decreased significantly (Klanderud and Totland 2005), and after four years of warming 44% of lichens had disappeared (Klanderud 2008). In another study conducted in Scotland, projected warming predicted that Arctic and alpine lichens will move upslope (Crabtree and Ellis 2010). My study contributes further evidence for the sensitivity of lichens to climate change, and adds a unique perspective on montane endemics in a global biodiversity hotspot.

The high-elevation ecosystems in the southern Appalachians are highly threatened by numerous anthropogenic forces (Rollins et al. 2010, McManamay et al. 2011, White et al. 2012). Our results further support the endangered status of these ecosystems, and specifically the threat posed by climate change in the coming century. All species investigated here are predicted to
lose considerable suitable area within their current distributions by 2070, regardless of the climate projection used. These findings have implications for the broader communities inhabited by these species, including other lichens, microorganisms that inhabit the lichens (Arnold et al. 2009, Hodkinsons and Lutzoni 2009), and plants and animals that inhabit these ecosystems with similar ecological tolerances regardless of their interactions with the lichens. Importantly, the results of this study point to the need for further investigation into the impacts of climate change on other species and groups of organisms in this ecosystem to identify patterns and differences in the predicted impact of climate change and to develop a more holistic view of the future of this region. This will likely prove challenging as many species in this ecosystem are rare, narrowly endemic, or both. As distribution modeling techniques progress, continually revising predictions, and monitoring communities throughout the region simultaneously will provide essential information for the conservation of these ecosystems and contribute to broader understanding of changing montane ecosystems in the coming century.

3.6 Acknowledgements

We thank Drs. Robert Anderson, Richard Harris, and Erin Tripp for helpful discussion regarding this project. We would also like to thank Sean McKenzie and Jenna Dorey for their field work assistance. We appreciate the work of Gary Kauffman (USFS), Paul Super (GSMNP), Ed Corey (NCSP), and The Blue Ridge Parkway for the issuance of permits. This research was supported by the National Science Foundation Graduate Research Fellowship, Highland Biological Station Science and Society Fellowship, the Southern Appalachian Botanical Society, and the City University of New York Doctoral Student Research Grant.
3.7 Figures and Tables

Figure 3.1. Targeted high-elevation, endemic lichen species. A) *Arthonia kermesina* (scale=0.5 mm), B) *Arthopyrenia betulicola* (scale=1 mm), C) *Buellia sharpiana* (scale=1 mm), D) *Cladonia appalachensis*, E) *Graphis sterlingiana* (scale=1 mm), F) *Lecanora masana* (scale=1 mm), G) *Lepraria lanata* (scale=1 mm), H) *Hypotrachyna virginica* (scale=2 mm).

Figure 3.2. Sites sampled for this study. Orange circles show the 99 sites that were sampled for this project. Gray shading shows the elevation, with darker gray areas indicating higher elevation.
Figure 3.3. Examples of distribution models for *Graphis sterlingiana* E.A. Tripp & Lendemer. The top left shows populations documented before 2014 (white circles) and during 2014 field work (black circles). All other maps show species distribution models for *G. sterlingiana* under different climate change models and RCPs. Habitat suitability ranges from unsuitable (lightest orange) to highly suitable (darkest orange).
Figure 3.4. Larger geographic area highlighting suitable areas for multiple species, the disappearance of suitable habitat in the southern Appalachians, and the agricultural (yellow) and developed areas (red) that will likely act as dispersal barriers among forested areas (green). Top left, land use and land cover for region of interest, showing agricultural and developed areas that are potential barriers to dispersal for southern Appalachian endemics (Jin et al. 2013). The remaining three maps were created by converting all species distribution models to binary and summing results. These maps highlight areas that are not suitable for any species (lightest orange) and areas that are suitable for the most species (darkest orange), along with protected areas (green shading).
### Table 3.1. Records and land area estimates by species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Pre-2014 Records</th>
<th>2014 Records</th>
<th>2015 (km²)</th>
<th>2050 (km² / %)</th>
<th>2070 (km² / %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CCSM 4.2.6</td>
<td>CCSM 4.8.5</td>
<td>CCSM 4.2.6</td>
</tr>
<tr>
<td>Arthania kermesina</td>
<td>12</td>
<td>4</td>
<td>2470</td>
<td>21 / 99.1%</td>
<td>2.1 / 99.9%</td>
</tr>
<tr>
<td>Arthropycenia betulicola</td>
<td>6</td>
<td>7</td>
<td>1217</td>
<td>0 / 100%</td>
<td>0 / 100%</td>
</tr>
<tr>
<td>Buellia sharpiana</td>
<td>1</td>
<td>1</td>
<td>NA</td>
<td>NA NA NA NA NA</td>
<td>NA NA NA NA NA</td>
</tr>
<tr>
<td>Cladonia appalachensis</td>
<td>3</td>
<td>1</td>
<td>NA</td>
<td>NA NA NA NA NA</td>
<td>NA NA NA NA NA</td>
</tr>
<tr>
<td>Graphis steringiana</td>
<td>7</td>
<td>6</td>
<td>1331</td>
<td>99 / 92.6%</td>
<td>2.8 / 99.8%</td>
</tr>
<tr>
<td>Hypotrachyna virginica</td>
<td>15</td>
<td>7</td>
<td>584</td>
<td>64 / 89.0%</td>
<td>47 / 92.0%</td>
</tr>
<tr>
<td>Lecanora masana</td>
<td>20</td>
<td>38</td>
<td>2442</td>
<td>204 / 91.7%</td>
<td>99 / 95.9%</td>
</tr>
<tr>
<td>Lepraria lanata</td>
<td>11</td>
<td>9</td>
<td>1624</td>
<td>6 / 99.7%</td>
<td>0 / 100%</td>
</tr>
</tbody>
</table>
Chapter 4. Quantifying the impacts of sea-level rise on coastal biodiversity: A case study on lichens in the Mid-Atlantic Coast of eastern North America

4.1 Abstract

Preliminary, large-scale assessments of global sea-level rise (SLR) have predicted significant impacts to coastal and island biodiversity. Region-specific estimates of SLR impacts that incorporate accurate species distributions and local rates of SLR are now required for effective conservation planning. Here I use a dataset of >13,000 occurrence records for lichens, obligate symbiotic fungi, in the Mid-Atlantic Coastal Plain of eastern North America to model distributions of 193 species. The resulting models were used to quantify the amount of each species’ distribution that is occupied by unsuitable land use types, along with the potential area that will be lost to SLR. We show that species have likely already lost an average of 32% of their distributional area to development and agriculture, and are predicted to lose an average of 12.4 and 33.7% of their distributional area with one foot (~0.3 m) and six feet (~1.8 m) of SLR, respectively. Functional and taxonomic groups were compared to identify specific effects of SLR. We show that species reproducing with symbiotic propagules have significantly larger distributions than species that reproduce sexually with fungal spores alone, and that the sexually reproducing species are predicted to lose greater distributional area to SLR. *Cladonia* species occupy significantly less area in the MACP than *Parmotrema* species and are predicted to lose more of their distributions to SLR. We further examined patterns of total species diversity and found that the area with the highest diversity is the Dare Peninsula in North Carolina, which is also predicted to lose the most land area to SLR. The workflow established here is flexible and applicable to estimating SLR impacts worldwide and can provide essential insights for local
conservation planning. This chapter was originally published in Biological Conservation (Allen and Lendemer 2016b).

4.2 Introduction

Sea-level rise (SLR) poses a considerable threat to human population and infrastructure, and both island and coastal biodiversity (Peters et al. 1985, Dobson et al. 1989, Lovejoy and Hannah 2005, Menon et al. 2010). The global average sea level has risen 0.19 m since 1901 (IPCC 2014), and has already negatively impacted coastal communities and biodiversity through loss of land area and increased flooding (FitzGerald et al. 2008, Nicholls et al. 2011). Estimates for how much further the sea will rise globally by 2100 range from 0.26—0.82 m (IPCC 2014), to 0.5—1.4 m (NRC 2012), and 0.7—1.4 m (Schaeffer et al. 2012). Halting greenhouse gas emissions immediately is not expected to slow SLR within the next 50 years, and would likely only lead to a moderate reduction in the SLR rate by 2100 (Schaeffer et al. 2012). As such, SLR poses a threat to coastal regions regardless of greenhouse gas emission scenarios or reductions therein. Island biodiversity is particularly threatened because many islands host high numbers of narrowly endemic species and will lose significant land area under even conservative SLR estimates (Menon et al. 2010, Bellard et al. 2014). However, coastal continental species are also at risk (Menon et al. 2010, Garner et al. 2015).

Previous research on SLR impacts on biodiversity has provided key insights for large-scale threat patterns, but has been limited by incomplete knowledge of species distributions (Menon et al. 2010, Bellard et al. 2014). Natural history collections contain vast quantities of information about the Earth’s past and present biodiversity that can be used to accurately model species distributions (Graham et al. 2004). Recent advances in computing have led to a suite of tools that are used to build species distribution models (SDMs) based on natural history
collection data (Phillips et al. 2009). These techniques have been used to study diverse topics including agriculture (e.g., Davis et al. 2012), biogeography (e.g., Besnard et al. 2016), and conservation (e.g., O’Connell et al. 2004, Bartomeaus et al. 2013), unlocking a new dimension of natural history collection utility. One of the most widespread uses of these models has been to predict how species distributions will be affected by changes in temperature and precipitation (Elith et al. 2010). However, these techniques have only recently begun to be applied to studies of SLR impacts on biodiversity (Garner et al. 2015).

The Mid-Atlantic region of eastern North America has one of the highest rates of SLR globally, and rapid SLR acceleration has been documented over recent decades (Kemp et al. 2009, Boon et al. 2012, Sallenger et al. 2012, Miller et al. 2013). Data collected at coastal stations from Nova Scotia to Florida over the past 75 years found a significant, non-linear acceleration in SLR from Virginia to Nova Scotia, and projected a 0.2—0.9 m increase in mean sea-level within the next 50 years (Boon et al. 2012). Evidence for similar SLR rates in the region was found in salt marshes in North Carolina, where an abrupt 2.2 mm/year increase in SLR began between 1897 and 1915 (Kemp et al. 2009). The underlying geology in the region also influences SLR rates. In the Mid-Atlantic the sea-level will rise 0.1—0.12 m more in coastal plain areas, which are not underlain by bedrock, than in those that are underlain by bedrock, with a total rise of 0.76—1.80 m predicated by 2100 (Miller et al. 2013). Ultimately, in the Mid-Atlantic south of Boston the sea-level is rising three to four times faster than the global average (Sallenger et al. 2012).

Hurricanes and severe storms in the Mid-Atlantic compound the impacts of SLR and lead to even greater habitat loss for coastal species. Sea-level rise of 0.5 m would lead to a 4—13% increase in inundation during storms and a rise of 0.82 m would result in 7—20% increase
(Maloney and Preston 2014). More frequent and severe tropical cyclones are also predicted due to increases in sea-surface temperature, though these predictions are less certain than predictions for SLR (IPCC 2014). In the Mid-Atlantic Coastal Plain (MACP), a subset of the entire Mid-Atlantic region comprised of a vast, low-lying area spanning from southern New Jersey through South Carolina (Michener et al. 1997). A unique maritime forest type occurs on barrier islands and supports a distinct community of lichens (Lendemer et al. 2016). If the barrier islands migrate more quickly than the maritime forests are able to, or if they are breached at the few remaining stands of older maritime forest, it would likely have negative consequences for the associated biota as revegetation may or may not be possible depending on multiple interacting biophysical processes (Vincent and Moore 2015). Together, the impacts of SLR and changes in hurricane frequency and intensity are poised to drastically reshape this landscape and the biodiversity that inhabits it (FitzGerald et al. 2008).

Here we built SDMs using a dataset derived from a large-scale inventory and natural history collections to 1) quantify the estimated regional impacts of SLR on biodiversity, 2) identify local biodiversity hotspots, 3) locate subsets of the region that are most threatened by SLR, and 4) identify functional traits or taxonomic subgroups that are most imperiled. The MACP is an ideal region to conduct this study because SLR poses a serious threat and a high quality data set was established through our recently completed large-scale inventory to document the lichen diversity, an important yet understudied components of coastal systems. We used >13,000 occurrence records to build SDMs for 193 lichen species and used National Oceanic and Atmospheric Administration SLR models to calculate how much area each species will lose to one foot (~0.3 m) and six feet (~1.8 m) of SLR. The resulting estimates were analyzed to identify the most species-rich and most threatened areas, and trait or taxonomic
groups that will likely be disproportionately impacted. Though there is uncertainty in both the species distribution and SLR models, the novel application of current knowledge and technical abilities to predict and quantify impacts on biodiversity provides useful insights and working hypotheses.

4.3 Methods

Sampling and Dataset Assembly

An inventory of the lichen diversity of the MACP was conducted between 2010 and 2015. During the inventory total lichen species diversity was documented at 215 sites using floristic habitat sampling (Newmaster et al. 2005), wherein a voucher specimen was collected for each species at every site (Lendemer et al. 2016). All vouchers were databased and deposited in the New York Botanical Garden herbarium (NY). A dataset was assembled that comprised all lichen collection records from NJ, MD, DE, VA, NC, and SC between 1870 and 2015 recorded in the NYBG database, clipped spatially to the Mid-Atlantic Coastal Plain ecoregion (EPA 2013). A total of 608 species in 213 genera and 16,982 records contributed by 66 collectors remained. Because modeling species distributions with very few localities presents a number of challenges (Elith et al. 2006, Wisz et al. 2008, Elith and Graham 2009), all species with fewer than 20 records were removed from the dataset, leaving 193 species and 13,921 records.

I used a standard set of lichen traits that have been utilized frequently in previous studies of the community and functional ecology of lichens (Giordani et al. 2012, Nelson et al. 2015 a,b, Giordani et al. 2016). Growth form, dominant reproductive mode, photosynthetic symbiont, and substrate were recorded for each of the 193 species. There are three main lichen growth forms: 1) crustose species, which are completely attached to their substrate and have only one surface interfacing with the atmosphere, 2) foliose species, which are leaf-like, have an upper and lower
surface, and variably attached to their substrate, and 3) fruticose species, which are erect, coral-like, and attach to the substrate at one point or rest on the ground. Hydration and water retention rates among the growth forms depend on the relationship between surface area and mass, as well as cortical structure (Palmqvist 2000). Generally, foliose species dehydrate the slowest followed by crustose then fruticose species. Lichen reproduction encompasses a variety of sexual and asexual, symbiotic and aposymbiotic modes. Most species have one dominant reproductive mode, though they may employ numerous different modes. Here we used the following categories for dominant reproductive mode: sexual reproduction via fungal ascospores or basidiospores, fungal asexual reproduction through conidia (i.e., non-lichenized mitotic propagules), and lichenized asexual reproduction through structures such as soredia and isidia. Conidia and spores are almost always much smaller than lichenized, asexual structures, and thus can travel further, however they lack the photosynthetic symbiont which they must find to re-establish a thallus. Photosynthetic symbionts fall into four main categories: coccoid green algae, Trentepohlia, cyanobacteria, or none, all which vary in their CO₂ acquisition and concentration modes, along with response to differences in light conditions (Palmqvist 2000). In this region lichens grow on tree bark (corticolous), on lignin (lignicolous), and on humus or soil (terricolous). Very little rock is available in the MACP as substrate, so there were no saxicolous species in the dataset.

Modeling and Spatial Analyses

First, distribution models were built for each species that were then used for all subsequent analyses. The methods for building the SDMs included specific steps to reduce the impacts of sampling bias and overfitting models, and all analyses were run in R (R Development Core Team 2008). To reduce the effects of sampling bias, which was likely to be a confounding
factor in our dataset (Fig. 4.1), species occurrence datasets were thinned using a randomization algorithm that created a resampled data set in which all species occurrences were at least 20 km apart (spThin 0.1.0, Aiello-Lammens et al. 2014). Species distributions were related to bioclimatic data using the worldclim variables dataset which includes 19 variables related to temperature and precipitation projected to a 30 arc-second (~1 km) grid and clipped to the MACP level III ecoregion (Hijmans et al. 2005, Anderson and Raza 2010, EPA 2013). To assess what level of complexity produced the best model for each species an algorithm was used that builds models with a suite of parameters and calculates a set of evaluation metrics (ENMEval, Muscarella et al. 2014). In this case I tested regularization multipliers ranging from 0.5 to 4 at 0.5 intervals, and feature classes L, LQ, LQH, LQHP, LQHPT, and H. Localities were divided into four equal blocks based on their latitude and these blocks were used to create training and testing datasets. Models were trained with three of the four blocks and tested with the final block. This process was iterated so that each block of localities was used to test the model at each set of parameters. From this suite of models the best level of complexity was chosen based on the Akaike Information Criterion corrected for sample size (AICc). Then, the final model was generated using Maxent v. 3.3.3k (Phillips et al. 2004, Phillips et al. 2006) for each species by including all thinned localities with the regularization multiplier and feature class settings that resulted in the lowest AICc score.

The SDMs for each species were used to calculate current distributions sizes, the amount of area they are likely to lose with one and six feet of SLR, and the amount of their distributions occupied by agriculture and development. First, models were converted from continuous probabilities to binary (suitable/unsuitable) based on the equal training selectivity and sensitivity threshold (Liu et al. 2013), where all values above the threshold were converted to one and all
below were converted to zero. Then, the area of cells with the value of one was calculated, which corresponds with the species estimated distribution size. The binary model was then clipped using shapefiles from the National Oceanic and Atmospheric Administration (NOAA) for the potential area that will be inundated with one and six feet rise above the current mean high high water level (NOAA 2015), the lowest and highest SLR estimates available from NOAA. The area that was clipped out of the model was then measured, and subtracted from the total estimated species distribution size. To calculate the suitable area for each species that is occupied by development and agriculture these land use types were extracted from the National Land Cover Dataset (Homer et al. 2015) converted to shapefiles, clipped out of the binary SDMs, and measured. The resulting values were subtracted from the original species distribution size estimate.

Statistical Analyses

The mean and standard error were calculated for all species, each trait category, and the four genera with nine or more species for: 1) predicted suitable area, 2) predicted suitable area with agricultural and developed areas removed, 3) percent loss with one foot SLR, and 4) percent loss with six feet of SLR. The sample size for cyanobacterial-associated, non-lichenized, and fungal asexual reproductive species were too low for further analyses. Substrate groups were not analyzed further because nearly all species were corticolous (89%). The distributions of values for all remaining trait categories and genera were tested for homogeneity of variance using Bartlett’s test and normality of distributions using Shapiro-Wilks test. The only distributions that were non-normal were for all values for lichens that have Trentepohlia as their photobiont, crustose species, and for the genus Pertusaria s. str. Analysis of variance was used to test for differences in predicted total distribution size, predicted realized distribution size, percent loss
with one foot SLR, and percent loss with six feet of SLR among the growth forms and remaining three genera (*Cladonia, Parmotrema, and Lecanora*), followed by a Tukey’s test for comparison of means. A two-tailed t-test was used to test for differences in the predicted total distribution size, predicted realized distribution size, percent loss with one foot SLR, and percent loss with six feet of SLR between fungal sexual reproduction and lichenized, asexual reproduction. Kruskal-Wallace tests were used to test the difference in predicted total distribution size, predicted realized distribution size, percent loss with one foot SLR, and percent loss with six feet of SLR among photobiont and growth form groups. Maps were created in QGIS (QGIS Development Team 2014). Spatial interpolation was used to create collection heatmaps in QGIS. Diversity distribution maps were created by merging rasters in ArcGIS 10.2. All statistical analyses were conducted in R (R Development Core Team 2008).

4.4 Results

Six-hundred and eight species were collected in the study area. Nineteen percent of species were represented by a single record and 57% of species were represented by 10 or fewer records. Only 193 species, comprising 32% of the lichen species diversity for the region, were represented by over 20 records and were thus treated as suitable for modeling. Sampling for the floristic inventory resulted in hotspots of collection effort, high collection density areas, spread throughout the region (Fig. 4.1). There was one highly-collected area on the Delmarva Peninsula, three along the Virginia-North Carolina border, one on the North Carolina Outer Banks, and one in the center of the South Carolina coast. Collections previous to the inventory that are held at NY, which maintains the largest and most comprehensive holdings of relevant vouchers, filled in many sampling gaps (Fig. 4.1). The average AUC of the final models was 0.795 (SE±0.0059).
The average distribution size for all species was 19,698 km$^2$ (SE±362), which was reduced to 13,399 km$^2$ (SE±279), a 32% loss of area, when agricultural and developed areas, that are unsuitable for most lichens, are removed (Lendemer et al. 2016). The analyses predict that species distributions will be reduced by an average of 12.4% (SE±0.3) and 33.7% (SE±0.7) with one (~0.3 m) to six feet (~1.8 m) of SLR, respectively.

The four genera with the most species diversity were *Cladonia*, *Lecanora*, *Parmotrema*, and *Pertusaria* with 19, 9, 17, and 9 species, and average distribution sizes 17,335 km$^2$ (±1279), 19,754 km$^2$ (±1958), 22,041 km$^2$ (±1184), and 18,409 km$^2$ (±1528), respectively (Fig. 4.2). *Cladonia* species were predicted to lose 14—36% of their ranges, *Lecanora* species 13—35%, *Parmotrema* species 10—29%, and *Pertusaria* species 14-36%, with one or six feet of SLR. Of the taxa studied, *Parmotrema* species had significantly larger distributions than *Cladonia* species (p<0.05 for total predicted suitable area and p<0.01 for predicted suitable area with agriculture and development removed), and were predicted to lose less of their distributional area with one foot of sea level rise (p<0.05).

The functional traits analyzed here were substrate, photobiont, dominant reproductive mode, and growth form. Almost all species were corticolous (171 species, 89% of total diversity), 11 were both lignicolous and corticolous, nine were terricolous, and two were lichenicolous. Because of the strong skew in the number of species in each substrate group no further statistical analyses were conducted using this trait. Two green algal photobionts were dominant: 143 species associated with coccoid photobionts, and 44 species associated with *Trentepohlia*. Only three species were associated with cyanobacteria and three were non-lichenized fungi, both of which were removed from further analysis due to small sample size. Average distribution size and area lost to SLR were compared between green coccoid- and
Trentepohlia-associated species and there was no significant difference between any of the values (Fig. 4.3). Most species reproduced predominantly through sexual fungal spores (n=106), followed by asexual, lichenized diaspores (n=84), and very few species by fungal asexual spores (n=3). Species that reproduce via lichenized asexual diaspores had significantly larger range sizes compared to sexually reproducing species (p<0.01), and were predicted to lose significantly less suitable area than sexually reproducing species under both SLR scenarios (p<0.01) (Fig. 4.3). Most species were crustose (n=115), followed by fruticose (n=53), and foliose (n=25). There was no significant difference among the range sizes or proportion of area loss to SLR among growth forms (Fig. 4.3).

I examined the spatial distribution of total species diversity in relation to SLR, and compared diversity distribution patterns among major genera. The low-lying swamps in the Albemarle-Pamlico Sound host the highest species diversity and will lose the most land area to SLR (Fig. 4.4). The barrier islands all along the coast also host a high percentage of the species diversity. The four largest genera had distinct distribution patterns (Fig. 4.5). *Cladonia* diversity was concentrated on the Delmarva Peninsula, and *Lecanora* was also diverse on Delmarva, with additional areas of high diversity spanning throughout the coastal regions of North Carolina. *Parmotrema* and *Pertusaria* were most diverse in low-lying regions from the Albemarle-Pamlico peninsula south through South Carolina.

### 4.5 Discussion

Here I used data from >13,000 lichen collections to: 1) estimate the amount of area common species in the MACP will lose to SLR, 2) investigate if certain taxonomic or morphological groups will be disproportionately negatively impacted by SLR, and 3) identify how the diversity is distributed throughout the region. We found that the average predicted
regional area loss is 12.4% per species with one foot of SLR and 33.7% with six feet of SLR, but that 32% of the species-area distributions are already rendered largely unsuitable by agriculture and development. These results suggest that habitat destruction has already reduced species distributions at least as severely as SLR will in the coming century. This finding is not surprising given that it is well documented that lichens, particularly epiphytic species, are highly sensitive to urban and suburban development, agriculture, climate change, and resource extraction (Scheidegger et al. 1995, Zotz and Bader 2009).

Distinct distributions of diversity and SLR threat were identified in the region. Low-lying land areas in the Albemarle-Pamlico Sound host the highest species diversity to future land loss ratio, supporting our previous results examining total species diversity by site throughout the region (Lendemer and Allen 2014). This peninsula encompasses many of the largest protected areas in the region (Lendemer and Allen 2014), which are inhabited by a number of endangered and sensitive species, such as the wood stork (*Mycteria americana*) and the marsh rabbit (*Sylvilagus palustris*) (NCWRC 2005). As the shoreline moves there is potential for inland areas to become suitable for coastal species (Menon et al. 2014). However, the inland areas of the MACP are currently largely occupied by development and agriculture, and will require large-scale changes to the current land use to become equivalent habitat for species currently inhabiting the outer peninsulas (Fig. 4.4).

Functional traits are measurable attributes of species that affect their fitness and influence on the surrounding environment (Violle et al. 2007, Giordani et al. 2016). Identifying functions that are more susceptible to environmental change provides data for considering how the ecosystem processes and biotic interactions may change. Conversely, studying threat impact on taxonomic diversity takes into account evolutionary history, important information that is lacking
from the trait perspective. These two complementary measures of diversity can be used to more fully understand the impacts of environmental change on ecosystems. The majority of taxonomic and functional groups examined here showed no significant difference in response to SLR. However, significant differences were found between two groups: 1) sexual fungal spores vs. asexual lichenized diaspores, and 2) *Cladonia* vs. *Parmotrema*.

The results of the functional diversity analyses largely agreed with previous studies of lichen functional traits, where dispersal ability (reproductive mode) was the most important trait. Species that reproduce asexually primarily through lichenized diaspores have larger ranges and will lose less of their distribution to SLR than species that reproduce primarily sexually through fungal spores alone. Many previous studies have recovered a similar pattern in community ecology analyses. For instance, Hale (1955) found that the majority of common species reproduce via propagules containing both symbionts, while most rare species reproduce via sexual, fungal spores. He also found that sorediate species, lichenized asexual propagules that consist of algal cells surrounded by fungal hyphae, were found in more plots than sexually reproducing species, a pattern that has been noted in other recent studies (Nelson et al. 2015a, b). This pattern appears to hold true on a continental scale, with sorediate species typically occurring on more continents than their fertile counterparts (Bowler and Rundel 1975). These findings suggest that symbiosis resynthesis is a limiting factor in sexually reproducing species establishment, resulting in sexually reproducing symbiotic organisms being more threatened by environmental change than species that predominantly reproduce through symbiotic propagules. A somewhat comparable pattern has been observed in plants, where clonal, vegetative reproduction is the most successful reproductive strategy to recolonize highly disturbed habitats (e.g., Fahrig et al. 1994, Ledo and Schnitzer 2014), though this pattern does not necessarily take
into account the added impact of an obligate symbiosis. Further research on dispersal ability, reproductive mode distributions, and differential response of symbionts to environmental change is essential for effective conservation of symbiotic organisms.

Of the four genera examined here, *Parmotrema* species have larger distributions and are predicted to lose a smaller percentage of their distributional area to SLR than *Cladonia*. This result is unsurprising given that species with smaller distributions are consistently found to be more threatened by environmental change and more likely to be extirpated or go extinct than species with larger distributions (Thomas et al. 2004). A simple comparison of their distribution patterns suggests why this is the case (Fig. 4.5). *Cladonia* is most diverse in montane and boreal habitats and many species in the genus reach the southern edge of their distribution in the MACP (Brodo et al. 2001). Conversely, *Parmotrema* is most diverse in the tropics and subtropics (Hale 1965), and species are widespread in the MACP. Due to the distinct ecological and biogeographic affinities of both genera, this same pattern is almost certainly not applicable in most other regions, but must be evaluated on a case-by-case basis.

There are several limitations to this study as well as sources of uncertainty that can be addressed through future research. First, we focused on a single threat, SLR, and did not quantify other changes, such as human population growth, and species distribution shifts with temperature and precipitation change. Spatially explicit projections of changes in land use over large geographic areas are not currently available, but will prove a powerful addition to assessments in the future. There are data available for multiple climate change scenarios, however, we did not project our models into the future because most species treated here are not restricted to the MACP and thus the entirety of their distributions, and by extension ecological tolerances, are not included in the modeling extent. One key assumption that is made when projecting models is that
the full ecological gradient occupied by a species is accurately sampled (Elith and Leathwick 2009), and projecting most models built here would violate that assumption. Many previous studies of SLR have effectively and informatively taken this single factor approach as we did here (Morris et al. 2002, Menon et al. 2010, Armitage et al. 2015), however, a multi-factor approach would prove fruitful in future studies (Osland et al. 2016). Second, due to difficulty modeling distributions of species with few occurrences (Elith et al. 2006, Wisz et al. 2008, Elith and Graham 2009), no rare species were included in this study, though many of them will likely be more severely impacted by SLR because narrowly endemic coastal species are known from in the MACP (Lendemer et al. 2016). Third, there is uncertainty in both SLR estimates and SDMs. Sea-level rise estimates vary globally and locally, and local estimates range from 0.2—0.9 m by 2050 (Boon et al. 2012) and 0.8—1.8 by 2100 (Miller et al. 2013). Here we chose to estimate the impacts of one foot (0.3048 m) and six feet (1.8288 m) of SLR because they are the extreme ends of SLR estimates for the coming century. Uncertainty and inaccuracy in SDMs can stem from modeling methods such as incorrect modeling extent (Anderson and Raza 2010) and sampling bias (Kramer-Schadt et al. 2013). Here we employed the current best practices for using Maxent to model species distributions from natural history collection data (e.g., reducing sampling bias (Aiello-Lammens et al. 2015)), and the environmental layers we chose are based on data collected from weather stations that are densely spaced in the eastern United States (Hijmans et al. 2005). However, there is some sampling bias in our dataset. Specifically, there is one large, unsampled area remaining in South Carolina (Fig. 4.1). Despite this bias, distinct patterns of diversity were uncovered for different genera (Fig. 4.5), and the patterns match the current understanding of their ecology and biogeography. We can address this sampling gap in future studies.
Conclusion

Studying the distributions of lichens and their threats provides essential insights into to the overall conservation of coastal biodiversity because of their utility as environmental indicators, markers of geological history, and ease of collection. It is well established that lichens are sensitive to environmental changes, including logging, habitat loss, and air pollution (McCune et al. 1997, Nimis et al. 2002, Will-Wolf et al. 2006). The sensitivity of lichens has been utilized worldwide by establishing monitoring protocols that primarily or solely use lichens communities and species as indicators of environmental health (Conti and Cecchetti 2001, Nimis et al. 2002, Szczepaniak and Biziuk 2003). Lichen species distributions largely follow well-established biogeographic patterns that correspond to interspecific and intergenic biogeographic distributions in plants (Galloway 2008). Studying the distributions of lichen species thus inherently incorporates information on the contribution of geological history to currently observed diversity patterns. Finally, from a more practical perspective, lichens are not seasonal, so they are available to sample at all times of the year. Surveys for total diversity do not suffer from the incomplete sampling due to variation in flowering or fruiting times that affect inventories of plants and other fungi, or low detectability due to unusual weather patterns that can impact animal surveys. While lichens are often overlooked or disregarded, they are in fact ideally suited to studies of biodiversity patterns and conservation.

Here I have implemented one workflow that can be applied at regional scales to quantitatively predict SLR impacts on species and functional groups, and identify biodiversity and threat hotspots. This approach can easily be applied to diverse organismal groups and spatial scales to address the current paucity of research on the specific threat SLR poses to biodiversity (Menon et al. 2010, Bellard et al. 2015). Most importantly, this study addresses the problem of
insufficient knowledge of coastal species distributions. While this same workflow could potentially be followed using available locality data from online repositories, data from detailed surveys and inventories provide more complete, accurate, and timely characterization of regional biotas. In this study, the aggregated lichen collections at the NYBG herbarium helped to demonstrate the gaps in collections in the region, but this study would not have been possible without the data gathered through a recent, intensive inventory of the area that made a deliberate effort to sample lichens throughout suitable habitats in the region. Continued fieldwork to document species occurrences, coupled with SDMs and spatial analyses are a powerful toolkit to address the threat SLR poses to species and to produce essential results for conservation planning.

4.6 Acknowledgements

I greatly appreciate the work of Drs. Richard Harris, William Buck, and James Lendemer, all of whom contributed significant collections to the inventory. The comments of an anonymous reviewer were very helpful and improved the manuscript. I would also like to thank Dr. Barbara Thiers for her comments on the manuscript. This work was funded by NSF DEB#114452 and the NSF Graduate Research Fellowship.
4.7 Figures and Tables

**Figure 4.1.** Distribution (left) and heatmaps of sampling sites from regional biodiversity survey (center) and collections held at NYBG combined with survey sites (right).
Figure 4.2. Distribution size and predicted loss of distribution for the most speciose genera in the MACP (* indicates $p \leq 0.05$ and ** indicates $p \leq 0.01$).
Figure 4.3. Distribution size and predicted area loss of functional groups (* indicates p≤0.05 and ** indicates p≤0.01).
Figure 4.4. Total species diversity in the MACP, spatial distribution of unsuitable land use types, and two levels of SLR, 1 foot and 6 feet.

Figure 4.5. Distribution patterns for most diverse genera in the MACP.
Chapter 5. Testing Lichen Transplant Methods for Conservation Applications in the Southern Appalachians

5.1 Abstract

Lichens species are threatened by land use change, air pollution, and climate change worldwide. Lichen transplant techniques can be used to take conservation actions, such as reintroducing extirpated populations, or bolstering existing populations. Three experiments were conducted to test new and established methods for lichen transplantation for conservation. First, small fragments of *Graphis sterlingiana*, *Hypotrachyna virginica*, and *Lepraria lanata* were placed on medical gauze attached to each of the species most common substrate to test the feasibility of transplanting narrowly endemic species. Second, burlap, cheesecloth, medical gauze, and a plastic air filter were directly compared for their use as artificial transplant substrates with *Lepraria finkii* as the test lichen. Third, transplants of *Usnea angulata* were established to test its amenability to transplantation via hanging fragments on monofilament. The first two experiments were established on Roan Mountain, North Carolina and the third experiment at Highlands Biological Station, North Carolina. In the first two experiments medical gauze did not withstand local weather conditions and nearly all pieces fell from the trees within 6 months. The plastic air filter and burlap performed best as artificial substrates for transplants, with a 100% and 80% success rate, respectively. Cheesecloth remained attached to the trees, but only 20% of lichen fragments remained attached to the substrate after one year. In the third experiment *U. angulata* grew 3.5±1.4 cm in 5 months, exceeding previously reported growth rates for this species. These results advance methods for conservation-focused lichen transplants, and expand established methods to a new region and new species.
5.2 Introduction

There is now broad scientific consensus that anthropogenic change to the environment increasingly results in greater extinction risk for species worldwide (Dirzo and Raven 2003, Butchart 2010, Ceballos et al. 2015, IUCN 2016). While in situ conservation is the ideal approach to preserving species, fragmentation and degradation of the natural landscape forces consideration of diverse actions (Chivian and Bernstein 2008, De Baan 2013). In the face of these threats translocations, movement of species to establish, re-establish, or bolster populations, are increasingly considered and conducted as a means to improve the conservation status of threatened and endangered species (IUCN/SSC 2013, Guerrant 2013, Brichieri-Colombi and Moehrenschlager 2016). North American animals illustrate the rapid increase in conservation translocations, with only one study published on the topic in 1974 compared to 84 such studies in 2013, and a total of 279 species over the 39-year period (Brichieri-Colombi and Moehrenschlager 2016). The ethics of species translocations have been thoroughly discussed. Major concerns include the potential for failure, negative impacts to source populations, impacts to the area where the species is introduced, and the resources required to successfully translocate a single species (McLachlan et al. 2006, IUCN/SSC 2013, Brichieri-Colombi and Moehrenschlager 2016). The most complete guide to address challenges and issues with conservation translocations was published by the International Union of Nature (IUCN/SSC 2013). Regardless of the issues surrounding the practice, conservation translocations are likely to become increasingly important for all groups of organisms as resource extraction, loss of diverse ecosystems, and climate change progress in the coming century (Gallagher et al. 2015).

Lichen translocations, more commonly referred to as transplants, have been successfully carried out for numerous species. These studies have investigated diverse questions about lichen
biology, including the impacts of air pollution on lichen physiology and survival (Brodo 1961, Ferry and Coppins 1979, Galun and Ronen 1988, Piccotto et al. 2011), morphology and development (Honegger 1996), and growth rates (Muir et al. 1997). Numerous examples of lichen translocation specifically for conservation purposes also exist (Smith 2015). Some have been notable for their large-scale approach, with transplants involving over 1,000 individuals (Sillett and McCune 1998, Hazell and Gustafsson 1999). Others have been notable for their small scale, specifically focusing on transplanting thallus fragments and asexual reproductive propagules (e.g., Scheidegger 1995, Hilmo and Ott 2002, Kon and Ohmura 2014). The vast majority of the conservation studies have, however, focused on transplanting foliose, epiphytic species, especially from the genus Lobaria, while very few studies of crustose lichen transplants have been published (Smith 2015).

There are three main groups of transplant methods, most of which were designed and implemented for foliose and fruticose species. The simplest approach is to attach thalli or propagules directly to the substrate using an adhesive or staples (Scheidegger et al. 1995, Honneger 1996, Lidén 2009). There have been reports of negative impacts to the lichens where they are directly in contact with synthetic adhesives (Gilbert 1977). To avoid this, one study used only water as the adhesive for attaching lichen propagules and thallus fragments (Sillett et al. 2000). Another strategy to avoid negative impacts of adhesives is to move the underlying substrate along with the thallus, and directly attach the original substrate to a similar substrate at the new location. This has been accomplished by moving bark fragments or whole branches (Hilmo 2002), or taking circular portions of bark out of trees and inserting them into a hole of the same size on the target tree (Brodo 1961). The third method involves attaching the lichen to an artificial substrate that is then attached to, or hung from, the target substrate. The two methods
used most frequently in this category are attaching sterile medical gauze to trees (Scheidegger 1995), and hanging lichens from monofilament (Denison 1988, McCune et al. 1996). The advantage of this method is that fragments of any size can be used. However, these methods do involve placing plastic and other synthetic materials into natural systems, and do not necessarily lead to the attachment of the transplanted lichens to the target substrate.

Few studies have investigated transplantation of crustose lichens (Hale 1954, 1959, Brodo 1968, Seaward 1976). This is likely due to the challenging nature of crustose lichen transplants, and is evidenced by the mixed success of the few published studies. Two species, *Lecanora muralis* and *Opegrapha lithyrga*, that grew on man-made substrates, were successfully translocated and survived for at least three years and one year of monitoring, respectively (Seaward 1976, Smith 2015). *Dibaeis baeomyces* thalli that were moved on intact soil plugs from Newfoundland to Connecticut disappeared over the course of a nine year monitoring study (Hale 1954, 1959). The technical difficulty of transplanting crustose species, rather than limitations of their physiology, is often a hindrance to their establishment, precluding studies of growth (Brodo 1968). Transplanting crustose species is clearly one area of lichen translocation research that warrants further effort and attention to test and establish methods.

In this study I used established protocols and tested new materials to transplant five lichen species, including three crustose, one foliose and one fruticose species, in the southern Appalachian Mountains of eastern North America (Table 5.1). The southern Appalachians are a well-known lichen diversity hotspot, including many rare and narrowly endemic species (Lendemer et al. 2013). The high-elevations of this region host a unique assemblage of organisms, including an endemic fir, *Abies fraseri*, the spruce-fir moss spider, *Microhexura monitvaga* (Harper 1948, Manos and Meireles 2015, Seaborne and Catley 2016), and many
lichens (Allen and Lendemer 2016a). This area has historically been impacted by logging, acid rain and fog, and sweeps of invasive pests (Milgroom and Cortesi 2004, Jetton et al. 2008, White et al. 2012). Now it faces the potential of warming and shifts in seasonality with climate change (Ingram et al. 2013). Because this ecosystem hosts such an incredible diversity of species, including numerous endemics, and is at the same time facing significant threats, it is an ideal place to test and develop transplant methods. These methods will then likely be useful in other regions with similarly wet and humid climates. Here I transplanted three species that are narrowly endemic to the region, Graphis sterlingiana, Lepraria lanata, and Hypotrachyna virginica (Lendemer and Allen 2015, Allen and Lendemer 2016a), one, Lepraria finkii, is common and widespread (Lendemer 2013) and one, Usnea angulata, has a multi-continental distribution but has declined substantially since the 1930s in eastern North America (Lendemer, unpub. data).

5.3 Methods

Sterile Gauze Transplants of Narrow Endemics

In the first experiment 2” X 2” (5.08 cm X 5.08 cm) pieces of sterile medical gauze were used as an artificial substrate and inoculated with fragments of Graphis sterlingiana, Hypotrachyna virginica, and Lepraria lanata. Portions of one or two thalli of each species were collected as source material to ensure that none of the source populations or individuals was negatively impacted. Graphis sterlingiana was collected from the Pisgah National Forest, near the intersection of US 215 and the Blue Ridge Parkway, Haywood County, North Carolina. Hypotrachyna virginica was collected from the Pisgah National Forest in the Black Mountains on Potato Hill, Yancey County, North Carolina. Lepraria lanata was collected from the Pisgah National Forest on Roan Mountain, Mitchell County, North Carolina. Roan Mountain was
chosen as the transplant location because forests similar to the source populations occur there, and SDMs predicted the mountain to be highly suitable for all of the species (See Chapter 3). 

Transplants of *Graphis sterlingiana*, *Hypotrachyna virginica*, and *Lepraria lanata* were established on Roan Mountain, North Carolina in May 2015 (Fig. 5.1). Three locations were established for each species on different slopes and aspects, at different altitudes. For *Graphis sterlingiana* and *Hypotrachyna virginica* at each site, one piece of sterile medical gauze was stapled at chest height and one was stapled two inches above the initial piece on the north, east, south, and west side of trees following the methods described by Scheidegger (1995). On each side of the tree a small fragment of source material was secured to the gauze, and the other piece was left blank as a control to test for independent lichen establishment and growth. The treatment and control pieces alternated in the upper and lower position. *Graphis sterlingiana* transplants were established on *Betula alleghaniensis* and *Hypotrachyna virginica* transplants were established on *Abies fraseri* or *Picea rubens*. *Lepraria lanata* transplants were established in a similar fashion, but gauze was attached to sheltered boulders and rock outcrops using silicon waterproof sealant. Thirty-six transplants of *Hypotrachyna virginica* and *Graphis sterlingiana*, and nine transplants of *Lepraria lanata* were established, for a total of 81 transplants and an equal number of controls. Photographs of each transplant were taken using a Nikon D3100 camera with Nikkor 105 mm macro lens. Transplant monitoring was conducted in November 2015, May 2016, and November 2016. Monitoring consisted of photographing any remaining transplants and controls, along with scanning the material for living lichen fragments using a hand lens and noting their presence or absence.

*Testing Alternative Transplant Substrates with Lepraria finkii*
A second transplant study was established in November 2015 to directly compare the quality of a variety of materials for establishing crustose lichen transplants (Fig. 5.1). The materials compared were 3” X 3” (measured at longest lengths) triangle pieces of burlap, 2” X 2” pieces of cheesecloth, 2” X 2” sterile medical gauze, and 2” x 2” pieces of Honeywell Filter A HRF-AP1 Universal Carbon Air Purifier Replacement Pre-Filter (referred to as ‘air filter’ throughout text). For this portion of the study the common species Lepraria finkii was used, and source material was collected from Roan Mountain, North Carolina. The site was established near Carver’s Gap on Roan Mountain. On a single Betula alleghaniensis one piece of each type of material was stapled to the north, east, south, and west side of the tree in a vertical line. Below the vertical line of treatment materials one extra piece of one of the substrate types was attached as a control, with each side having a different material as a control. Small fragments of L. finkii were attached to each of the treatment patches on each side of the tree. On a second tree each of the four materials were stapled to the north side of the tree in a vertical line, along with an extra patch of medical gauze as a control. Again, L. finkii fragments were attached to each of these materials. Photographs of each transplant were taken using a Nikon D3100 camera with Nikkor 105 mm macro lens. Monitoring was conducted in May 2016 and November 2016. Monitoring consisted of photographing any remaining transplants and controls, along with scanning the material for living lichen fragments using a hand lens and noting their presence or absence.

*Monofilment Transplants of Usnea angulata*

To test the potential for re-establishing populations of Usnea angulata, transplants were established following the protocols used by McCune et al. (1996) for U. longissima (Fig. 5.1). On July 7th, 2016 one, large thallus of Usnea angulata was collected from the ground where it had recently fallen from a tree along Turtle Pond Rd. on the south shore of the Cullasaja River in
Macon County, North Carolina. The next day the thallus was cut into pieces between 19.5 — 63.5 cm long and attached to monofilament loops with silicon sealant. These loops were allowed to dry for 1.5 hours before being hung on a wooden dowel and secured at even spacing using silicon sealant to attach the monofilament to the wood. Each individual was numbered from 1 — 16 using a Sharpie marker to write directly on the dowel. The initial length of each lichen fragment was measured as the distance between the silicon attachment point and the end of the axis furthest from the attachment point. The dowel was then hung on the Highland Biological Station campus between two branches of a large *Tsuga caroliniana* at the edge of a pond (Fig. 5.1), roughly simulating the microhabitat the species is observed in naturally. On November 28, 2016, ~5 months later, the lengths of the transplants were remeasured.

### 5.4 Results

The outcomes of the three transplant studies established varied. The three southern Appalachian endemics established on sterile medical gauze had a 98.8% failure rate due to gauze detaching from trees. Direct comparison of four different transplant substrates showed that burlap and plastic air filters performed better than cheese cloth and medical gauze. Monofilament transplants of *Usnea angulata* resulted in rapid growth over a five-month period. Great detail for the results of each experiment are provided below.

**Sterile Gauze Transplants of Narrow Endemics**

Small fragments of *Graphis sterlingiana*, *Hypotrachyna virginica*, and *Lepraria lanata* were used to inoculate pieces of medical gauze stapled to trees in May 2015. Initially, 162 pieces of medical gauze were established, half as treatments and half as controls. When the transplants were monitored six months later in November 2015, only 34 (21%) pieces of gauze remained attached to the trees and only seven (8.6%) of the remaining treatments still retained lichen.
fragments. There were three remaining observable *Lepraria lanata* transplants, two *Graphis sterlingiana*, and two *Hypotrachyna virginica*. In May 2016, 20 (12.3%) of the gauze pieces remained, and 7 (8.6%) of these still hosted the same lichen fragments from the previous monitoring time point. During the final monitoring in November 2016, seven (4.3%) of the pieces of gauze remained and only one (0.6%) piece of gauze still retained a fragment of *Graphis sterlingiana* (Table 5.1).

*Testing Alternative Transplant Substrates with Lepraria finkii*

In November 2015, a second transplant study was established to test the quality of different artificial substrates for transplanting small fragments of lichens. *Lepraria finkii* fragments were used to inoculate plastic air filter, burlap, cheesecloth, and medical gauze. During the first monitoring point in May 2016 only one of the trees hosting transplants could be found for monitoring due to dense fog conditions and the presence of a bear. In November 2016 both trees were located and monitored, and all the observations from the May monitoring were the same except for one cheese cloth transplant that lost its lichen fragment between May and November (Table 5.1). The six established pieces of air filter were all present, and there were still observable lichen fragments on all five of the treatments. The burlap performed second best, with all six pieces of fabric remaining after one year, and four of the five treatments still retaining visible granules. The cheesecloth was resilient, with all six pieces of material remaining, but the lichen did not stick well to the fabric and after one year only one piece of material still retained observable granules. The medical gauze performed poorly as well. Only two of the six pieces of medical gauze remained attached to trees after one year, but both pieces had observable lichen granules attached.

*Monofilment Transplants of Usnea angulata*
The *Usnea angulata* fragments were 19.5—63.5 cm long when first established, averaging 33.3 ± 14.1 cm long. After five months they ranged from 22—56 cm long, averaging 34.3 ± 12.4 cm long. The maximum length decreased because two of the thalli were shorter than when they were originally established and their ends were blunt, while the growing ends of the other transplants tapered to a point, suggesting that the two either broke or were eaten by animals. One lost 2 cm and the other lost 29.5 cm in length. The two broken thalli are excluded from the remaining results. On average, the transplants grew 3.5 ± 1.4 cm longer, gaining 1 — 6 cm in length. This represented a 5.6 — 17.7% length increase with an average of 11.7%.

Fourteen of sixteen thalli displayed noticeable blackening to the outer cortex, potentially indicating the damage to, or senescence of, the thalli. If they are unhealthy it is likely due, in part, to extreme drought conditions in the area throughout much of 2016 (Fig. 5.2), or a negative reaction to the sealant.

### 5.5 Discussion

Established and new methods to transplant lichens were tested with mixed success for five species representing the three major lichen growth forms. The first established transplants involved attaching fragments of *Graphis sterlingiana*, *Hypotrachyna virginica*, and *Lepraria lanata* to sterile medical gauze. This method largely failed due to the gauze not withstanding the weather conditions on Roan Mountain, which is characterized by near 100% humidity year-round and almost daily precipitation (Martin et al. 2015, Ulrey et al. 2016). Under these very wet conditions most of the gauze fell from the trees within six months of transplant establishment. What remained were six pieces of gauze still attached to a very protected rock face with silicon sealant but devoid of lichens, and one *Graphis sterlingiana* transplant on a protected face of a *Betula alleghaniensis* (Fig. 5.1). There was no measurable growth of the *G. sterlingiana*
transplant for the duration of this project, but it does seem to have attached itself firmly to the gauze along one edge (Fig. 5.1). Previous studies that used gauze as a substrate for lichenized propagules were frequently successful (Scheidegger 1995, Kon and Ohmura 2014), so the suitability of this substrate likely depends on the transplant location.

The second transplant experiment directly compared the utility of four potential transplant materials: medical gauze, plastic air filter, burlap, and cheese cloth, for establishing thalli from asexual lichenized propagules of *Lepraria finkii*, and testing their resilience to the weather along with their ‘stickiness’ for the lichen material. As in the previous experiment, the medical gauze was not resilient to the weather conditions and over half had fallen off within a year. Cheesecloth, while remaining attached to the tree, was not a good substrate for the lichen to stick to, and only one of five transplants still retained lichen material after one year. Burlap was resilient to the weather conditions and the granules maintained attachment to it for the duration of the project. Based on observations there was noticeable lichen growth, but it was not quantifiable with the available photographs. The final material was a plastic air filter, which performed the best as it was resilient to the weather and maintained the most granules of *L. finkii* for the duration of the project. However, this material is not biodegradable. If the goal of the project is for the lichen to eventually grow onto the underlying substrate and establish itself while the artificial substrate degrades, then burlap is a far better material choice. The large gaps between strands of material in the burlap leaves sufficient space for the lichen to grow through and onto the underlying substrate while the burlap slowly degrades. This could provide a viable method for transplanting crustose lichens, especially those that produce lichenized asexual propagules. Furthermore, in dry habitats the burlap will hold moisture, providing a higher humidity microhabitat for the transplanted fragments. The few previous studies reporting
successful crustose lichen transplants were grown on artificial substrates (Seaward 1976, Smith 2015). This study supports the use of artificial substrates for crustose lichen transplants, though more studies are required to thoroughly compare all methods.

The final transplant reported here was of *Usnea angulata*, a large, pendulous species that was once widespread in eastern North America but is now reduced to a few known populations (Lendemer, unpub. data). The species predominantly grows on dead or dying hemlock, *Tsuga caroliniana*, and as the tree continues to decline due to the invasive hemlock wooly adelgid (Jetton et al. 2008), it is unclear whether or not *U. angulata* will transfer to a different substrate. Translocating this species from fallen, dead hemlocks to protected areas on healthy trees is one clear conservation action that can be taken to address the ongoing decline of the species in eastern North America. Attaching lichen fragments to monofilament with silicon sealant, as has been shown to be effective for *Usnea longissima* in the past (McCune et al. 1996, Marks et al. 2015). This method was also successful for *U. angulata*. During five months the fragments grew 1 — 6 cm and averaged 3.5 cm of growth. A previous transplant study that measured the growth of *U. angulata* in Argentina found an average yearly growth rate of 2.35 cm (Rodriguez et al. 2010). It is not yet possible to state whether or not *U. angulata* grows faster in the southern Appalachians as monitoring has not been conducted for a full year and seasonal differences in growth rate are likely (Muir et al. 1997). Longer monitoring and experimental attachment to diverse tree species are now needed to fully assess the translocation potential for this species.

Here I present three studies on lichen transplant methods with the aim of testing and expanding methods to a new region and new species. While the technical difficulty of transplanting lichens is likely to be the largest challenge to implementation, a number of other questions and considerations must be addressed by lichen researchers regarding translocation and
reintroduction studies (Guerrant 2013, Brichieri-Colombi and Moehrenschlager 2016). First, we must consider what constitutes success. Survival of the transplanted material is the most obvious, but growth of the material, development of reproductive structures, and recruitment would also be viable measures (Guerrant 2013). Second, the time frame of monitoring required to determine if a transplant is truly successful needs to be determined. The time frame will vary by species and regions, but in many cases consistent monitoring for over 10 years and up to 30 is likely required to assess the average growth rate and life span of many lichens (Gilbert 1991, 2002). Finally, we must investigate what factors are the most important to ensure success of a transplant study. There are the technical aspects associated with the transplant methods, but other variables that must be considered include the depth of knowledge about the species’ biology and ecology (Benedict 1990, Antoine and McCune 2004), size of the transplanted thallus fragment (Gauslaa et al. 2009, Coxson and Stevenson 2011), and the protection status of the site to which lichens are moved. A full assessment of factors that lead to successful transplants is reliant on reports of failures, as well as successes. Continued focus on lichen transplant methods are essential as the need to reinforce, reintroduce, and translocate species for conservation increase.

5.6 Acknowledgements

I am grateful for the contributions of the following people to this work: Gary Kauffman for aid with permitting and alternative substrate brainstorming, Bruce McCune and Christoph Scheidegger for helpful discussion and guidance on lichen transplant methods, Sean McKenzie and Alex Cecil for their assistance in the field, James Lendemer for acquiring the *Usnea angulata* thallus, Thaiyeba Jallil for her work with photograph processing, Lisa Campbell for suggesting and providing the plastic air filter to use as a substrate, and the Highlands Biological Station Lichen Biology and Conservation Class for help setting up the *Usnea angulata*
transplants. This project was funded by grants and support from the American Bryological and Lichenological Society, Highlands Biological Station, and the National Science Foundation Graduate Research Fellowship.
Figure 5.1. Diversity of substrates and species used in transplant studies. A) *Graphis sterlingiana* on sterile medical gauze, B) sterile medical gauze continually attached to protected boulder >2 years with silicon sealant as adhesive, C) *Lepraria finkii* granules on burlap, D) *L. finkii* granules on air filter, E) *Usnea angulata* hanging on *Tsuga caroliniana* branches, and F) attachment of *U. angulata* to monofilament.
Figure 5.2. Total annual precipitation at Highlands Biological Station 1961-2016 from http://highlandsbiological.org/weather-data/.

Table 5.1. Results of *Graphis sterlingiana*, *Hypotrachyna virginica*, *Lepraria lanata*, and *Lepraria finkii* transplants. Success rates reported as number of transplant substrate pieces with observable lichen fragments over the initial number established.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Graphis sterlingiana</em></td>
<td>Medical Gauze</td>
<td>Est.</td>
<td>2/36</td>
<td>2/36</td>
<td>1/36</td>
</tr>
<tr>
<td><em>Hypotrachyna virginica</em></td>
<td>Medical Gauze</td>
<td>Est.</td>
<td>2/36</td>
<td>2/36</td>
<td>0/36</td>
</tr>
<tr>
<td><em>Lepraria lanata</em></td>
<td>Medical Gauze</td>
<td>Est.</td>
<td>3/9</td>
<td>3/9</td>
<td>0/9</td>
</tr>
<tr>
<td><em>Lepraria finkii</em></td>
<td>Medical Gauze</td>
<td>-</td>
<td>Est.</td>
<td>3/5</td>
<td>2/5</td>
</tr>
<tr>
<td><em>Lepraria finkii</em></td>
<td>Burlap</td>
<td>-</td>
<td>Est.</td>
<td>3/5</td>
<td>4/5</td>
</tr>
<tr>
<td><em>Lepraria finkii</em></td>
<td>Cheesecloth</td>
<td>-</td>
<td>Est.</td>
<td>1/5</td>
<td>1/5</td>
</tr>
<tr>
<td><em>Lepraria finkii</em></td>
<td>Plastic Air Filter</td>
<td>-</td>
<td>Est.</td>
<td>5/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>
Supplement 1

Independent Test of Model Quality

To test the predictive ability of Maxent modeling with very few localities in this region I divided the locality data into a training set and a test set. The training set consisted of all known localities for all species before the fieldwork for this project began in May 2014. The test set included all newly discovered populations. Modeling methods were followed as detailed above. The models were projected to the southern Appalachian region in the present and converted to binary suitable and unsuitable based on the equal training sensitivity and specificity threshold. The omission rate was then calculated for each species by summing all discovered localities that fall outside of the predicted suitable area and dividing by the total number of localities in the test set. The commission rate was calculated by summing all visited localities that were predicted to be part of the species distribution, but where the species was not detected, and dividing this value by the total number of visited sites.

Predictive Ability of the Models

Models were built using only localities known before surveys were conducted for these species, and evaluated using the omission and commission rate with the independent survey conducted in 2014. The resulting omission rate was zero for all species, while the commission rate varied as follows: Arthonia kermesina was 0.85, Arthopyrenia betulicola was 0.66, Graphis sterlingiana was 0.71, Hypotrachyna virginica was 0.77, Lecanora masana was 0.15, and Lepraria lanata was 0.79.
Bibliography


Høistad F, Gjerde I (2011) Lobaria pulmonaria can produce mature ascospores at an age of less than 15 years. Lichenologist 43:495–497.


Lendermer JC, Allen JL (2015) Reassessment of Hypotrachyna virginica, an endangered, endemic Appalachian macrolichen, and the morphologically similar species with which it has


Martinez-Meyer E (2005) Climate change and biodiversity: some considerations in forecasting shifts in species’ potential distributions. Biodiversity Informatics. doi: 10.17161/bi.v2i0.8


