Mycologia Obscura: Hidden and Layered Realms of Fungal Diversity

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MYCOLOGIA OBSCURA: HIDDEN AND LAYERED REALMS OF FUNGAL DIVERSITY

A dissertation presented

by

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A dissertation submitted in partial fulfillment of the requirements for the Doctor of Philosophy Degree State University of New York College of Environmental Science and Forestry Syracuse, New York August, 2020

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ACKNOWLEDGEMENTS

My PhD journey has been one of tremendous growth and transformation and I am thankful for everyone who helped me, pushed me, challenged me, and comforted me along the way. My transformation into a scientist was primarily shepherded by my advisor, Dr. Alex Weir. Alex cherishes naturalism, the wild and obscure, and can recognize beauty in what others may overlook—all values that I think are both rare and important in today’s world. I am grateful that he saw my potential and stoked my curiosities. I am grateful for his continual understanding and patience during my education. Thank you, Alex. My other committee members, Dr. Melissa Fierke and Dr. Tom Horton, are sources of great inspiration during this journey. Melissa has been a role model as a woman in science. Her dynamicism, expertise, kindness, and rigor were essential to my success in my program. Tom’s lab was my second academic home, and a place of impressive research and important camaraderie. Thank you, Melissa and Tom. Thank you to Dr. Robin Kimmerer, who began inspiring me an entire decade ago with her writing in Orion Magazine. Robin’s ability to live in multiple frameworks has given me courage in my journey of decolonizing my worldviews. Thank you, Robin. Thank you to Dr. Lauren Goldmann for her expertise and insights on Laboulbeniales and academia, and early guidance given to me as a new graduate student and now again in the final stages. And thank you to all of my professors at ESF, all of whom I found to be so very impressive.

In my personal life, I have benefitted from a powerful support network, comprised mostly of my very large family and a few dear friends. My parents, Regina and Jim Kaishian, have been some of my most devoted advocates and I could not have done this without them. My siblings Maryanne, Masoud, Jackie, Jim, Emma, and JJ were always there to provide much needed humor, perspective, and motivation. My dear friends Claudia Victoroff, Sam Cott, Michael Czajkowski, Samrawit Genet, Akemi Inamoto, Hasmik Djoulakian, Liv Monko (and many others!) have supported and championed my academic journey, while reminding me that life is also about love, friendship, and community. I would like to thank Fortune Ogochukwu Ononiwu for his love, humor, comfort, wisdom, and devotion. Fortune, I learn from you every day. These human beings are all so dear to me, but this little acknowledgement does not do my appreciation justice. Thank you all from the bottom of my heart!

Lastly, but of course not least, I would like to thank my fungal kin. I thank them for their wild ways of knowing, for their endless giving, for their lessons in reciprocity, and for their companionship in both moments of joy and hardship. At times when I wanted to give up, I thought about them, their quiet and unassuming power, and I felt restored.
# Table of Contents

Abstract .......................................................................................................................... viii

INTRODUCTION ............................................................................................................. 1

  GENERAL INTRODUCTION ....................................................................................... 1

  HISTORY OF LABOULBENIALES RESEARCH ......................................................... 2

  MORPHOLOGY OF LABOULBENIALES ...................................................................... 5

  CLASSIFICATION SYSTEMS ...................................................................................... 11

  SYMBIOSIS AND PARASITOLOGY ............................................................................. 14

  PARASITIC MECHANISMS OF LABOULBENIALES .................................................. 18

  HOST SPECIFICITY ..................................................................................................... 20

  BIODIVERSITY OF INSECTS AND FUNGI ............................................................... 22

  RESEARCH METHODS ............................................................................................. 26

  MOLECULAR STUDIES .............................................................................................. 31

  NATURAL HISTORY COLLECTIONS ........................................................................ 32

  DISSERTATION OBJECTIVES ................................................................................... 33

  LITERATURE CITED .................................................................................................. 35

CHAPTER 1 ...................................................................................................................... 44

  Abstract ..................................................................................................................... 44

  INTRODUCTION ....................................................................................................... 45

  MATERIALS AND METHODS .................................................................................... 46

  TAXONOMY ................................................................................................................ 47

  DISCUSSION ............................................................................................................... 58

  LITERATURE CITED .................................................................................................. 62

CHAPTER 2 ...................................................................................................................... 64

  Abstract ..................................................................................................................... 64

  INTRODUCTION ....................................................................................................... 65

  MATERIALS AND METHODS .................................................................................... 66

  TAXONOMY ................................................................................................................ 67

  DISCUSSION ............................................................................................................... 75

  LITERATURE CITED .................................................................................................. 79

CHAPTER 3 ...................................................................................................................... 81

  Abstract ..................................................................................................................... 81
List of Figures

Introduction
Figure 1: Laboulbenia morphology
Figure 2: Prolixandromyces blackwelliae and Ceratomyces ansatus morphology

Chapter 1
Figure 1.1: Illustrations of Prolixandromyces sp
Figure 1.2: Photographic plate of Prolixandromyces sp.

Chapter 2
Figure 2.1: Illustrations of Laboulbenia sp.
Figure 2.2: Photographic plate of Laboulbenia sp.

Chapter 3
Figure 3.1: Species richness plots and habitat types
Figure 3.2: Species richness plots and feeding ecology
Figure 3.3: NMDS: community assemblage and habitat types
Figure 3.4: NMDS: community assemblage and feeding ecology
Figure 3.5: NMDS: community assemblage and tendency to aggregate.
Figure 3.6: Photographic plate of Laboulbenia sp.

Chapter 3 Appendix:
Figure 3.1A: Combined morphological and molecular phylogeny of Heteroptera

Chapter 4
Figure 4.1: Map of Lake Eustis, Lake Griffin and surrounding area of Lake County, FL
Figure 4.2: Lake Eustis collection site
Figure 4.3: EMCA collection site
Figure 4.4: Ceratomyces sp.
Figure 4.5: Hydrophilomyces sp.
Figure 4.6: Insect family richness, abundance, and infection from EMCA
Figure 4.7: Insect family richness, abundance, and infection at Lake Eustis
Figure 4.8: Insect family richness, abundance, and infection at Lake Eustis and EMCA

Appendix
Figure A.1: Ceratomyces filiformis attahed to host (Tropisternus sp.) claw
Figure A.2: Hydrophilomyces gracilis attached to host, Cercyon sp.
Figure A.3: Insect family richness, abundance, and infection at Lake Eustis and EMCA
Figure A.4: EPA National Lakes Assessment: A Collaborative Survey of the Nation’s Lakes
List of Tables

Chapter 3 Appendix
Table 3.1A: Insects scanned at AMNH
Table 3.2A: Ecological data matrix

Chapter 4
Table 4.1: Species of Laboulbeniales recorded from Eustis in 1897 and 2018, EMCA in 2018

Chapter 4 Appendix
Table 4.A.1: Insect order, family, count, and infection count at Eustis.
Table 4.A.2: Insect order, family, count, and infection count at EMCA.

Appendix
Table A.1: Year 1 sampling schedule for FL.
Table A.2: Year 2 and 3 sampling schedule for 8 ecozones
Abstract


Collectively, this dissertation explores taxonomy, biodiversity studies, natural history collections, ecology, and theory. The first chapter is focused on the genus Prolixandromyces, in which 4 new species are described, representing the first records of this genus in South America. The genus is emended and a key to the genus is provided. The second chapter is focused on the genus Laboulbenia, in which four new species are described on a new host family, Gerridae (Heteroptera) or water striders. The third chapter also includes the description of 4 new species of Laboulbenia on Heteroptera, and targets significant gaps in the literature on Heteroptera associated Laboulbeniales. By utilizing the entomological collection at the American Museum of Natural History, this chapter explores host utilization patterns in the group and tracks insect infection rate at the family level. These three chapters all emphasize the scientific value of maintaining our natural heritage in accessible, research oriented biological collections. The fourth chapter is a field-based ecological pilot study focused on exploring how Laboulbeniales and their insect hosts are impacted by urbanization at two lakes in central Florida. Using the historical records of Laboulbeniales diversity from 1897 of mycologist Roland Thaxter, a comparison is drawn to modern (2018) diversity. A rapid biodiversity assessment was conducted on insects and fungi at a protected area and a developed area in 2018 and the results are compared. This study highlights the potential relevance of Laboulbeniales as environmental health indicators and a proposal for future directions is included in the appendix. Lastly, the fifth chapter approaches the field of mycology through a theoretical framework rooted in queer and feminist theories, as well as philosophy of science and Traditional Ecological Knowledge. This chapter is relevant as it challenges, pushes, and explores central tenets of institutional science and functions to socially and historically situate current research dilemmas in mycology. By excavating and laying bare ingrained, systemic biases in scientific institutions, this chapter seeks to disarm fallacious assertions of “purity” in science. Additionally, this work reiterates themes introduced in the preceding chapters, such as the value of taxonomy and biodiversity studies, the importance of biological collections, and the urgent need for expanded and imaginative conservation practices in the age of climate change.

Key words: Laboulbeniales; taxonomy; biodiversity; natural history; collections; philosophy of science

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INTRODUCTION

General Introduction

The class Laboulbeniomycetes is a group of arthropod-associated fungi in the Ascomycota. Laboulbeniomycetes are sister to the Sordariomycetes, and currently contain three strongly supported, named orders: Laboulbeniales, Herpomycetales, Pyxidiophorales (Blackwell et al., 1986; Blackwell and Malloch, 1989; Blackwell, 1994; Henk et al., 2003; Goldmann and Weir, 2018; Haelewaters et al., 2019) and, in addition, a clade comprising the poorly understood asexual and filamentous genera Chantransiopsis and Tetrameronycha (Thaxter, 1914, 1920; Goldmann and Weir, 2018; Blackwell et al., 2020). Pyxidiophorales, which contains the single genus Pyxiophora, are hyphal, arthropod dispersed, and are likely all mycoparasites (Blackwell and Malloch, 1989). All members of the orders the “thallus forming” orders, Laboulbeniales and Herpomycetales, are unified by the following characteristics: 1) non-hyphal, 2) obligate ectoparasites on arthropods, 3) bicellular ascospores, 4) determinate growth, 5) deliquescent asci (Blackwell et al., 2020).

This dissertation is focused on the order Laboulbeniales, although some relevant comparisons to Herpomycetales will be discussed. The order Laboulbeniales is the most diverse fungal lineage associated with arthropods and the largest order in the class. Currently, there are ~ 2,200 described species in the order (Santamaria et al., 2020). These fungal parasites are recorded from the following groups of insects: Coleoptera, Dermaptera, Diptera, Heteroptera, Hymenoptera, Isoptera, Mallophaga, Orthoptera, and Thysanoptera. In addition, millipedes (Diplopoda), Opiliones, and mites (Arachnida) are known to carry infections (Weir and Beakes, 1995; Santamaria et al., 2017). The majority of known species of Laboulbeniales, approximately
80%, are found on Coleoptera (Weir and Hammond, 1997). Fewer species have been described from the other groups, such as the Hemiptera (true bugs), with only 96 described species occurring on the aquatic, semi aquatic, and terrestrial families of Hemiptera (Santamaria, 2008; Lee and Na, 2009; Kaishian and Weir, 2018; Kaishian et al., 2020). As suggested by Tavares (1985), early evolution of Laboulbeniomycetes likely occurred in association with an ancestor of both Blattodea and Coleoptera during the Devonian. Recent evidence that orders Laboulbeniales and Herpomycetales are not monophyletic, however, suggests the possibility that the thalloid structure may have arisen independently in both groups (Blackwell et al., 2020). Despite being poorly studied, Laboulbeniales have been described and recorded from every continent excluding Antarctica, and are widely distributed on hosts occupying numerous habitat types, from fully terrestrial to fully aquatic (Weir and Hammond, 1997; Weir, 2001; Haelewaters et al., 2018).

**History of Laboulbeniales Research**

Beginning in 1849, there was a small flurry of references made in the literature to the Laboulbeniales. This early work was characterized by much confusion with regard to the basic nature of what we now recognize of fungi in the Ascomycota. In 1849, French entomologist Rouget made reference to these organisms, but considered them to be “supernumerary antennal segments” (Rouget, 1850). In the same year, Schmidt (1850) posited these to be “parasitic plants that resemble setae.” Of course, during this time, fungi were still considered to be plants, so it is unclear based on his work whether or not he considered these to be fungal, or the outdated term “lower plants.” Two other French entomologists, Laboulbène (after whom the group is named) and Follin, reported what they believed to be fungal spores on the integument of beetles in 1848 (Laboulbène and Follin, 1848). In 1853, Mayr suggested Laboulbeniales to be chitinous outgrowths of insect integument, although he also described different developmental stages
Robin (1852, 1853) properly established these organisms as fungi and erected the genus *Laboulbenia*. While this put the group on the right track, Robin did also make some incorrect assertions about the fungi: he placed them in Pyrenometeaceae and put forth that nutrition of the group was aerially derived. Following this, there was then a series of records that were incorrect. For example, Kolenati (1857) considered Laboulbeniales to be acanthocephalan worms and Knoch (1868) implicated *Laboulbenia baeri*, which occurs on house flies, as cause to the cholera outbreak.

In 1869, Karsten made important discoveries to Laboulbeniales ecology and morphology. He noticed the relationship between occurrence of these fungi and insect copulation (Karsten, 1869). He also described the trichogyne and identified the antheridia as reproductive structures. In 1871, Peyritsch started making a series of substantial contributions towards understanding Laboulbeniales, perhaps conducting the most important work prior to the contributions of Roland Thaxter (Peyritsch, 1871). Peyritsch introduced the family name “Laboulbeniaceae” (Peyritsch, 1873), at the time recognizing twelve species in five genera. Additionally, Peyritsch accurately identified and described the asci and perithecia, detailed mechanisms of spore discharge and germination, detailed the formation of haustoria, reported the maturation rate of *Stigmatomyces baeri* (10-14 days); noted the lack of resting spores and the lack of fungal growth on larvae and pupae (Peyritsch, 1873, 1875).

In 1890, Roland Thaxter entered the fray and published a series of comprehensive, foundational papers in which he made detailed contributions to the understanding of the fungal life cycle, the intricacies of fertilization, and basic morphology (Thaxter, 1891, 1892, 1893, 1894, 1895). His first monographic volume, *Contributions towards a Monograph of the Laboulbeniaceae*, was published in 1896. With this volume, 152 species from 28 genera in total
were published (Benjamin, 1971). Thaxter then went on to publish four more volumes and in total, described over 100 genera and approximately 1260 species (Thaxter, 1896, 1908, 1924, 1926, 1931; Benjamin, 1971).

Thaxter’s work sparked additional work by other researchers, most notably from Picard, Spegazzini and Cépède. Cépède and Picard (1908) investigated host and position specificity of Laboulbeniales, finding high specificity in both cases was the norm. Cépède (1914) also proposed the classification of Laboulbeniales in the Phycascomycetes, in agreement with the Floridean hypothesis. This hypothesis, which was first put forth by Sachs (1874), claims that Ascomycetes are derived from red algae by loss of photosynthetic pigments and conversion to parasitism or saprophytism. As summarized by Weir (2001), this hypothesis has been supported over time by numerous mycologists (Dennison and Carroll, 1966; Kohlmeyer, 1973, 1975, Demoulin, 1975, 1985), however modern researchers do not currently favor this hypothesis on the basis of conflicting molecular evidence (Weir, 2001; Blackwell et al., 2020). Montagne and Robin (1853), Peyritsch (1873), and Thaxter (1896) recognized Laboulbeniales as belonging to Ascomycota. Faull (1906a, 1906b, 1911, 1912) contributed evidence for the placement of Laboulbeniales in Ascomycota by describing syngamy and three nuclear divisions in the asci. Placement in the Ascomycota is now secure based on more recent ultrastructural studies and discovery of simple septal pores, flattened spindle pole bodies, and Woronin bodies (Hill, 1977; Hughes, 2008) and molecular work using SSU rDNA (Weir, 2001; Weir and Blackwell, 2001b). Spegazzini (1902, 1912, 1914b, 1917) contributed substantially to the study of Laboulbeniales, especially towards Argentinian taxa, describing eight genera and approximately 270 species and varieties. Considerable taxonomic work was carried out by Benjamin, including the creation of keys to genera (1971, 1973) and his descriptions of approximately forty species of

**Morphology of Laboulbeniales**

Thallus ontogeny begins with a two-celled ascospore. Each genus within the group will follow a unique and definitive sequence pattern of mitotic cellular divisions (Rossi et al., 2016). Most genera in the group exhibit strict definitive growth patterns, such as *Laboulbenia* (Fig.1), while others, such as *Zodiomyces*, will exhibit minor plasticity in growth patterns (Rossi et al., 2016). The number of cells and their relative position in the thallus are diagnostic at the generic level. There are monoecious thalli (*mono* meaning one, *ecious* meaning house) where the female and male sexual reproductive structures are contained within one body; and there are dioecious (“two houses”) where the female and male reproductive structures are housed in separate bodies (Tavares, 1985; Benjamin, 1986). In dioecious groups, the male and female thalli will typically develop in close proximity on the same host, arising from unique ascospores (Thaxter, 1908; Benjamin, 1986). About 20% of genera are dioecious, and dioecious groups have independently arisen multiple times (Benjamin, 1971; Tavares, 1985). The Herpomycetales are an “archaic dioecious group,” suggesting that the Laboulbeniales too were originally a dioecious order (Tavares, 1985). The Laboulbeniales have no known asexual stage or resting stage and ascospore transmission is typically direct between hosts and subsequent development is immediate (De
Kesel, 1993). However, asexuality is known in the class, within the Pyxidiophorales (Blackwell and Malloch, 1989a) and within the clade comprising *Chantransiopsis* and *Tetrameronycha* (Goldmann and Weir, 2018).

**Ascospores.** The ascospores of Laboulbeniales are consistently spindle-shaped, composed of two unequally sized cells, and hyaline. The two cells in the spore are separated by a septum, which is often persistent and frequently remains visibly apparent in mature thalli. The spores are enveloped in a mucilaginous adhesive substance which facilitates attachment to the host and resists desiccation. The mucilaginous envelope is thicker around the base of the larger of the two cells (Weir and Beakes, 1996). Ascospores develop within deliquescent asci located within the perithecia, and at maturity are positioned with the larger of the two cells oriented upwards, therefore exiting the perithecium first, and initiating contact with the host, forming what is referred to as the “foot” (discussed more below). Pressure on the ostiole, often from contact with another (potential) host will trigger spore release through the ostiole (Tavares, 1985; Weir and Beakes, 1996).

**Receptacle.** The primary receptacle is the base of the thallus which is connected to the host by a holdfast known as the “foot” (Thaxter 1896, 1908). Both the foot and the receptacle develop from the larger cell of the two-celled ascospore. The receptacle forms the primary axis of the thallus and includes cell I, (or basal cell) cell II (or suprabasal cell; formerly sub-basal cell), and cell III, which are formed from cells mitotically dividing from the lower spore segment. The size, shape, and relative arrangement of these cells are diagnostic taxonomic features. Whether or not these cells undergo subsequent subdivisions is also diagnostic and varies greatly across genera.
Fig. 1. Thalli of Laboulbenia spp. A) whole, mature thallus showing the foot, and antheridium, per apex = perithecial apex, pa = primary appendage; B) upper portion of mature thallus showing wavy branches of the appendage, upper receptacle indicated by roman numerals IV and V, per = perithecium, per apex; C) whole, immature thallus showing five cells of the receptacle indicated by roman numerals I – V, stalk cell of the per = IV, developing per, and 2 antheridia; D) whole, mature thallus receptacle, per, per apex; E) upper portion of immature thallus showing developing per, upper receptacle, 2 antheridia, pa, branches, ins = insertion cell.
**Perithecium.** The perithecium is typically elongate and somewhat ellipsoid structure with a terminal ostiole. The perithecium is always an outgrowth of the receptacle, typically produced by cell II or its derivatives. The perithecium consists of stalk cells and a procarp, or the derivative cells thereof. The perithecium contains one inner and one outer layer of wall cells, with each layer composed of four vertical rows of cells. The more ancestral groups, such as Ceratomycetaceae and Herpomycetales, possess perithecia that are formed by four vertical rows of outer wall cells which then contain numerous cells. More phylogenetically advanced groups, such as Laboulbenia, typically have four vertical rows of outer wall cells, which then contain fewer (often four or five) Tavares (1985) extensively investigated the taxonomic importance of the perithecial wall cells, and Goldmann & Weir (2018) used molecular data to confirm that perithecial characters are reliable taxonomically and that phylogenetically, the perithecial morphology trends toward reduction in the Laboulbeniomycetes. The perithecium develops by upgrowth of the wall cells around the carpogonium in the true Laboulbeniales and by intrusion of the carpogonium upward between rows of wall cells in the Herpomycetales. Four vertical rows are typical in the more phylogenetically recent genera, with reduced number of outer wall cells, and with the lower cells being taller than the upper cells. A lack of distinct development of wall cells typically accompanies reduction in the overall number of cells, and seems to correspond with more recently evolved groups (Tavares, 1985). The perithecium may follow different sequences for development (Benjamin, 1971). The first pathway is the most common pathway in the Laboulbeniales, with only two known genera exhibiting different pathways. In this pathway, the receptacle laterally bears a cell which further divides into an upper and lower cell. From the lower cell, five cells will arise: the perithecial stalk cell (cell VI), the secondary stalk cell (VII),
and the basal cells m, n, and n’. From the upper cell, three cells will form: the basal carpogenic cell, trichophoric cell, and terminal trichogyne, which collectively form the female sexual organ. The genus *Coreomyces* Thaxt. is known to follow a unique sequence (Thaxter, 1908; Benjamin, 1971). In this case, wall cells develop from one of two initial intercalary cell and the female organ develops from the other initial intercalary cell. *Herpomyces*, which is the only genus in the now formally distinct order (Herpomycetales) within the third type of perithecial development, which was elucidated by Tavares (1965). In this pathway, the supra-basal cell forms an outgrowth which in turn gives rise to the perithecium. The outer wall cells of the perithecium generate the carpogonial upgrowth (as opposed to outgrowth) and inner wall cells.

The trichogyne is an ephemeral structure that facilitates fertilization by receiving spermatia from the antheridia. The trichogyne is an outgrowth of developing perithecia, and it may be branched or simple. Prior to maturation of the perithecium, the trichogyne will break down, sometimes leaving behind a scar where the base was attached to the perithecium. During fertilization, a spermatium containing the male nucleus is received by the trichogyne and guided into the carpogenic cell, initiating the dikaryotic phase of the life cycle. While there are early reports of spermatia being “attached” to the the trichogyne (Faull, 1911), there is currently no evidence of spermatia fusing to or penetrating the trichogyne. The fusion of the male and female nuclei occurs, which leads to development of the diploid mother ascus. This initiates formation of the ascogenous cell(s), which in turn give rise to asci via mitotic division. Asci may be 4 or 8-celled. Most genera likely have four spores per ascus, whereas *Herpomyces* and *Compsomyces* have 8 spores per ascus (Thaxter, 1908; Tavares, 1985).

*Antheridia and Appendages.* The upper (smaller) cell of the spore produces the primary appendage system and the secondary appendage system arises from the lower cell of the spore.
Many genera cannot produce this, such as *Stigmatomyces*. Breakage of appendage may lead to proliferation, and it is necessary to distinguish between broken and unbroken appendage systems, and secondary proliferation. These features are taxonomically important, such as in the genus *Laboulbenia*. Antheridia may be simple or compound, solitary, terminal, clustered, seriate and sessile/subsessile. Antheridia usually mature and produce spermatia as the trichogyne finishes developing. Antheridia may also begin to disappear after the trichogyne deteriorates and the perithecium fully matures. The primary and/or secondary appendages may bear spermatia.

Spermatia may be produced exogenously or endogenously (Fig. 2). Exogenous spermatia production is found in the Ceratomycetaceae and the spermatia in these species are very small and rodlike (Benjamin, 1973). Germination of spermatia never gives rise to thalli. Most species produce spermatia endogenously, possessing either simple, flask-shaped antheridia, or more complex compound antheridia. Within a given species, antheridia may be definitive in number, such as in some species of *Stigmatomyces*, or these numbers may be plastic, such as in some species of *Corethromyces* (Hughes, 2008). At the generic level, there is often great variation in number of antheridia. Compound antheridia, such as in *Cantharomyces, Dimeromyces, Dimorphomyces, Eumonoicomyces*, and *Monoicomyces*, are comprised of a cluster of antheridial
Fig. 2. A) Prolixandromyces blackwelliae with endogenous spermatia; B) Ceratomyces ansatus showing the appendages upon which exogenous spermatia would be found.

cells from which spermatia are released into a single common cavity, prior to being disseminated. Variation in the structure of the compound antheridia exists, with some species bearing flask-shaped, elongate, rounded, or in rows (Benjamin, 1971; Hughes, 2008). Goldmann and Weir (2018) provide evidence for multiple independent origins of compound antheridia, challenging reliance on these characters in classical taxonomy.

Classification Systems

In Thaxter’s first volume of Contributions towards a Monograph of the Laboulbeniaceae (1896) antheridial characters were emphasized as being of primary taxonomic significance.
Thaxter divided what was then the family Laboulbeniaceae into two categories of gamete formation and antheridial morphology: Endogenae and Exogenae, based on endogenous and exogenous formation of gametes on the appendages. In the Endogenae, the spermatia are formed within the antheridia; in the Exogenae, the spermatia are formed from intercalary cells or terminally on the branches. The larger group was the Endogenae, which at the time contained 26 genera and from two “orders,” the Laboulbenieae, which possessed simple antheridia, and Peyritschielleae which possessed compound antheridia. The Exogenae contained only two genera: *Ceratomyces* and *Zodiomyces*. In Thaxter’s second monographic volume (1908), he erected Laboulbeniinae and Ceratomycetinae in lieu of the groups Endogenae and Exogenae, respectively, as subordinal names. The two “orders” originally contained within the Endogenae, Laboulbenieae and Peyritschielleae, were replaced with the families Laboulbeniaceae and Peyritschiellacea, respectively. Maire (1916) first proposed the family name Ceratomycetaceae, which was later published by Colla (1934).

Within her classification system, Tavares (1985) shifted taxonomic emphasis away from antheridial characters towards perithecial characters. For a thorough record of her taxonomic changes, Tavares (1985) provides a table (Table 1, p. 92) comparing her classification system to Thaxter’s 1908 system. Noteworthy changes were made to family organization, as Tavares recognized four total families within the group (including Herpomycetaceae, which was placed in what she established as the suborder Herpomycetineae). She classified three families within the suborder Laboulbeniinae: Ceratomycetaceae, Euceromycetaceae, and Laboulbeniaceae. Ceratomycetaceae is unified by morphological characters including a singular series of superposed cells forming the primary receptacle, and cells VI and VII are intercalary in the primary receptacle. This family contains 12 genera: *Autoicomyces, Ceratomyces,*
Drepanomyces, Eusynaptomyces, Helodiomyces, Phurmomyces, Plectomyces, Rhynchophoromyces, Synaptomyces, Tettigomyces, Thaumasiomyces, and Thripomyces, most of which are found living on aquatic hosts, excepting *Tettigomyces* which is found on Gryllotalpidae (Orthoptera). Tavares placed *Tettigomyces* in Ceratomycetaceae (1985), and Goldmann and Weir confirmed support for this placement (2018). The Euceratomycetaceae is unified by cells VI and VII successively forming the lateral secondary appendage which arises from the primary appendage. This family contains five genera: *Cochliomyces*, *Colonomyces*, *Euceratomyces*, *Euzodiomyces*, and *Pseudoecteinomyces*, which are found on terrestrial hosts. but Goldmann and Weir (2018) later found that the family was unsupported, with the placement of *Cochliomyces* unresolved, and *Euzodiomyces* coming out with Ceratomycetaceae. The Laboulbeniaceae are unified by the presence of outer wall cells in the perithecia which are arranged in tiers with 4 to 5 rows. This family contains about 120 genera, including the class’ largest genus (*Laboulbenia*) that occur on hosts in a range of environments, from terrestrial to aquatic.

Goldmann and Weir (2018) used SSU rDNA to construct the first molecular phylogeny of the Laboulbeniomycetes. They concluded that the class is monophyletic and related most closely to the Sordariomycetes. The class includes *Chantransiopsis* and *Tetrameronycha*, which are asexual genera with linearly superposed cells in the thalli. Herpomycetales was confirmed to lie outside the main clade, as posited by Tavares (1985) and further supported by Haelewaters et al. (2019). Within the Laboulbeniomycetes, Goldmann and Weir (2018) posit that the class could be divided into four or five orders. They put forward 8 clades: Clade A containing *Laboulbeniopsis*, Clade B containing *Pyxidiophora*, Clade C containing *Chantransiopsis* and *Tetrameronycha*, Clade D containing *Herpomyces*. The following clades contain what most
would consider Laboulbeniales proper and include: Clade E, containing Cochliomyces, Clade F, containing the “aquatic” Laboulbeniales such as Ceratomyces and Euceratomyces, Clade G, containing Chitonomyces and Coreomyces and Clades H and I which contain the “terrestrial” Laboulbeniales. Numerous groups in Tavares (1985) classification scheme are evidenced to be polyphyletic, and despite Thaxter’s emphasis on antheridial structures in taxonomic classification, Goldmann and Weir provide evidence that these structures are homoplastic, instead emphasizing perithecial characters, which trend towards reduction, as diagnostic taxonomic features.

**Symbiosis and Parasitology**

Coined by A.B. Frank in 1885, the term “symbiotism” is defined as a spectrum of interspecies interactions, ranging from parasitism to commensalism to mutualism, with foundational contributions by de Bary (1878). While some researchers have used the term symbiosis to refer only to mutualistic, or mutually beneficial relationships, there is precedent within mycology to deploy the term when referring to a spectrum (Trappe, 2005; Horton, 2015) and is the meaning invoked here in this text. That being said, there is additional confusion around the term symbiosis. The terms mutualism, commensalism, and parasitism contained within the spectrum of symbiosis also engender amorphous and shifting meanings, challenging the discrete categories biologists often seek to ascribe when understanding biological systems. Original hypotheses on mutualisms of mycorrhizal networks put forward by A.B. Frank in the late 19th century were considered revolutionary when introduced and were therefore highly contested. Around the same time, the biology of lichens was being fiercely debated, with mycologists such as de Bary and Schwendener first probing into notions of symbiosis between fungi and algae (Plitt, 1919). A century and a half later, these ideas are only just becoming well-
integrated into scientific literature and lexicon, and remain poorly understood and under-
recognized outside of mycology (Trappe, 2005).

Even more confounding is the term “parasite,” as parasites are a polyphyletic group
cheekily defined as “organisms studied by parasitologists” (Brooks and McLennan, 1993). E.O.
Wilson (2014) attempted to quantify the definition of parasite by saying, “parasites eat prey in
units less than one.” Alternatively, parasites can be defined as organisms that “find their
nourishment and habitat on other living organisms, without destroying it as predators do prey”
(Brooks and McLennan, 1993). While that definition may seem appropriate at first blush, there
are a number of organisms that do not fit that definition but have been traditionally considered
parasites, such as Trichostrongylidae nematodes which feed on intestinal bacteria and protozoa
rather than host tissue. Or, alternatively, there are a number of organisms that meet this
definition, but have not traditionally been considered parasites, such as vampire bats,
mosquitoes, and perhaps most strikingly, herbivores. Other proposed definitions, summarized by
Brooks and McLennan (1993), include: 1. A form of symbiosis in which one species lived “at the
expense of the other” in the association (Chandler and Read, 1961; Dogiel, 1962; Henry, 1966);
2. “An organism living in or on another living organism, obtaining from it part or all of its
organic nutriment, commonly exhibiting some degree of adaptive modification, and causing
some degree of real damage to the host” (Webster’s Third New International Dictionary, 1961;
and somewhat comically, 3. “A particular lifestyle suited to a particular environment, requiring
certain adaptations and endowing certain advantages.” (Brusca and Brusca, 1990). In order to
understand this dilemma, it is useful to consider the origins of the discipline of parasitology,
which are situated in the context of disease biology.
The original definition of “parasite” came from the Latin *parasitos*, with *para* meaning beside and *sitos* meaning food or grain. The word was applied negatively to a person who gleaned meals from the wealthy in exchange for flattery (Harant, 1995; Brooks and McLennan, 1993). Parasitology emerged in the early twentieth century, around the same time bacteriology, linking with research of pathogenicity and widely viewed through a non-Darwinian framework (Brooks and McLennan, 1993). Thus, parasites were historically approached and understood as unwanted, harmful organisms that disrupted the “natural” order of otherwise discrete and independent individuals. We now understand that it is an impossible task to definitionally bind these organisms by the shared trait as “harmful” or “pathogenic,” as if the parasitological essence is something that can be located in the organism’s DNA. Hegner et al. (1938) argue that, “the principles that govern the structure, life cycles, habitats and activities of free-living and parasitic animals are really the same.” Indeed, it is increasingly apparent that these definitions are contextual, and depend greatly on perceived value of the organism(s) in question. For example, nematodes are a ubiquitous group that fill a vast array of niches. The aforementioned Trichostrongylidae living in guts of mammals, feeding not on host tissue but on bacteria and protozoa are, by definition, commensalists. However, their nature as worms predisposes humans to view them as “lesser than,” and their presence within the bodies of mammals is understood negatively.

A similar but opposite example of this conflict exists in the tension between the biology and perception of the red-billed oxpeckers (*Buphagus erythrorhynchus*). These birds live on the bodies of large African mammals, such as cattle, feeding on ticks, dry skin, and exudates such as sweat and mucus (Weeks, 2000). Because ticks are understood to be harmful to their hosts, causing disease, infection, and metabolic drag, the relationship between the red-billed oxpeckers
and the mammals was thought to be mutualistic: the mammals benefit from having the ticks removed and in turn, the birds benefit with a meal (Weeks, 2000). Birds—due to their status as popular, beloved organisms—are not typically associated with parasitism. Therefore, the red-billed oxpecker’s positive status as “mutualist” in this system went widely reported but uninterrogated in the literature (Weeks, 2000). Weeks conducted the first quantitative study of the relationship between oxpeckers and ticks on host cattle, finding that adult tick loads on the cattle were not impacted by the presence or absence of the birds. Furthermore, the presence of the birds actually prolonged the healing time of wounds on cattle. These findings significantly complicate the categorization of the red-billed oxpecker as a mutualist, and in fact may even suggest that the bird is parasitic.

Numerous similar examples exist across the discipline, in which scientists are forced to reckon with the pliable, amorphous, and sometimes contradictory webs of ecology. Likewise, the burgeoning field of microbiology has substantially altered perceptions of individualism in nature. Scientific investigations have begun to attach quantitative data to interdependencies that have been recognized outside of Western culture for millenia, and biologists are remediating ourselves from notions of inherent pathogenicity of microbes. The hologenome concept asks, what is the relevance of an isolated human genome? Bodies are, in fact, communities, there are more fungal and bacterial cells in the human body than there are human cells, upending conceptions of autonomous individuals. The porous boundaries between individual organisms begins to strain our functional, biological definitions. In the context of evolutionary biology, attempts to unify organisms by anything other than common ancestry are bound to be fraught with conditionals and limitations. A polyphyletic group such as parasites are unified on historical associations rather than common ancestry, but nonetheless the collective works amassed by parasitologists
offer fascinating insights into the fields of ecology, evolutionary biology, taxonomy and medicine.

Parasitic Mechanisms of Laboulbeniales

The current notion is that most Laboulbeniales typically have little to no measurable impact on the well-being of their hosts (Richards and Smith, 1956; Whisler, 1968; Scheloske, 1969; Benjamin, 1971; Tavares, 1985). A small handful of studies have demonstrated measurable negative impact on infected hosts (Bro Larsen, 1952; Benjamin, 1971; Strandberg and Tucker, 1974; Gemeno et al., 2004; Nalepa and Weir, 2007; Báthori et al., 2015), particularly when subjected to environmental stressors. For example, in an experimental study of *Rickia wasmanii* on ants, the researchers demonstrated that infected ants were more likely to die when subjected to water and/or nutritional deprivation than their uninfected counterparts (Báthori et al., 2015). Similarly, Haelewaters et al. (2020) demonstrated that lady beetles of the species *Olla v-nigrum* that are co-infected with both *Hesperomyces virescens* and either *Beauveria bassiana* or *Metarhizium anisopliae* have higher mortality than a co-infected nonnative (to North America) species of lady beetle, *Harmonia axyridis*. These studies suggest that there is a metabolic cost to the host, but that host fitness, whereby fitness is defined as the contribution of an individual’s genotype to succeeding generations, has not been measurably impacted. More experimental studies conducted on an array of fungi and hosts are necessary to further clarify the degree of pathogenicity across the group.

On the mutualism-commensalism-parasitism spectrum of symbiosis, Laboulbeniales likely occupy an overlapping range of commensalism and parasitism. For this reason, some researchers prefer the term “ectobiont” to describe these fungi, suggesting a nonparasitic interaction, while others have maintained the use of terms such as “infect” and “parasite.”
Because there is evidence that many Laboulbeniales draw nutrients from their hosts, and because we lack any evidence to suggest that any Laboulbeniales provide any quantifiable benefit to their host, I believe it appropriate to refer to these organisms as parasites. As such, the parasitic mechanisms are as follows:

The appressoria of pathogenic and parasitic fungi are specialized hyphal “pressing” organs which may be simple, single-celled terminal swellings of germtubes, or more complicated “compound” appressoria which sometimes contain a discrete cell wall layer of melanin (Ryder and Talbot, 2015). Melanin has differential permeability to water and solute and thereby can generate internal hydrostatic pressure by creating a high solute concentration, of typically glycerol, within the cell. This pressure enables penetration of the host by creating a gasket-like effect. From the appressorium, an infection peg forms, entering the host via turgor pressure or is facilitated chemically by enzymes. In the case of Laboulbeniales, complete knowledge of nutritional modes and mechanisms are still lacking for this group and have been the subject of much speculation. Currently, it is believed to be likely that the vast majority Laboulbeniales produce haustoria, though recent histopathological study by Tragust et al (2016) found that four species of ant-parasitizing Laboulbeniales—*Laboulbenia formica, Rickia wasmannii Laboulbenia camponoti* and *Rickia lenoirii*—found no evidence of penetration of insect host nor development and haustoria (Tragust et al, 2016). The point of attachment to the host is the foot, which, in many cases, encompasses the appressorium which initially may be a “flattened pad,” and enlarges into a melanized “holdfast” (Weir and Beakes, 1996). In many groups, the penetration point is covered by the holdfast’s anterior section. The melanin appears to be restricted to the center of the foot, which itself contains a hyaline, narrow pore that facilitates passage of the haustorial apparatus (Weir and Beakes, 1996). Variation in haustoria size, precise
penetrative and/or attachment mechanisms, and penetrative depth into host tissue exist across the Laboulbeniales. The lice-parasitizing Trenomyces produce more extensive rhizomycelia and lack the typical melanized foot (Meola and DeVaney, 1976). Haustoria can range from 0.3–5 µ in length. Some are believed to penetrate the setal shafts of the host, while others may penetrate indiscriminately. The haustoria typically form or expand within the host’s epidermis, which is the layer of active, living cells beneath the host’s cuticular layer and above the body cavity. Scheloske (1969) performed a study on thalli attached to beetle elytra, and used nile blue sulfate dye to demonstrate the connection of the thalli to hemolymph conducting tissues of the host. More controlled studies of fungal pathogenicity are necessary.

**Host Specificity**

As discussed by De Kesel (1996b), Laboulbeniales exhibit a range of host specificity which can be categorized as three types:

1. Univorous, in which one host or, more rarely, two related hosts are parasitized. This is the most common degree of specificity.

2. Oligovorous, in which related host species from genera within the same family. Examples include Laboulbenia flagellata which occurs on multiple genera of Carabidae (Coleoptera) including the following genera: Agonum, Amara, Colpodes, Oxypselaphus, Patrobus, Platynus, Pterostichus (Huldén, 1983; Haelewaters, 2019); Laboulbenia vulgaris which occurs on Bembidion, Trechus, Bradycelus, Dyschirius, Brachinus, Duvalius (Coleoptera, Carabidae) (Huldén, 1983); and Laboulbenia pedicellata which occurs on Dyschirius, Bembidion, Tachys (Coleoptera, Carabidae) (Huldén, 1983). This degree of specificity is a minority of cases.
3. Plurivorous, in which parasitization occurs on phylogenetically distant host groups. Examples include *Misgomyces dyschirii* which occurs on *Dyschirius* (Coleoptera, Carabidae) and *Bledius* (Coleoptera, Staphylinidae) (Tavares, 1985; Santamaria, 1995) and *Euzodiomyces lathrobii* which occurs on *Lathrobium, Homeotarsus, Hemiquedius, Xantholinus*, (Coleoptera, Staphylinidae) as well as *Patrobus* (Coleoptera, Carabidae) (Huldén, 1983). This degree of specificity is the rarest.

Plurivorous groups, while occurring on phylogenetically distant host groups, are frequently found on spatially proximate host individuals, sharing either the same habitat or interacting in a predator/prey, or symbiotic relationship. Compatible body chemistry between cohabitating host groups, such as Coleoptera, Hymenoptera and Acarina which are host to *Laboulbenia ecitonis* may facilitate plurivory (Benjamin, 1971). Cohabitation can lead to successful host transitions, or may be an ecological deadend, such as in the case *Rickia* on a larva of an inquiline fly species, *Microdon myrmicae* (Syrphidae) (Pfliegler et al., 2016). The inquiline fly is postulated to be an alternative host and ecological deadend for the fungus. De Kesel (1996) investigated the relationship between host specificity and host habitat preferences. In an experiment with the univorous fungus *Laboulbenia slackensis* which occurs on *Pogonus chalceus* (Coleoptera: Carabidae), he found that soil conditions impact fungus development, and that conditions that are optimal for fungal development are the same conditions that a typical host would select for its optimal habitat. Host physiology alone does not dictate success of the fungus and the occurrence of atypical host infections is more probable in habitat conditions (such as soil chemistry) that are consistent with the habitat conditions of a typical host. De Kesel compared host populations to islands in biogeographical terms. Like islands, host specialization and the subsequent speciation there upon occurs through the ecological isolation of the host, and
the host’s specific habitat on the macro and micro level. Overall, infection is determined by the following factors: host morphology (specifically the integument) and thus the availability of nutrients; chemical and micro-climatological features of the host’s surface; biotic and abiotic habitat dynamics (De Kesel, 1996). High numbers of univorous Laboulbleniales on host groups such as the Carabidae and Staphylinidae are likely proportionate to the high numbers of unique life histories of the hosts. Weir and Hammond (1997) summarize and expand upon factors listed by Huldén (1983) thought to be essential to host distribution patterns. These factors include: general warmth of environment, overwintering hosts, overlapping host generations, intergenerational mating, large and/or dense host populations, interactions between host populations, stable host populations, and moisture (Huldén, 1983; Hammond, 1995; Weir and Hammond, 1997).

**Biodiversity of Insects and Fungi**

Assessing the biodiversity of insects and fungi presents challenges. Both groups are enormously diverse and suffer from a paucity of trained taxonomists. Knowledge of insects and fungi can be described as highly uneven, with representative members, often those associated with agriculture, industry or disease, receiving vastly more attention than other groups (Ainsworth et al., 2018; Kim, 1993). In both groups, millions of species remain undescribed (Hawksworth and Lücking, 2017; Grimaldi and Engel, 2004).

**Insect Diversity**

Insect diversity studies have yielded a range of estimates for global and site-specific studies, with a number of researchers trying their hand at different techniques and methods in order to arrive at sound estimates. Insects are so diverse that researchers do not even agree on estimates of currently described species. Grimaldi and Engel (2004) report estimates that range
from 750,000 to 1.4 million (Wilson, 1992; Hammond, 1992, respectively). Based on work by Gaston (1991) and Resh and Carde (2003), Grimaldi and Engel (2004) endorse the estimate of 925,000 for currently named species. While this discrepancy may seem surprising, it is also understandable given the lack of sufficient incentives for researchers to spend time scouring old literature, synonymizing, cataloguing and producing monographs.

Regarding estimates of living species, both described and undescribed, the estimates of insect richness are even more variable. The lowest estimate is about 2 million species (Grimaldi and Engel, 2004) and the largest is a staggering 30 million tropical insect species (Erwin, 1982). Erwin’s estimate was based on using fogging techniques on tree canopies in neotropical forests, upon which extrapolations were made for total insect diversity. Erwin recorded trees as having unique species of insects in their canopies and used the total tropical tree diversity of approximately 50,000 species to extrapolate. Most researchers now agree that this estimate is much too high, largely because the assumption that the insects found in the canopies would be highly host specific is likely erroneous (Grimaldi and Engel, 2004). Grimaldi and Engel endorse Gaston’s (1991) estimate that there are about 5 million total living insect species. This estimate was arrived at by surveying the collections held by systematists around the world. Despite this method having some potential shortcomings, such as collection biases of individual collectors and the presence of unexamined or unknown duplicates held across collections, the authors believe it is currently the best way to estimate global insect diversity. If this estimation is accepted, then the aforementioned figure of 925,000 named insects would represent nearly 20% of insect diversity.

In a quickly changing climate, there is increasing evidence of mass declines of insects and therefore a pressing need to monitor insect biodiversity and local and regional scales (Kim
and Byrne, 2006). Biodiversity studies focused on invertebrates and fungi are rare, and usually instead focus on vertebrate animals and vascular plants (Fiesler and Drake, 2016). Despite being ubiquitous and essential components of the biosphere, macro-invertebrates such as insects remain under-served with respect to their risk assessment and conservation status. As of 2006 less than 0.1% of described insects had been assessed by the Red List authored by the International Union for the Conservation of Nature (IUCN) (Rodrigues et al., 2006).

Because of the staggering diversity and abundance of insects, there exists feasibility concerns when designing biodiversity studies. Rapid biodiversity assessments of insects are frequently employed in order to glean broad but manageable data that can be, albeit tentatively, extrapolated as a flexible measure of communities and populations (Ward and Larivière, 2004). There is currently a concerted effort by entomologists to establish optimal sampling methods for assessing insect biodiversity by taxa, population, community, habitat, and region (Kim, 1993; Brown, 1997; Hughes et al., 2000; Ward and Larivière, 2004; Fattorini, 2013). Many scientists agree that establishing protected areas is the most effective way to protect multi-kingdom species diversity, particularly when considering understudied, vulnerable and uncharismatic groups, which includes many insects and fungi (Hughes et al., 2000).

**Fungal Diversity**

The state of knowledge of fungi is substantially behind that of the insects. A widely cited estimate of global fungal diversity is upwards of 1.5 million (Hawksworth, 1991). Mycologists including Hawksworth generally agree that this is a conservative estimate, in part because it was based primarily upon extrapolations from plant-fungus ratios (6:1) in temperate regions, and did not give due consideration to the hyper-diverse realm of insect associated fungi, such as the Laboulbeniales, or account for tropical species diversity (Hawksworth, 1991; Hawksworth and
Lücking, 2017). The most recent estimate (Hawksworth and Lücking, 2017) of fungi thought to be in existence is 2.2 to 3.8 million, and the updated fungus-plant ratio for temperate zones is 8:1. Of that, 135,000 species have been described (Hibbett et al., 2016). With only ~ 6% of the lower estimation being known to science, the remaining task is tremendous. Unlike the many groups within the plant and animal kingdoms, fungi do not broadly enjoy the benefits of being well studied and clearly understood. New species are most likely to be discovered by studying relatively understudied habitats and microhabitats, including insect bodies, lichen dwelling fungi, and cryptic species, and through environmental sequencing (Hawksworth and Lücking, 2017).

In addition to fungi being relatively poorly studied, the often sporadic, ephemeral, and unpredictable appearance of fruiting bodies complicates our ability to obtain thorough population data, and has constrained our ability to provide clear, objective assessments of fungi over time. The complex biotic and abiotic forces that lead to a species producing a fruiting body remains unknown in many cases, and likely involves the combination and interactions of degree days, soil temperature, precipitation volume, vegetation patterns, and so forth (Mihail et al., 2007). While some fungi, like some species of Morchella (the morels), can be reliably found in the same place during the same, small seasonal window every year, other species, such as Ionomidotus sp. (personal observation) or Hericium bembedjaense (Jumbam et al, 2019) may be seen once in a given location and then not again for years, if ever. While substantial efforts have been recently made in fungal conservation, this field remains in its early stages (Mueller, 2017). According to the State of the World’s Fungi (Ainsworth et al., 2018), only 56 species of fungi have been evaluated for placement on the IUCN Red List, with 43 species ending up on the list. Comparatively, 25,452 species of plants and 68,054 species of animals have been evaluated. It is therefore imperative that this field receives increased attention, concern and action.
Laboulbeniales Diversity

Laboulbeniales are considered to be the most diverse lineage of insect-associated fungi with about 2,200 described species in 142 genera, but current estimates indicate that there are at least 40,000 species awaiting description (Weir and Hammond, 1997). The impact of these fungi on their hosts is poorly understood and basic studies of their biology are still limited. Only a handful of scientists in the world specialize in the study of Laboulbeniales, and yet, because of the size, diversity, and uniqueness of this lineage, it is undoubtedly a cradle of novel taxonomic and ecological information.

Research Methods

Despite being relatively understudied compared to other groups of fungi, the Laboulbeniales possess certain qualities that lend themselves towards being a model group for fungal diversity studies and ecosystem health. Weir and Hammond (1997) lay out six features of Laboulbeniales that position them as such. These features are as follows: 1) Laboulbeniales display high host specificity in their association with arthropods, exceeding that of any other group of insect/arthropod-associated fungi; 2) these fungi are reasonably visually detectable ectoparasites and can be found in tact growing on living hosts or preserved in collections; 3) patterns of species richness can be assessed by studying the fruiting bodies (thalli) of these fungi and do not require culturing; 4) due to their obligate association with arthropods, entomological collections serve as a tremendous repository for these collections, allowing the researcher utilize an alternative source for novel taxa, host lists, or habitat associations; 5) similar to 4, previous systematic and spatial work by entomologists already exist and can be utilized for the study of this group; different studies from different localities or focused on different insect groups can be compared against one another; 6) Thaxterian taxonomic concepts are relatively solid, consistent,
and comprehensive, making this group particularly accessible and systematically reliable. Furthermore, as discussed above, some fungi, particularly those that form fleshy sporocarps, may only fruit during extremely narrow and/or sporadic windows of time. Because Laboulbeniales lack a known asexual or vegetative stage, and possess spores that are only briefly viable in the environment (if at all) (De Kesel, 1996a), the presence of their fruiting body in the environment is more reliable than many other groups of fungi. The combination of all these factors uniquely positions the Laboulbeniales as a focal group for fungal diversity studies.

Previous studies have begun to explore the potential of Laboulbeniales as indicators. Sugiura et al. (2010) conducted a study of Carabidae beetles and their associated species of Laboulbenia across different habitats in central Japan—riverside, secondary forest and farmland, and the microhabitats therein—to quantify insect-fungal interactions at the host assemblage level. This study found that 14/156 or 8.97% of Carabidae collected at the riverside site were infected with Laboulbenia, individuals; 2/214 or 0.93% in the forest and 0.161 or 0% at the farmland habitat, building evidence that the host habitat partly impacts the prevalence of Laboulbeniales. However, collection of insects in this study took place during December, and while Laboulbeniales are present on hosts overwinter, seasonal impacts of Laboulbeniales richness and abundance are not fully understood and may vary. This study expanded upon studies by Anderson and Skorping (1991) and De Kesel (1996b), which both demonstrated, via field work and experimentation respectively, that host microhabitat impacts occurrence of Laboulbeniales and the presence of host taxa alone does not guarantee presence (or detection) of fungal counterparts.

Hammond (1990) reports results of a beetle survey from a study in a 5 km² patch of land in northern Sulawesi, an island in Indonesia. As part of this study, 1.7 million Coleoptera were
examined over the course of one year (1985). Of the 1.7 million beetles, 80,000, representing the full range of morphotypes, were scanned for infection of Laboulbeniales (Weir and Hammond, 1997). Part of this study examined different trapping methods that have been employed for insect collection, as well as used to the end of collecting Laboulbeniales. These methods include: flight intercept traps, pitfall traps, Malaise traps, and ultraviolet light traps. Based on this study, the unbaited pitfall traps and the flight intercept traps were determined to be the most effective methods for obtaining groups of beetles that host infections. Of course, this data is focused solely on Coleoptera and does not deal with other orders. But because the majority of infections occur on Coleoptera, and within Coleoptera most infections (~50%) occur on Staphylinidae and Carabidae, this knowledge is highly functional (Weir and Hammond, 1997). This study also showed that different traps have certain biases towards different groups of insects. More specifically, the authors found that beetles with a habit of living on or amongst dung, fungal sporocarps, leaf litter, and carrion were more likely obtained via light intercept and pitfall traps. Malaise traps were more likely to trap insects that associate with wood and other fungi, and light traps were biased towards attracting insects with a riparian or aquatic habit. The study reported that beetles associated with wood or living plants tend to have disproportionately few infections, so methods that are biased towards collecting those groups would tend to yield fewer infected individuals than methods that are biased towards beetles that are associated with dung and decaying matter.

In the same study, the authors examined infection rates across the Coleoptera. Of the 80,000 beetle specimens scanned for infection, 158 species were found to be infected totalling 500 infected individuals. In total 0.6% of the screened individual beetles were found to be infected, and 2.7% of the estimated species were found to be hosts of Laboulbeniales. A subset
of the total collection from lowland areas was treated independently. In this subset, a higher total, 3%, of individual beetles were found to be hosts of Laboulbeniales. These results are compared with data from other studies, such as Huldén (1983), Majewski (1994), Weir (1996) and Santamaria (1991), the first two of which are discussed in more detail below. Based on their findings, the authors make two assertions: 1. Laboulbeniales diversity is greatest in moist tropical areas and 2. The majority of Laboulbeniales diversity is associated with Coleoptera hosts. From there, the authors argue that Coleoptera diversity in the tropics is likely a relevant baseline from which to inform estimates of Laboulbeniales diversity. If, given the modern estimate of existing beetles is 2 million, and 3% are infected at the regional or global scale, then there would be 60,000 species of Laboulbeniales on Coleoptera. If the host species:parasite species ratio is 2:1 (meaning that some species of Laboulbeniales infect multiple host species), then there would be 30,000 Laboulbeniales species on Coleoptera. Given that 75% of infections occur on Coleoptera, one could assume that there would be 10,000 species of Laboulbeniales occurring on the rest of the host groups across Arthropoda. This brings the total estimate of Laboulbeniales species diversity to 40,000.

There have been few other detailed studies of Laboulbeniales at a given site. Two in particular are cited by Weir and Hammond as being of value and will be summarized here in chronological order. The first is that of Huldén (1983) in which the Laboulbeniales of Finland and adjacent regions of what was formerly the U.S.S.R were surveyed. This study utilized museum collections from within this area and about 160,000 insects from 1,100 species were scanned for the presence of Laboulbeniales. This study resulted in 64 new occurrence records of Laboulbeniales within that area and 24 new species were described, some of which have since been relegated to synonymy. 166 insect species from Coleoptera and Diptera were found to be
host to a total of 88 species of Laboulbeniales. The frequency of infection for this study was approximately 1%. While comparable studies are extremely limited, this is thought to be low, and the author compares this frequency to frequencies reported from Central Europe by Scheloske (1969) which ranges from 10–35%. Huldén attributes this striking difference to a combination of factors including: changing climatic conditions leading to higher winter mortality, and smaller, more isolated host populations that less frequently overlap generationally.

The second study was conducted by Tomasz Majewski in 1994. This study was a survey conducted over the course of four years in Bialowieza National Park in northeast Poland. Unlike the aforementioned study by Huldén, Majewski conducted his own sampling of insects between 1987–1991. This study site is substantial in size, being about 154 hectares, but is substantially smaller than the spatial scale of Huldén’s study of Finland and adjacent areas. Bialowieza National Park is approximately 1,000 km south of Helsinki. The author recorded 78 insects from Coleoptera and 6 insects from Diptera as hosts in this study, with a total of 50 Laboulbeniales species found on 84 insect species.

Between 1998-2001, a series of surveys dedicated to Laboulbeniales of New Zealand were conducted by Hughes et al. (2008). Insects were collected and museum specimens were surveyed from the New Zealand Arthropod Collection. Previously, only 33 species had been reported from New Zealand. This work, which involved scanning hundreds of thousands of insects, led to 158 species records, from ~33 genera. Of those recorded, 39 species and 7 genera are new, although remain unpublished. Although the precise infection rate of insects examined was not recorded, most species were found on Coleoptera (91.2%) and all hosts recorded were true insects.
Molecular Studies

The advent of molecular studies has revolutionized our understanding of organismal relationships. These techniques are of particular value when investigating cryptic species and synonymies and could help sharpen estimates of species numbers within the group. In a few instances, molecular techniques have successfully clarified relationships at various taxonomic ranks (Goldmann and Weir, 2012; Haelewaters et al., 2018, 2019b), but unfortunately, application of these techniques have been difficult for this group due to their recalcitrance to culture, minute size, and melanized tissue (Weir and Blackwell, 2001a, 2001b; Haelewaters et al., 2015; Sundberg, 2018). As sequence-based identification continues to become commonplace, Laboulbeniales researchers have struggled to keep pace. Hibbett et al. (2016) noted that 64 species of Laboulbeniales were described between 2010–2016, but only two were accompanied with sequence data.

This dilemma necessitates consideration of the value of alpha-taxonomy. While researchers of certain groups, such as fleshy macrofungi, may challenge the validity of species descriptions made in the absence of DNA, morphological and ecological species descriptions have been critical in building foundational knowledge of Laboulbeniales. And while polyphasic or integrated taxonomy (alpha taxonomy combined with molecular data) is an ideal to which researchers strive, Hibbett et al (2016) also note that many researchers still lack access to sufficient funding or equipment to acquire molecular data, and these researchers are often working in tropical areas where most of the world’s undescribed species reside. Because millions of fungal species still remain undescribed, it seems prudent for taxonomists to continue to work with available resources and techniques, with the understanding that future molecular work may shift species limits.
Natural History Collections

Entomological collections are of tremendous value in the study of Laboulbeniales. Thalli of Laboulbeniales persist indefinitely on the host body whether the host is preserved in ethanol or dried and pinned as in standard museum grade preparation. Because collections often contain a wide array of taxa from a broad range of localities, researchers utilizing collections can pursue a scope focused on geography and/or taxa. With the infection rate of Laboulbeniales on insects being low, hundreds to hundreds of thousands of insects may be scanned in search of fungi, depending on the scope of the study. Therefore, utilizing collections for research as opposed to conducting new field work has logistical advantages, saving the researcher time and expense, and circumventing the need to kill hundreds of thousands of insects.

Collections have been used to investigate an array of questions on the subject of biodiversity, taxonomy, biogeography, host utilization patterns. Thaxter intensively utilized collections from the Harvard Museum of Comparative Zoology, European collections such as the Hope Collections at Oxford, the natural history museums of Britain, France, Germany, and others, as well as Central and South American Museum collections such as the Museo Nacional Buenos Aires, and the Sharp Collection in Mexico City. Haelewaters et al. (2015, 2019a) used collections of Coccinellidae, Staphylinidae, and Carabidae largely from the Harvard Museum of Comparative Zoology, as well as the American Museum of Natural History in New York, NY, Tupper Center of the Smithsonian Tropical Research Institute in Ancon, Panama, and Collection d’insectes du Québec, Ministère de l’Agriculture, des Pêcheries et de l’Alimentation du Québec, Québec City, QC, Canada. Hughes (2008) utilized the collections contained within in the New Zealand Arthropod Collection. Included in this work (Kaishian, 2018, 2020, and within) are 12 new species from the genera Laboulbenia and Prolixandromyces which were obtained first by Benjamin from the collection of John Polhemus which is currently held at the in the Department
of Entomology at the National Museum of Natural History, Smithsonian Institution, Washington, DC; Heteropteran insects at the American Museum of Natural History were studied in an exploration of host utilization patterns. Santamaria et al. (2020) utilized millipede collections from the Natural History Museum of Denmark in Copenhagen and the National Museum of Natural History in Paris to describe a new species, *Troglomyces twitteri*. Similarly, Báthori et al. (2017) reported a new record of *Rickia wasmanii* from Greece that was found and identified using the digital image collection on AntWeb.org. These recent studies speak to the timelessness of natural history collection and their continued value in modern research.

**DISSERTATION OBJECTIVES**

Collectively, this body of work threads taxonomy, biodiversity studies, natural history collections, ecology, and theory. Taxonomy and the taxonomists are in decline at a critical point in time where biodiversity is threatened by human behavior and global climate change (Kim and Byrne, 2006). The first two chapters therefore are focused on development of alpha taxonomic skills and the utilization of fungal collection at SUNY-ESF. The first three chapters are thematically bound by their focus on Laboulbeniales occurring on hosts in the Heteroptera, the true bugs. **CHAPTER 1** is focused on the genus *Prolixandromyces*, in which 4 new species are described, representing the first records of this genus in South America. The genus is emended and a key to the genus is provided. **CHAPTER 2** is focused on the genus *Laboulbenia*, in which four new species are described on a new host family, Gerridae (Heteroptera) or water striders. **CHAPTER 3** also includes the description of 4 new species of *Laboulbenia* on Heteroptera, and targets significant gaps in the literature on Heteroptera associated Laboulbeniales. By utilizing the entomological collection at the American Museum of Natural History, this chapter explores host utilization patterns in the group and tracks insect infection rate
at the family level. These three chapters all emphasize the scientific value of maintaining our natural heritage in accessible, research oriented biological collections. Natural history museums, like taxonomy, are currently suffering from declining social value and understanding, and the collections are at risk for liquidation or deterioration. Research programs focused on utilizing collections can raise awareness in the scientific community and to the public of the relevance and service of these collections -- all aims of these three chapters.

CHAPTER 4 is a field-based ecological pilot study focused on exploring how Laboulbeniales and their insect hosts are impacted by urbanization at two lakes in central Florida. Using historical records of Laboulbeniales diversity from 1897 of mycologist Roland Thaxter, a comparison is drawn to modern (2018) diversity. A rapid biodiversity assessment was conducted on insects and fungi at a protected area and a developed area in 2018 and results are compared. This study highlights the potential relevance of Laboulbeniales as biodiversity indicators.

CHAPTER 5 approaches the field of mycology through a theoretical framework rooted in queer and feminist theories, as well as philosophy of science and Traditional Ecological Knowledge. This chapter is important and relevant as it challenges, pushes, and explores central tenets of institutional science and functions to socially and historically situate current research dilemmas in mycology. By excavating and laying bare ingrained, systemic biases in scientific institutions, this chapter seeks to disarm fallacious assertions of “purity” in science. Additionally, this work reiterates themes introduced in the preceding chapters, such as the value of taxonomy and biodiversity studies, the importance of biological collections, and the urgent need for expanded and imaginative conservation practices in the age of climate change.
LITERATURE CITED


de Bary HA. 1878. Die Erscheinung der Symbiose. (‘de la Symbiose’) [The phenomenon of symbiosis]. Speech presented at the meeting of the German Natural Scientists and Physicians in Cassel, Germany.


CHAPTER 1

New species of *Prolixandromyces* (Laboulbeniales) from South America

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PUBLISHED IN 2018


Abstract

Four new species of the genus *Prolixandromyces* (Laboulbeniales, Ascomycota) found on Veliidae (Heteroptera) from Bolivia, Brazil, Peru, and Venezuela are described and illustrated. These four species, *Prolixandromyces anseris*, *P. tritici*, *P. blackwelliae*, and *P. bromelicola*, represent the first records of this genus from South America and their discovery requires emendation of the original generic circumscription. Newly described taxa are compared to known species and a new key to identification is provided.

KEY WORDS: hemipterans, *Stridulivelia, Paravelia*, neotropical, parasites, fungi.
INTRODUCTION

Prolixandromyces R. K. Benj. (1970) was erected for *P. veliae* R. K. Benj. and *P. corniculatus* R. K. Benj. both on species of Velia (Heteroptera: Veliidae) from Mexico. Later, Benjamin (1981) described three additional species in the genus (*P. lingulatus* R. K. Benj., *P. rhinoceralis* R. K. Benj., and *P. tenuis* R. K. Benj.), all parasites on species of *Velia* from Central America (Mexico, El Salvador, Nicaragua, Costa Rica), and provided a key for the identification of the five known species.

More recently, Santamaria (1988) described a new species, *P. triandrus* Santam. on *Velia* from Spain and Weir (2008) described two additional species, *P. benjaminii* A. Weir and *P. lanceolatus* A. Weir, both on *Rhagovelia* from the Philippine Islands (*P. benjaminii*) and from various countries in Africa and Asia (*P. lanceolatus*). Based on these latter collections, Weir (2008) also emended the original generic description of the genus. Benjamin (1970, 1981) had described the appendage of all known species as being composed of 3 superposed cells, the uppermost only, bearing 2 simple antheridia. The old world taxa described by Weir (2008) extended the appendage characteristics of the genus to include taxa with 3-11 superposed appendage cells, the basal 2 cells sterile, and with each of the remaining cells in the series bearing a single, simple antheridium, with the exception of the terminal cell, that bears 2 antheridia.

In this contribution we add to the known diversity, host, and geographical range of the genus *Prolixandromyces* with the description of four new species on *Paravelia* and *Stridulivelia* from South America. The slides for these new species were included in the Benjamin collection now housed at SYRF. The new taxa are compared to the 8 known species and a new key is provided to aid in the identification of the now 12 known species of the genus. Appendage characteristics
of one of our new species (P. bromelicola - with the uppermost cell of the series producing only a single, terminal antheridium), along with re-examination of material described by Weir (2008) to include the discovery of five tiers of perithecial outer wall cells in at least some of the vertical rows of outer wall cells, also necessitates emendation of the generic description of *Prolixandromyces*.

All of the known hosts of species of *Prolixandromyces* belong to the heteropteran family Veliidae, a globally distributed group of predatory semiaquatic bugs, with many species of Neotropical distribution (Moreira and Barbosa, 2011). These small to medium sized heteropterans are found on the surface of water bodies (Andersen, 1982) where they feed mostly on small arthropods, and are quite easily collected using standard entomological equipment.

**MATERIALS AND METHODS**

All of the hosts of the fungi described in this study were either collected directly in the field by John and/or Dan Polhemus, or obtained indirectly via the collection of John Polhemus. This means that some collections were made by other entomologists, but the insect hosts are in the Polhemus collection. Specimens were preserved in 70-80% ethyl alcohol although some had later been pinned, dried, and placed in museum boxes. None of these hosts were available for study as they had been returned by Benjamin and all host names were those determined by John/Dan Polhemus. Benjamin studied the hosts, removed fungal thalli and made microscope slides, and then returned the hosts to Polhemus. The Polhemus collection of aquatic Heteroptera is now housed in the Department of Entomology, National Museum of Natural History, Smithsonian Institution, Washington DC.
Fungal thalli were removed with a micropin and mounted in glycerine using previously described methods (Benjamin, 1971, 1986, 1993). Initial pencil drawings were prepared for two of the new species (*P. blackwelliae* and *P. anseris*) by Benjamin using a Leitz Dialux microscope fitted with differential interference contrast (DIC) optics, and new line drawings were prepared by us for the remaining two species (*P. bromelicola* and *P. tritici*) using a Nikon E-800 microscope also fitted with DIC optics. The final preparation and inking of all drawings was carried out by us.

Terminology and abbreviations used in describing the ascoma (used here for the entire perithecium-bearing thallus) are those used by Tavares (1985). Holotypes of the newly described taxa are deposited in the fungal herbarium at SUNY College of Environmental Science & Forestry, in Syracuse, New York SYRF.

**TAXONOMY**


Receptacle consisting of three superposed cells bearing distally on one side a free appendage and on the other side a stalked perithecium. The basal (I) and suprabasal (II) cells of the receptacle very strongly obliquely superposed, the latter subtending the primary stalk cell (VI) of the perithecium; the upper cell of the receptacle (III) subtending the appendage. Appendage consisting of 3–11 superposed cells. The basal two are sterile and each of the upper cells bear a single, simple antheridium. The uppermost cell in the series either producing a single simple terminal antheridium (in the 3 celled appendage), or more frequently (in the 4-11 celled appendage), giving rise to both a terminal and lateral antheridium. Antheridia with discharge tubes free and moderately to very elongate. Perithecium with two stalk cells (VI, VII), three
persistent basal cells \((m, n, \text{ and } n')\) and four vertical rows of outer wall cells with four (or five) cells of unequal height. Ascogenic cell single. Ascospores 1-septate.

Type species: *Prolixandromyces veliae* R. K. Benj.

**Key to species of Prolixandromyces**

1. Single terminal antheridium
   - 1. Two or more antheridia

2. Length of primary perithecial stalk cell (VI) exceeding length of perithecial body
   - 2. Primary perithecial stalk cell (VI) shorter than perithecial body

3. Perithecial apex with projecting acuminate appendage(s)
   - 3. Perithecial apex lacks such appendage, instead comprised of multiple, obtuse, lip cells

4. Perithecial apex with singular, acuminate appendage, which is about one third the length of perithecium
   - 4. Perithecial apex with multiple, acuminate appendage, which are extremely short

5. Primary appendage with two antheridia
   - 5. Primary appendage with more than two antheridia

*P. bromelicola*

*P. tritici*

*P. tenuis*

*P. anseris*
6. Perithecium with hooked, subterminal outgrowth  
   \textit{P. triandrus}

6. Perithecium lacking hooked, subterminal outgrowth  

7. Primary appendage separated from cell III of receptacle by a constricted, 
   externally blackened crosswall  
   \textit{9.}

7. Crosswall separating primary appendage from cell III not constricted and 
   not externally blackened  
   \textit{10}

8. Primary appendage long and flexuous, composed of 7-11 cells 
   bearing a series of 6-10 antheridia  
   \textit{P. benjminii}

8. Primary appendage composed of 5 cells, 
   bearing a series of 5 antheridia  
   \textit{P. lanceolatus}

9. Cell I of receptacle more or less clavate; primary stalk cell VI 
   approximately the same length as appendage including antheridia  
   \textit{P. veliae}

9. Cell I of receptacle reniform; primary stalk cell VI much shorter 
   than appendage including antheridia  
   \textit{11.}

10. Basal cell of primary appendage long and rectangular; cells in appendage 
    with transverse septa; light in coloration  
    \textit{P. blackwelliae}

10. Basal cell of primary appendage short, cells in appendage with oblique 
    septa; dark in coloration  
    \textit{P. lingulatus.}

11. Perithecial apex with multiple appendages, including at least one 
    ligulate appendage and one stout, sharply divergent, hornlike appendage  
    \textit{P. rhinoceralis}

11. Perithecial apex with single, narrower, hornlike appendage  
    \textit{P. corniculatus}

\textit{Prolixandromyces anseris}  A. Weir & Kaishian sp. nov.  
Figs. 1.1G, H; 1.2A

MycoBank: MB 823506
Typification: BOLIVIA, DEPT. LA PAZ, Sud Yungas Prov., rocky stream 3 km southeast of Sapecho, elevation 520 m, on upper surface of femur on left middle leg on male *Stridulivelia tersa* Drake & Harris (Heteroptera, Veliidae), 13 September 1989, D.A. & J.T. Polhemus (CL 2515), (holotype RKB 3646A in SYRF).

**Etymology.** From *anser* (Latin), goose; alluding to the overall appearance resembling a goose neck and head.

*Ascoma* overall shape elongate, with perithecium curving away from appendage. Yellow brown to brown, with cell III in receptacle and lower cells of appendage being darker amber brown. Total length from top of blackened foot to apex of perithecium 342–575 μm. *Receptacle* comparatively short 88–100 μm from top of foot to base of appendage. Basal cell I 25–40×5–10 μm at broadest point, clavate, rounded apically, tapering down towards foot with curve at base; suprabasal cell II and basal cell I obliquely superposed with cell II being much longer than cell I; cell II 80–88×10–13 μm, distally flattened, tapering slightly down to foot, subtending primary stalk cell VI; cell III slender, triangular, flattened distally, tapering to a point toward foot, adnate to both cell II and primary stalk cell VI, suffused with dark amber brown, 18–38×10–13 μm; subtending primary appendage. *Appendage* free, straight, elongate, total length 100–115 μm; basal cell of appendage 25–30×13 μm; rectangular, superposed by slightly smaller rectangular cell, 23–25×13 μm; these lowermost cells of appendage are sterile, superposed by smaller, triangular cell, 13–20×13 μm at broadest point, flattened at base and rising to a point, subtending two antheridia—terminal and lateral; antheridial venter 18–20×8–10 μm, discharge tubes 15–38×3 μm, elongate. *Perithecium* total length 148–213×45–75 μm primary stalk cell VI very long and narrow, 118–275×13–23 μm, fairly straight, may curve slightly near perithecium; perithecium slightly curved to almost perpendicular to stalk cell, bulging and tapering gradually
toward apex; ostiole blunt; apex with terminal projecting ligule and flap-like structure, ligule tapers to a point, measuring 13×3 μm; flap-structure 10×3 μm.

Other specimens examined. BRAZIL, DEPT. AMAZONAS, Igarape da Anta, 2.5 km east of Ducke Reserve, 25 km northeast of Manaus, on lower mid surface of femur of left anterior leg on male *Stridulivelia strigosa* Hungerford (Heteroptera, Veliidae), 25 August 1989, D.A. & J.T. Polhemus (CL 2472), RKB 3609B in SYRF.

Notes: *Prolixandromyces anseris* is described from specimens collected in two countries—Bolivia and Brazil. In total, five fungal specimens were examined. *Prolixandromyces anseris* is distinctive based upon the following combination of features: within the receptacle, cell I is half as long as cell II. In *P. tritici*, there is barely any overlap between receptacle cell II and cell III, unlike in *P. tenuis* where cell II extends up the length of cell III. In *P. anseris*, the second cell of the appendage is 2–3 times long as it is broad, being longer and more rectangular than *P. tenuis*. The perithecium of *P. anseris* lacks an “elongate acuminate appendage” as in *P. tenuis*, possessing instead a small, short projection from cell m and one projection from either n or n’ cells.

*Prolixandromyces tritici* A. Weir & Kaishian sp. nov. Figs. 1.1E, F; 1.2B

MycoBank: MB 823507

**Typification:** BOLIVIA, DEPT. LA PAZ, Sud Yungas Prov., rocky stream 3 km southeast of Sapecho, elevation 520 m, on lower surface of femur of right middle leg, on female *Stridulivelia tersa* Drake & Harris, (Heteroptera, Veliidae), 13 September 1989, D.A. & J.T. Polhemus (CL 2515), (holotype RKB 3647B-3 in SYRF).
Etymology. From *triticum* (Latin), wheat; alluding to the long, wheat-like body form and golden coloration.

Ascoma slender, erect, elongate; perithecium bulging slightly; measuring 400–625 μm long from top of melanized foot to tip of perithecium; yellow to yellow brown with the perithecium, receptacle and appendage being the darkest, and the primary stalk cell VI being hyaline.

Receptacle relatively short, 60 μm from top of foot to base of appendage; basal cell I clavate, 40×10 μm at broadest point, apically rounded and tapering downward toward foot; suprabasal cell II and basal cell I obliquely superposed; cell II 43×13 μm at broadest point; flattened distally, broadest at center, tapering downward toward foot; subtending primary stalk cell VI; cell III triangular 25×10 μm distally obtuse, flattened, tapering to a point towards foot; adnate against cell II and cell VI, subtending primary appendage. Appendage free elongate, 88–105 μm long; basal cell of appendage 18–25×13 μm, separated obliquely from second cell in appendage; second cell 15×13 μm and subtending obliquely third cell in appendage, nearly triangular, 15–20×13 μm, flattened at base and tapering to a point, giving rise to two antheridia; the antheridia are both equally terminal, creating a ‘Y’ shaped appearance; antheridial venter 15–23×8 μm; discharge tubes 20–38×3 μm, free, elongate. Perithecium primary stalk cell (VI) hyaline, much longer than broad, 178–300×23–28 μm at broadest point; broadest apically, being either flattened or rounded, tapering down to receptacle, subtending stalk cell VII; cell VII short, broad, rectangular. Body of perithecium 180–218×45–53 μm at broadest part of perithecial venter; consistent across specimens is a central bulge in venter, tapering gradually towards apex. Wall cells spiral around the perithecium. Apex formed from four cells, 2 cells derived from basal cell n are prominent; one is clavate, the other terminating with a finger-like projection with subterminal, flap-like swelling, somewhat tongue-shaped; terminal cells derived from cell m and
n’, lacking distinct features. Basal part of neck region with distinct, raised striations, sometimes causing a darker coloration to a region.

*Other specimens examined.* BOLIVIA, DEPT. LA PAZ, Sud Yungas Prov., rocky stream 3 km southeast of Sapecho, elevation 520 m, on tip of basal segment of left antennae and on lower surface of femur of right middle leg, on female *Stridulivelia tersa* Drake & Harris, (Heteroptera, Veliidae), 13 September 1989, D.A. & J.T. Polhemus (CL 2515), RKB 3647A-1, 3647A-2 in SYRF.

*Notes:* *Prolixandromyces tritici* is described from specimens from Bolivia. In total, 12 fungal specimens were examined. The long primary stalk cell (VI) distinguishes this species from other known species of *Prolixandromyces*, with the exception of *P. tenuis* and *P. anseris*. This species differs from *P. tenuis* in the breadth of cell VI (about three times as broad as cell VI in *P. tenuis*), and from both *P. tenuis* and *P. anseris* in the lack of one or more acuminate appendages at the tip of the perithecium. *P. tritici* has a blunt perithecial apex comprised of multiple lip-shaped cells. The basal cell gives rise to a more or less rounded terminal cell that does not project beyond the apex. Additionally, two terminal cells derived from basal cell n gives rise to clavate “lip.” In *P. tenuis*, cell II of the appendage is about half as long as broad compared to *P. tritici*, in which cell II is slightly longer than broad. In the receptacle of *P. tritici*, cell I reaches to distal end of cell II, and more than 40% of cell III lies above cell II.

*Prolixandromyces blackwelliae* A. Weir & Kaishian sp. nov. Figs. 1.1C, D, I; 1.2D

MycoBank: MB 823508
Typification: BRAZIL DEPT. AMAZONAS, small rain forest stream south of Walter Egler Reserve northeast of Manaus, elevation 70 m, on right side of meso- and metasternum above legs on female Stridulivelia alia Drake (Heteroptera, Veliidae), 30 August 1989, D.A. & J.T. Polhemus (CL 2479), (holotype, RKB 3615-10 in SYRF).

Etymology. Name in honor of the esteemed mycologist, Dr. Meredith Blackwell.

Ascoma slender, more or less erect, long. Perithecium may be angled away from appendage by way of a curved stalk cell VI; perithecium more or less bulging with undulations on the surface. Colors ranging from pale yellow, yellow, pale brown, yellow brown to darker amber brown; in some specimens the venter of perithecium, antheridial tubes, and/or basal cells of receptacle, particularly the perimeter of cell III, are darker brown in color; stalk cell VI may be hyaline. Total length from top of blackened foot to tip of perithecium 235–425 μm. Receptacle relatively short 33–50 μm from top of foot to base of appendage; basal cell I 25–43×10–13 μm, clavate, rounded or sometimes flattened apically, tapering downward towards foot. Suprabasal cell II and basal cell I obliquely superposed; cell II 28–38×10–15 μm subtending primary stalk cell VI. Cell III 10–18×5–10 μm; shape is variable, most often distally obtuse and flattened, tapering down to a point towards foot, though may be rounded apically or be trapezoidal or nearly square, laterally adnate to cell II, subtending primary appendage above. Appendage free, long, 100–175 μm, from top of cell III to tip of appendage. Basal cell 30–50×13 μm, rectangular; superposed above by shorter rectangular cell 20–38×10–13 μm at broadest point. The two lowermost cells in appendage are sterile. The uppermost cell of the the appendage is small, 13–30×8–13 μm, flattened at base, rises to sharp point, almost triangular in shape, subtending two antheridia—terminal and lateral; antheridial venter 15–25×5–8 μm at broadest point; discharge tubes ranging 13–50×3 μm; long, free. Perithecium primary stalk cell much longer than broad, 50–138 × 13–25
μm at broadest point, widest at apex with slight, descending taper, erect to strongly curved. Secondary stalk cell VII rounded with a diameter of 13–15 μm. Perithecium 150–250×38–63 μm at broadest point; ellipsoid, slender; surface undulating/bumpy; tapers above venter towards apex. Ostiole blunt; apex with terminal projecting ligule, 10–38×5–10 μm, tapering abruptly toward tip.

*Other specimens examined.* BOLIVIA, DEPT. LA PAZ, Sud Yungas Prov, rocky stream 3 km SE of Sapecho, elevation 520 m, on anterior left margin of abdomen near pronotum; on female *Stridulivelia tersa* Drake & Harris, (Heteroptera, Veliidae), 13 September 1989 RKB 3647C-13 in SYRF; BRAZIL, DEPT. AMAZONAS, Igarapé da Anta, 2.5 km east of Ducke Reserve, 25 km northeast of Manaus; on right surface of pronotum, rear of center; on posterior inflexed left margin of pronotum; on female *Stridulivelia strigosa* Hungerford (Heteroptera, Veliidae), 25 August, 1989, RKB 3609A-4, RKB 3609A-6 in SYRF; BRAZIL, DEPT. AMAZONAS, Igarapé da Anta, 2.5 km east of Ducke Reserve, 25 km northeast of Manaus, on upper right metasternum adjacent to margin of pronotum; on left lateral surface of mesosternum adjacent to margin of pronotum; on male *Stridulivelia strigosa* Hungerford (Heteroptera, Veliidae), 25 August, 1989, RKB 3609A-2; RKB 3609A-8 in SYRF; BRAZIL DEPT. AMAZONAS, small rain forest stream south of Walter Egler Reserve northeast of Manaus, elevation 70 m, on right side of meso- and metasternum above legs; on female and male *Stridulivelia alia* Drake (Heteroptera, Veliidae), 30 August 1989, RKB 3615-11, RKB 3615-12 in SYRF; VENEZUELA: ESTADO AMAZONAS, slow tributary to Rio Mavaca at Alto Mavaca base camp, on left lateral median surface of pronotum; on female *Stridulivelia tersa* Drake & Harris (Heteroptera, Veliidae), 31 January 1989, RKB 3606 in SYRF.
Notes: Prolixandromyces blackwelliae is described from specimens collected in three countries—Bolivia, Peru, and Venezuela. In total, 23 fungal specimens were examined. P. blackwelliae is most similar to P. lingulatus, but can be distinguished by the very pale coloration of cell II and cell III in the receptacle, compared to very dark coloration of those cells in P. lingulatus. Primary stalk cell VI is shorter and broader in P. lingulatus compared to P. blackwelliae, which is longer than it is broad. In P. blackwelliae the basal cells of the appendage are much longer than broad, and not obliquely separated, as in P. lingulatus. Thalli of P. blackwelliae (RKB 3609A; RKB 3647C) were found in the same collections of host insects as P. anseris (RKB 3609B) and P. tritici (RKB 3647A, B). Detailed mapping of positions of growth of thalli on different sexes of host, combined with information on copulatory positions in related Heteroptera (Hoffman, 1932; Hungerford, 1920; Polhemus and Chapman, 1979) led us to the conclusion that there is no evidence in support of the hypothesis that these two species-pairs could be different morphs of a single species as found in the genus Chitonomyces (Goldmann and Weir, 2012). The positions of occurrence of thalli of P. blackwelliae on male and female insects of S. strigosa do, however, show a pattern that could support within-species transfer during copulation of hosts.

Prolixandromyces bromelicola A. Weir & Kaishian sp. nov. Figs. 1.1A, B; 1.2E

MycoBank: MB 823509

**Etymology:** bromelicola referring to the bromeliad habitat in which the host species are found.

*Ascoma* erect to slightly curved away from appendage, slender with a more or less bulging perithecium. Pale brown to yellow brown with venter of perithecium a darker amber brown; junction of cell III of receptacle (particularly around perimeter) and basal cell of appendage suffused with darker coloration. Total length from top of foot to tip of perithecium 300–338 μm. *Receptacle* relatively short, 38–70 μm from top of foot to base of appendage. Cell I 30–33×8–13 μm at broadest point, clavate, rounded apically and tapering down to foot. Suprabasal cell II and cell I obliquely superposed. Cell II 38–45×15–18 μm at broadest point, also clavate, apically rounded, and tapering towards foot; subtending primary stalk cell VI of perithecium. Cell III short, 15–20×8–13 μm, lachrymiform or flattened apically, tapering down toward foot; suffused with darker amber coloration, particularly at junction with primary appendage; subtending primary appendage above; adnate with base of stalk cell VI. *Appendage* free, long, whip-like, flexuous, 163–170 μm long. Basal cell 33–38×15–18 μm, broadest at middle; superposed above by a smaller cell, 23–25×18 μm, broadest at its base. The lowermost cells are sterile, the uppermost cell of appendage is very small 10–15×8–11 μm, broadest at base, obliquely separated below from the middle cell of the appendage, subtending above a single terminal antheridium. Antheridial venter 13–25×8 μm; discharge tube 78–87×4 μm; free, elongate.

*Other specimens examined.* PERU. DEPT. PASCO: Pan de Azucar, in bromeliads, on right margin of pronotum of female *Paravelia recens* Drake & Harris, (Heteroptera, Veliidae), 13 July 1961 RKB 3666–2 in SYRF; BOLIVIA: DEPT. LA PAZ, Rio Coroico gorge, 28 km west of Caranavi, elevation 745 m, in bromeliad on rock wall, on upper surface of femur, right anterior leg near base on female *Paravelia manausana* J. & D. Polhemus (Heteroptera, Veliidae),
14 September 1989, RKB 3654 in SYRF BRAZIL. DEPT. AMAZONAS: Manaus, Rio Branco, on right middle margin of pronotum of female Paravelia recens Drake & Harris (Heteroptera, Veliidae), 4 Dec. 1961, E.J. Fittkau (A439) RKB 3667-4 in SYRF.

Notes: Prolixandromyces bromelicola is described from specimens collected from three countries—Peru, Bolivia, and Brazil. In total, nine fungal specimens were examined. This species is readily distinguishable from other members of the genus based upon its single, terminal antheridium, whereas all other members possess two or more antheridia. The host species, Paravelia recens (Drake and Harris), is a bromeliad-inhabiting veliid recorded from terrestrial and arboreal bromeliads throughout the Neotropics (Rodrigues and Moreira, 2016).

DISCUSSION

With the addition of these four new species to the eight already known, the status of the genus Prolixandromyces as a valid taxon seems secure. The collection of these new taxa in South America also adds considerably to the known geographical range for the genus. What is still uncertain, however, is the placement of the genus at a higher taxonomic rank. Benjamin (1970), in his original description of the genus, recognized a close relationship with a complex of genera constituting what Thaxter (1908) had termed the Stigmatomyceteae. This assemblage included at least 10 genera characterized by a simple receptacle composed of three superposed cells, the basal cell (I) forming the foot (attachment to host), the suprabasal cell (II) subtending the primary stalk cell of the perithecium (VI), and the uppermost cell (III) subtending an appendage. More recently, Tavares (1985) in her overview of Laboulbeniales, recognized the then 5 known species of Prolixandromyces and placed the genus in her newly erected subtribe Stigmatomycetinae, that now includes more than 40 genera. This subtribe has come under
scrutiny following development of a method for DNA extraction and PCR amplification (Weir and Blackwell, 2001) and has been found to be polyphyletic (Goldmann, 2015). The genera comprising Stigmatomycetinae are distributed in at least 3 separate clades (Goldmann, 2015), with *Prolixandromyces* occupying an unresolved position but close to the genera *Acompsomyces*, *Bordea*, *Ilyomyces*, and *Hesperomyces*. This placement has led us to re-examine some of the morphological characters of the genus, in particular the number of wall cell tiers in each of the four vertical rows of the perithecium. All four of the above genera have 5 cells in each of the vertical rows of outer wall cells. For the four newly described species of *Prolixandromyces* in this paper we have been unable to distinguish with certainty a fifth tier, however, looking back at some of our previously published material (e.g. *P. lanceolatus*) (Weir, 2008) we notice that we illustrated a fifth cell in at least some vertical rows (see Fig 17, Weir 2008 and Fig. 2C [this paper]). Perhaps these divisions are very late and hard to observe. In support of this, Tavares (1985) recorded that in her study of material loaned by Benjamin, in both *P. corniculatus* and the type species, *P. veliae*, that the fourth tier of outer wall cells may divide at maturity, although the septa are extremely thin and could only be detected by careful focusing. Obviously, more work needs to be done to document the diversity, host utilization patterns, and to clarify the relationships of this interesting genus.
Figure 1.1. A. Mature thallus of *P. bromelicola* with single antheridium (an), RKB 3654. B. Perithecium of *P. bromelicola*, RKB 3666. C. Mature thallus of short form of *P. blackwelliae* showing shortened primary stalk cell of perithecium (VI), secondary stalk cell (VII), one of the perithecial basal cells (m), trichogyne scar (tr), and detail of terminal perithecial appendage arising from basal cell n (ex n); RKB 3609A-1. D. Detail of perithecial tip of *P. blackwelliae*, RKB-3609A-1. E. Mature thallus of *P. tritici* showing spiraled outer wall cells of perithecium, RKB 3647B-3. F. Detail of perithecium of *P. blackwelliae*, RKB 3647B-3. G. Mature thallus of *P. anseris*, RKB 3609B. H. Perithecium of *P. anseris* showing secondary stalk cell (VII), one of the perithecial basal cells (m), trichogyne scar (tr), and detail of perithecial apex with shorter perithecial appendage arising from basal cell m row (ex m); RKB 3609B. I. Mature thallus of long form of *P. blackwelliae* with two antheridia (an), RKB 3609A-2. All specimens in SYRF. Bars = 50 μm.
Figure 1.2. A. Mature thallus of *P. anseris*, RKB 3609B. B. Mature thallus of *P. tritici*, RKB 3647B-3. C. Detail of perithecium of *P. lanceolatus* showing five perithecial wall cells, RKB 3587. D. Mature thallus of short form of *P. blackwelliae*, RKB 3609A-1. E. Mature thallus of *P. bromelicola* with single antheridium, RKB 3654. All specimens in SYRF. Bars: A, B, D, E = 50 μm; C = 25 μm.
LITERATURE CITED


CHAPTER 2

New species of *Laboulbenia* (Laboulbeniales, Ascomycota) on Gerridae (Hemiptera, Insecta), a new host family.

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PUBLISHED IN 2020


Abstract

Four new species of *Laboulbenia* (Laboulbeniales, Ascomycota) occurring on Gerridae (Hemiptera, Insecta), a new host family, are described from six Central and South American countries: Bolivia, Brazil, Ecuador, Panama, Peru, and Venezuela. The new species are *Laboulbenia brachymetrae*, *L. cylindrostethi*, *L. neogerris*, and *L. tachygerris*.

KEY WORDS: neotropical, parasites, fungi, 4 new taxa
INTRODUCTION

The order Laboulbeniales (Ascomycota) is the most diverse fungal lineage associated with arthropods, with about 2,200 described species. These fungal parasites are recorded from the following groups of insects: Blattodea, Coleoptera, Dermaptera, Diptera, Heteroptera, Hymenoptera, Isoptera, Mallophaga, Orthoptera, and Thysanoptera. In addition, millipedes (Diplopoda), Opiliones and mites (Arachnida) are known to carry infections (Weir and Beakes, 1995; Santamaria et al., 2017). The majority of known species of Laboulbeniales, approximately 80%, are found on Coleoptera (Weir and Hammond, 1997). Fewer species have been described from the other groups, such as the Hemiptera (true bugs), with only 92 described species occurring on the aquatic, semi aquatic, and terrestrial families of Hemiptera (Santamaria, 2008; Lee and Na, 2009; Kaishian and Weir, 2018). Of the 92 described species, 21 are of the genus Coreomyces which is restricted to the host family Corixidae (Sundberg, 2018). Within Hemiptera, all Laboulbeniales currently described occur on hosts within the suborder Heteroptera, with other suborders–Auchenorrhyncha, Sternorrhyncha, Coleorrhyncha–having no documented parasites among these fungi (Benjamin, 1967; Santamaria, 2008).

To date, only 12 (Anthocoridae, Corixidae, Cydnidae, Hebridae, Hydrometridae, Lygaeidae, Macroveliidae, Mesoveliiidae, Pentatomidae, Plataspidae, Rhyparochromidae, Veliidae) of the 92 recognized families within Heteroptera have been recorded as hosts to Laboulbeniales. A small group of mycologists have contributed to the knowledge of Laboulbeniales on Heteroptera and only about a dozen publications dedicated to these taxa exist. Between 1967 and 1999, Richard K. Benjamin described approximately 40 species within six genera occurring on semi aquatic Hemiptera. Contributions were also made by Thaxter, 1912, 1931; Majewski, 1981; Rossi, 1994; Santamaria, 2008; Santamaria and Rossi, 1999; Weir, 2008; Rossi, 2016; Rossi and Bernardi,
Currently, there are 11 genera of Laboulbeniales described on Heteroptera: *Coreomyces, Corethromyces, Cupulomyces, Laboulbenia, Majewskia, Monoicomyces, Polyandromyces, Prolixandromyces, Rhizopodomyces, Tavaresiella, and Triceromyces*. The genus *Autophagomyces* once contained several species found on Heteroptera, but Benjamin reassigned those species to *Monandromyces* and to *Triceromyces* (Benjamin, 1999). The genus *Monandromyces* is now synonymized with *Prolixandromyces* (Hyde et al., 2019).

Here we document the occurrence of Laboulbeniales on Gerridae, a new host family in Heteroptera, and we describe four new species in the genus *Laboulbenia*. The genus *Laboulbenia* is the largest genus in the order Laboulbeniales, with approximately 650–700 known species, of which only 11 were previously described from Heteroptera (Santamaria, 2008). Our new taxa are broadly Neotropical, collected from localities in Bolivia, Brazil, Ecuador, Panama, Peru, and Venezuela. Gerridae, commonly referred to as “water striders,” or “water skimmers” is a family of true bugs with worldwide distribution. Gerrids live on the surface of still or slow-moving water, where they feed on living and dead insects. In Gerridae the hind femurs are longer than the abdomen, which distinguishes this family from other families within the infraorder Gerromorpha (Henry, 2009).

**MATERIALS AND METHODS**

All of the hosts of the fungi described in this study were either collected directly in the field by John and/or Dan Polhemus, or obtained indirectly via the collection of John Polhemus. Specimens were preserved in 70–80% ethyl alcohol although some had later been dried, pinned, and placed in museum boxes. All the host-insects were identified by John and Dan Polhemus.
Fungal thalli were removed with a micropin and mounted in glycerine using previously described methods (Benjamin, 1971, 1986, 1993). Initial pencil drawings were prepared by Benjamin using a Leitz Dialux microscope fitted with differential interference contrast (DIC) optics. The final preparation and inking of all drawings were carried out by us.

Terminology and abbreviations used in describing the ascoma (used here for the entire perithecium-bearing thallus) are those used by Tavares (1985). Holotypes of the newly described taxa are deposited in the mycological herbarium at SUNY College of Environmental Science & Forestry, in Syracuse, New York (SYRF).

TAXONOMY

*Laboulbenia brachymetrae* A. Weir, W. Rossi & Kaishian, sp. nov.

FIGS. 2.1A, B, C, 2.2A.

MycoBank MB833796


*Etymology:* Referring to the name of the host genus, *Brachymetra*.

*Ascoma* olive-brown except cell I, which is nearly hyaline for most of its length. Cell I very slender, almost completely hyaline, curved at the base, slightly and gradually enlarging from the base upwards, 47.5–75 × 17.5–25 μm at broadest point. Cell II olivaceous, distinctly shorter, broader, and darker than cell I, 30–37.5 × 22.5–27.5 μm. Cell III and IV replaced by a single, relatively large and broadly squarish cell, 25–30 ×15–22.5 μm (cell III+IV). Cell V small, triangular, oblique, 8–32 × 8–58 μm, placed between the inner surface of the insertion cell and
the perithecium. **Appendage** Insertion cell compressed, broad, oblique, 22–37.5 \( \times \) 5 \( \mu \)m and positioned slightly below the middle of the perithecium. Basal cell of the outer appendage small, irregularly triangular to lacrymiform or rectangular, 7.5–10 \( \times \) 5 \( \mu \)m, giving rise through a darkened basal septum to a long hyaline inner branch extending to 175 \( \mu \)m, and from its upper outer angle, to a forked, usually shorter, branch that is typically broken at maturity. The inner basal cell of the appendage quadrate and then becoming flattened, 16–40 \( \times \) 5–9 \( \mu \)m, forming a crest-like series of up to six subequal cells laying beside each other above it. Each of these cells approximately 7.5 \( \times \) 5 \( \mu \)m, giving rise distally to a single, short, stout antheridium, 12.5 \( \times \) 5 \( \mu \)m. At maturity the antheridia become brownish with a dark basal collar and are sometimes displaced laterally by the growth of a single, relatively long, and unbranched hyaline appendage with brownish base, measuring up to 182.5 \( \mu \)m long. **Perithecium** deep olivaceous below, paler above, tapering to the tip, inner lip elongate and blackish, extending beyond the distinctly shorter and hyaline outer lip, 87.5 –100 \( \times \) 27.5 \( \mu \)m at broadest point, which is near the center of the perithecial body. Ascospores from these specimens were not visible outside of the thalli therefore measurements were not taken.

**Other specimens examined:** ECUADOR. NAPO: Aguarico, small stream, 16 Aug. 1975, A. Langley coll. on femora of anterior legs (A), on antennae (B), on femora of rear legs (D) of *Brachymetra* sp. RKB 3536A,B, D; Panama: Colon Prov., limestone hill stream E of Ft. Shaman between Ft. Shaman gate and Gatun Locks, sea level–80 m, 18 Jan. 1993, CL 2843, J.T. Polhemus & A. R. Gilloghy, on the legs of *Brachymetra albenerva* (Amyot & Serville) 1 female, 1 male. RKB 3829.

**Notes:** The description of this species is based on 70 thalli obtained from insects collected in Ecuador and Panama. *Laboulbenia brachymetrae* is distinguished by the following combination
of characters: the replacement of cells III and IV with a single large cell and the horizontal array of up to six cells above the flattened inner appendage basal cell. *Laboulbenia brachymetrae* shares some similarities to *L. truxalii* R.K. Benj. in the single cell III+IV of the receptacle, in the structure of the appendages and in overall size, but can be separated by the absence of the rounded protuberance at the tip of the perithecium which is found in *L. truxalii*. Additionally, the cells arising from the flattened basal cell of the inner appendage are only 2 in *L. truxalii*, this trait is also found in *L. tachygerris*, *L. microveliae*, *L. drakei*, and *L. truxalii*. *Laboulbenia brachymetrae* possesses shorter branches in the appendage as compared to *L. tachygerris* and *L. cylindrostethi*. *Laboulbenia brachymetrae* is similar to *L. cylindrostethi* and *L. tachygerris* in that they all possess rectangular and unequally sized basal cells of the appendage and differs from *L. neogerris*, which has hemispherical and more or less equally sized basal cells of the appendage.

*Laboulbenia cylindrostethi* A. Weir, W. Rossi & Kaishian, sp. nov.

FIGS. 2.1D, 2.2B

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*Typification:* VENEZUELA. AMAZONAS: swift tributary to Rio Siapa, 1 43 N/64 30 W, alt. 635m, 5 Feb. 1989, D.A. Polhemus CL 8007, on the lower surface of the abdomen, 1<sup>st</sup> to 4<sup>th</sup> segments of *Cylindrostethus palmaris* Drake & Harris. (holotype, RKB 3537A).

*Etymology:* Referring to the name of the host genus, *Cylindrostethus*.

*Ascoma* very pale yellow to light brown. Cell I slender, curved at the base, three to four times longer than broad, 62.5–87.5 × 17.5–32.5 μm. Cell II elongate, three to four times longer than broad, about the same length or slightly longer than cell I, 75–100 × 25–35μm. Cell II, giving
way distally to cells III and VI, which are side by side; the former slightly longer but distinctly broader than the latter. Cell III 20–37.5 × 25–30 μm; cell IV about as long and broad as cell III, 22.5–50 × 15–25 μm. Cell V, small, shield-shaped to rounded, distinctly rising from the upper inner angle of cell IV, thus making the orientation of the insertion cell oblique, 5–12.5 × 5–10 μm. Appendage insertion cell dark and thick, 20–25 × 5–10 μm. Basal cell of outer appendage unusually elongate, 17.5–25 × 7.5–12.5 μm, giving rise terminally to a very long, slender, flexuous, hyaline to pale yellow undivided branch on the outer side. A similar branch is on the inner side, which is, however, bifurcate near the base. There are two basic morphs of this species with reference to the length of these branches. The “branches-long” morph measures up to approximately 1,500 μm in length, whereas the “branches-short” morph measures up to 242.5 μm in length. In some thalli, the second cell in the outer appendage bears an antheridium, which may then give way to another branch. The basal cell of the inner appendage is distinctly shorter than the outer, 10–12.5 × 5 μm. The basal cell subtends a second cell in the appendage, which in turn gives rise to 1 or 2 flask-shaped, yellowish to hyaline antheridia, 15–25 × 7.5 μm. These antheridia are laterally displaced by the growth of a long branch similar to those of the outer appendage. Perithecium broadly elliptical, dark olive-brown, the lower third connected to the receptacle, 100–120 × 37.5–50 μm. The tip subconical, with large hyaline lips, the apex turned outwards. Ascospores from these specimens were not visible outside of the thalli therefore measurements were not taken.

Other specimens examined: VENEZUELA. AMAZONAS: swift tributary to Rio Siapa, 1 43 N/64 30 W, alt. 635m, 5 Feb. 1989, D.A. Polhemus CL 8007, tip of femur of left middle leg of Cylindrostethus palmaris Drake & Harris. RKB 3537C; Bolivia: Dept. Santa Cruz, blackwater tributary to Rio Chore at Chore Forest Reserve, alt. 425m, 19 Sept. 1989, D.A. and J.T.
Polhemus CL2532, on the middle of the second abdominal sternite of *C. palmaris*. RKB 3676A; Santa Cruz; 5 Oct. 1957, C. Pinckert coll., on all parts of *C. bilobatus* Kuitert. RKB 3678; Brazil: Manaus, Paraná Urariá, 12 Jan. 1941, H. Sioli, on all parts of *C. erythropus* (Herrich-Schaeffer). RKB 3677.

*Notes:* The description of this species is based on 278 thalli obtained from insects collected in Venezuela, Bolivia and Brazil. *Laboulbenia cylindrostethi* is distinctive based upon the following combination of characters: the long to extremely long branches separate this fungus from *L. brachymetrae* and *L. neogerris*. *Laboulbenia tachygerris* also possess extremely long branches, but *L. cylindrostethi* can be separated from the latter for the position of cell IV, which is adnate to perithecium in *L. cylindrostethi*, but perpendicular to axis of perithecium in *L. tachygerris*. Additionally, cell V is short in *L. cylindrostethi*, while the same cell is very narrow and elongate in *L. tachygerris*.

*Laboulbenia neogerris* A. Weir, W. Rossi & Kaishian, sp. nov.

FIGS. 2.1G, 2.2D

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*Typification:* VENEZUELA. AMAZONAS: small blackwater tributary to Rio Mavaca, alt. 228m, 21°N/65°W, 11 Feb. 1989, D.A. Polhemus, CL 8005, on the middle of the femur of the right mid leg of *Neogerris celeris* (White), (holotype, RKB 3534).

*Etymology:* Referring to the name of the host genus, *Neogerris.*
Ascoma with cell I elongate, almost the same width throughout its length, hyaline, forming a pedicel-like structure curved at the base, 50 × 12.5–15 μm. Cell II much shorter, broader, and darker, colored olive-brown, broadly pentagonal, 25–30 × 25–30 μm. Cell III + IV forming a single large, quadrate to pentagonal cell, 20–22.5 × 15–17.5 μm. Cell V relatively small, triangular, with the longer side lying along the perithecial margin, 20 × 12.5 μm. Cell VI flattened, 12.5 × 5 μm. Appendage insertion cell triangular to very oblique, short, only slightly darker than the cells below, free from the perithecium, 16 × 3 μm. Basal cells of appendage similar in size, hemispherical, tinged with yellowish-brown, approximately 6 × 6 μm. Inner and outer appendages also similar, each consisting of four or five relatively short, pale yellowish, branchlets, measuring up to 64 μm in length. Perithecium oblong, olive-brown below, gradually becoming paler distally, 64–72 × 24 μm, adherent to the receptacle on the inner side for slightly more than half of its length, the wall cells spirally twisted. The tip slightly bent inwards, ending in two rounded lips, the outer of which is bigger and subtended by a subterminal contrasting black spot. Ascospores from these specimens were not visible outside of the thalli therefore measurements were not taken.

Other specimens examined: BOLIVIA. BENI: Prov. Gral Jose Ballivian, savannah pond 2km E of San Borja, alt 220m, 10 Sept. 1989, D.A. and J.T. Polhemus, CL 2505, near the base of the femur of left mid leg of Neogerris sp. RKB 3670.

Notes: In total, 4 thalli were examined. Laboulbenia neogerris is distinctive based upon the following combination of characteristics: the hemispherical, and more or less equally sized basal cells of the appendage separates L. neogerris from L. brachymetrae, L. cylindrostethi, and L. tachygerris, which all possess rectangular and unequally sized basal cells. Cell II of the receptacle is distinctly pentagonal. This species resembles L. hemipteralis which is described on
*Paravelia platensis* (Berg) (Veliidae, Hemiptera), but can be distinguished based upon cell II, which is distinctly pentagonal in *L. neogerris*. *Laboulbenia hemipteralis* also possess a distinct cell IV, which is lacking in *L. neogerris*.

*Laboulbenia tachygerris* A. Weir, W. Rossi & Kaishian, sp. nov.

FIGS. 2.1E, F, 2.2C

MycoBank MB 833799

Typification: PANAMA. SAN BLAS: waterfall ca 5km E on road past Nusagandi station toward Carti, alt. 450m, 2 Jan. 1993, J.T. Polhemus and A.R. Gillogly CL 2775, on the mid lower surface of thorax behind anterior legs of a male of *Tachygerris opacus* (Drake & Harris), (holotype, RKB 3828A).

Etymology: Referring to the name of the host genus, *Tachygerris*.

Ascoma cells I and II, very elongate and almost of the same width throughout, though cell II sometimes narrower. Cell I usually paler and ranging from about one-third the length of cell II to about nearly equal in length to cell II. Cells I and II are 42.5–125 × 12.5–20 μm and 37.5–205 × 7.5–12.5 μm respectively. Cell III+IV small, elongate, wedge-shaped at its distal end, laying alongside the basal portion of the perithecium, 15–27.5 × 7.5–10 μm. Cell V very narrow and elongate, vertically-oriented, laying along the outer margin of the perithecium for most of the length of the latter, 27.5–55 × 5–7.5 μm. Cell VI distinctly broader than long, and sometimes very compressed, 5 × 15–25 μm. Appendage insertion cell very small, usually oriented vertically 7.5–12.5 × 2 μm. The appendage is relatively simple, comprised of one or two basal cells arising from the insertion cell. The basal cell when there is one cell, and the inner basal cell when there
are two, is 7.5–10 × 5 μm, is more or less rectangular, about twice as long as broad. This then gives rise either to a vertical series of superposed cells forming a single, long, flexuous appendage or to a 1–3 times branched appendage. These branches may or may not fork. In some specimens, a third branch can be seen arising from the inner side of the inner basal cell. The outer basal cell is typically slightly larger than the inner basal cell, 17.5–50 × 7.5 μm. This cell subtends one very long and narrow cell, which then forks and forms an appendage nearly identical to the afore described branch arising from the inner basal cell. The longest appendage observed measured 600 μm. Few functional antheridia have been observed because all specimens were too immature or fully mature. One observed antheridium is present on a Brazilian specimen, arising from the inner basal cell of the appendage, is elongate and flask-shaped, 22.5 × 5 μm. In the specimens from Panama the remains of a tiny branch are still visible just above the insertion cell. *Perithecium* olive-brown, oblong, rather narrow, tapering gradually to the tip, 62.5–70 × 25 μm. The apex is slightly enlarged, ending in very unequal lips, the inner of which is distinctly longer, darker and narrower, while the outer is flattened and almost hyaline; as a consequence, the apex is distinctly turned outwards. Ascospores from these specimens were not visible outside of the thalli therefore measurements were not taken.

*Other specimens examined:* Brazil: Para, Rio Xingua Camp, ca 60km S of Altamira, 52 22 W/ 3 39 S, 3 Oct. 1986, P.J. Spangler and O. Flint coll., on mid surface of mesosternum near the base of anterior legs of *Tachygerris adamsoni* (Drake). RKB 3535; Peru: Dept. Huanuco, jungle near Leonpampa, alt 800m, 11-30 Dec. 1937; F. Woytowski coll # 3811, on the mesothorax near anterior legs of male of *T. adamsoni*. RKB 3675C.

*Notes:* This species is described based upon material collected from Brazil, Peru and Panama. In total, 32 thalli were examined. *Laboulbenia tachygerris* can be distinguished at first sight from
all the other species of *Laboulbenia* occurring on Hemiptera for its slender and elongate habitus and the very long branch(es) of the appendage. Thalli with unbranched appendage bear a superficial resemblance with *L. longipilis* Haelewaters & W. Rossi, which however has a more fusiform perithecium and, above all, cells III and IV clearly divided (Haelewaters and Rossi, 2015).

**DISCUSSION**

Tavares (1985) separated *Laboulbenia* into structural groups based on particular morphological arrangements. While these groups are not considered to be infallible phylogenetically, they are useful in comparing members of this large genus. As described here, cells III and IV in *Laboulbenia brachymetrae* and *L. tachygerris* are undivided. This is true in several other species of *Laboulbenia* on bugs: *L. microveliae, L. drakei, L. truxalii*. These species are all contained in what Tavares refers to as the *Laboulbenia hemipteralis* group that share a single ancestral form, likely derived from the *Laboulbenia luxurians* group. Two other of Tavares’ structural groups, *Laboulbenia muscariae* and *L. pectinulifera*, also share this trait, but occur on Diptera. *Laboulbenia cylindrostethi* and *L. neogerris* do not share this character, and instead cells III and IV are distinct. This variation suggests that these four newly described species are not a monophyletic group.

These four new species and new host family adds to the record of known occurrences of Laboulbeniales on Heteroptera. Families of Heteroptera from aquatic, semi aquatic, and terrestrial environments are all known to host Laboulbeniales. Similarly, Laboulbeniales from distinct clades have been recorded on Heteroptera (Goldmann and Weir, 2018). This suggests that, evolutionarily, infection of this insect group by Laboulbeniales has arisen independently.
numerous times. This raises the question of how these host groups are uniquely suited for parasitism if at all, and what common characteristics may be correlated with parasitism. That only about 9% of Heteroptera families are known to host Laboulbeniales is more likely due to sampling biases amongst entomologists and mycologists than to a general lack of infection across this group. Systematic sampling across all Heteroptera, proportionate to that of the Coleoptera, would undoubtedly reveal many new species and new host records, as well as help clarify host utilization patterns across the group. The fungal specimens were removed from insects that were collected between 26–82 years ago, and therefore it was not possible to obtain DNA sequences. Even when fresh, DNA sequencing on Laboulbeniales tends to be challenging, as the thalli are extremely minute, often few in number, and recalcitrant to culture. Additionally, some groups, including *Laboulbenia*, are heavily melanized which further complicates DNA extraction. In these cases, alpha-taxonomic approaches are of value. This material was obtained from insect collections dating back to 1937, speaking to the continual value of museum collections for biodiversity research.
Figure 2.1. A. Immature thallus of *L. brachymetrae*, RKB 3536. B. Immature thallus of *L. brachymetrae*, RKB 3536. C. Mature thallus of *L. brachymetrae*, RKB 3536. D. Immature thallus of *L. cylindrostethi*, RKB 3537A. E. Mature thallus of *L. tachygerris*, RKB 3536C. F. Perithecium of mature thallus of *L. tachygerris*, RKB 3536C. G. Mature thallus of *L. neogerris*, RKB 3670. Bars = 50 μm
Figure 2.2. A. Mature thallus of *L. brachymetrae*, RKB 3536. B. Mature thallus of *L. cylindrostethi*, RKB 3536. C. Mature thallus of *L. tachygerris*, RKB 3536C. D. Mature thallus of *L. neogerris*, RKB 3670. Bars = 50 μm.
LITERATURE CITED


CHAPTER 3

Laboulbeniales (Ascomycota, Fungi) on Heteroptera (Hemiptera, Insecta) at the American Museum of Natural History

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Abstract
A survey of Heteroptera specimens held in the entomology collections at the American Museum of Natural History was conducted along with a literature meta-analysis in order to explore Laboulbeniales fungi occurring on Heteroptera. The aim of this study was to assess host utilization patterns and infection rate across the insect suborder, explore evolutionary trends between Laboulbeniales and Heteroptera, and report new occurrence records. Non-metric Multidimensional Scaling (NMDS) was conducted on community and ecological matrices assembled from the literature using the vegan package R in order to visualize community assemblage of Laboulbeniales as they relate to ecological factors. Permutational multivariate analysis of variance using distance matrices was carried out using the ‘Adonis2’ function in the vegan package to determine the statistical significance of ecological factors on host parasitization. Four new species of Laboulbenia are described from the collection of Dr. Richard Benjamin, which were obtained from Veliidae specimens within the collection of John Polhemus. This work was cut short due to the COVID-19 pandemic and will be updated and published following completion of all intended goals.

KEY WORDS: taxonomy; biodiversity; collections; fungi; Gerromorpha; Veliidae.
INTRODUCTION

Laboulbeniales (Ascomycota) are minute, obligate ectoparasites on a variety of arthropods. They are the most diverse lineage of insect-associated fungi, with 40,000 species estimated to exist (Weir and Hammond, 1997). Only about 2,200 species have been described to date, as there have been relatively few researchers dedicated to the study of Laboulbeniales. Of the described species, 80% are recorded from Coleoptera hosts (Weir and Hammond, 1997). The remaining 20% are found on Dermaptera, Diptera, Heteroptera, Hymenoptera, Isoptera, Mallophaga, Orthoptera, Thysanoptera, and non-insect arthropod groups including Diplopoda, Opiliones, Arachnida (Weir and Beakes, 1995; Santamaria et al., 2017).

Hemiptera, also known as the true bugs, is the largest clade of non-holometabolous insects (Weirauch et al., 2018). Heteroptera, suborder of Hemiptera, is an enormous and diverse group, with more than 42,000 described species. This group occupies an array of niches, with groups found in a range of terrestrial and aquatic habitats, including the only known marine insect group, Halobates (Gerridae) (Weirauch et al., 2018; Mahadik et al., 2019). Their feeding strategies are similarly diverse, with mycophagy, phytophagy, predation on insects, and hematophagy on vertebrates found throughout the group (Henry, 2009; Weirauch et al., 2018).

Previously, only 96 of the approximate 2,200 species of Laboulbeniales have been recorded on the Heteroptera (Kaishian et al., 2020). The addition of these four new species of Laboulbenia occurring on two genera within Veliidae, brings the total number to 100. All known Laboulbeniales species occurring on Heteroptera have been recorded from 13 out of the 92 recognized families: Anthocoridae, Corixidae, Cydnidae, Gerridae, Hebridae, Hydrometridae, Lygaeidae, Macroveliidae, Mesoveliidae, Pentatomidae, Plataspidae, Rhyparochromidae, Veliidae (Kaishian et al., 2020). There are 11 genera of Laboulbeniales described on
Heteroptera: Corethromyces, Coreomyces, Cupulomyces, Laboulbenia, Majewskia, Monoicomyces, Polyandromyces, Prolixandromyces, Rhizopodomyces, Tavaresiella, and Triceromyces (Thaxter, 1912, 1931; Majewski, 1973; Rossi, 1994; Santamaria, 2008; Santamaria and Rossi, 1999; Weir, 2008; Rossi et al., 2016; Rossi and Bernardi, 2018; Rossi and Leonardi, 2018; Kaishian and Weir, 2018; Kaishian et al., 2020). Laboulbeniales occur on heteropteran families from a range of environments, including aquatic, semi-aquatic, and terrestrial. Because Laboulbeniales from distinct clades have been recorded on Heteroptera (Goldmann and Weir, 2018), it is likely that Laboulbeniales host-switched from Coleoptera to Heteroptera numerous times throughout their evolutionary history (Kaishian et al., 2020).

Most of these species are clustered within the infraorder Gerromorpha, with 28 species occurring on Mesoveliidae and now 59 species occurring on Veliidae. Within Nepomorpha, the fungal genus Coreomyces contains 21 species, and occurs strictly on Corixidae (Sundberg, 2018). Gerromorpha is a monophyletic infraorder containing semi-aquatic bugs, including “water striders,” which are water-surface dwelling (Weirauch et al., 2018). All members of this group exhibit some degree of predation on insects and other invertebrates. Nepomorpha is also a monophyletic infraorder, and contains true aquatic bugs, as well as some species that are secondarily terrestrial. Like Gerromorpha, all members of this group exhibit some degree of predation (Weirauch et al., 2018).

The majority of previous work focused on Laboulbeniales on Heteroptera was conducted by Dr. Richard K Benjamin, who described approximately 40 species within six genera occurring on semi-aquatic Heteroptera (Benjamin, 1967, 1970, 1971, 1981, 1986, 1993, 1999). Much of Benjamin’s work utilized material collected by the entomologists John and Dan Polhemus, most of which has now been incorporated into collections at the Smithsonian National
Museum of Natural History, as well as at the American Museum of Natural History. John Polhemus’ collection contains a relatively high number of semi-aquatic Heteroptera, for which he had a personal interest (Dr. Randall Schuh, personal communication).

Previously, no systematic approach to surveying the Heteroptera for Laboulbeniales had been recorded. Benjamin did not record sampling efforts across the Heteroptera surveyed during his studies. Therefore, it was unclear if the clustering of infections on certain groups within the Heteroptera was due to biological or investigative constraints, given that the Polhemus collection contains a disproportionate abundance of semi-aquatic Heteroptera. This study was designed to further explore Laboulbeniales on Heteroptera in order to: 1. Determine infection rate across the Heteroptera, 2. Assess host utilization patterns by examining what common host group characteristics may be associated with parasitism, 3. Report occurrence records for Laboulbeniales. I hypothesized that clustering of infections on semi-aquatic insects was disproportionately high due to investigative constraints, and a systematic approach would reveal a more even, albeit patchy, distribution of fungi across the Heteroptera.

The four new species of *Laboulbenia* described here were obtained first by Benjamin from the collection of John Polhemus, and are currently held at mycological herbarium at SUNY College of Environmental Science and Forestry, in Syracuse, New York (herbarium code SYRF).

**METHODS**

A total of 7,728 Heteroptera specimens from the American Museum of Natural History (AMNH) were examined. All specimens were from the subsection of the collection stored in ethanol. Ethanol collections are preferable to dried and pinned collections when conducting
Laboulbeniales studies because fungal thalli are more visible when in liquid suspension, and the whole insect body is accessible (as opposed to when insects are pinned or carded), increasing the speed at which insects can be scanned. Insects were examined for the presence of Laboulbeniales infection in small trays of 75% ethanol under a dissecting microscope. The ethanol collections at AMNH are sorted by different taxonomic ranks, with many collections not identified beyond the rank of family. Therefore, a dataset was created to sort scanned insects at family level. Representatives were scanned from the majority of families present and identified within the ethanol collection, though families with fewer than 10 representative collections were typically omitted. All specimens examined came from tropical or subtropical localities in both the Old and New world, but were otherwise selected arbitrarily. Had COVID-19 not interrupted research, more families would have been scanned and all locality information would have been specifically recorded. Prior to publication of this work, a return visit will be made to gather this data. In total, representatives from at 22 identified families and five undetermined but putatively distinct families (27 total) were scanned with at least one representative family from five infraorders within Heteroptera: Cimicomorpha, Gerromorpha, Leptopodomorpha, Nepomorpha and Penatomomorpha (Chapter 3 Appendix, Table 3.A.1). Fungal thalli were removed with a micropin and mounted in glycerine using previously described methods (Benjamin, 1971, 1986, 1993). All fungi were then identified to species.

A meta-analysis of all species and occurrence records of Laboulbeniales on Heteroptera was conducted from literature. From this meta-analysis, a binary community data matrix was constructed showing presence/absence data for known species of Laboulbeniales infections across the 92 families of Heteroptera. Based upon the habitat and lifestyles data overlaid with the combined morphological and molecular phylogeny provided by Weirauch et al. (2018) (Chapter
Appendix, Fig. 3.A.1), an ecological data matrix was constructed for the families of Heteroptera indicating the following characters: habitat (aquatic, water-surface dwelling, riparian, leaf litter, living plants, ground dwelling, and tree bark), feeding ecology (phytophagous, arthropod predation, mycetophagous, mixed, root feeder), size range, aggregate (yes, no, unknown), and host status (known, unknown) (Chapter 3 Appendix, Table 3.A.2) (Schuh and Slater, 1995; Henry, 2009; Weirauch et al., 2018). A total of 29 families from across the following infraorders were included in the community data matrix: Cimicomorpha, Gerromorpha, Leptopodomorpha, Nepomorpha and Penatomomorpha. Families were added to the data matrix if they were known hosts from the literature and/or if they were scanned during this work from the AMNH collection. Non-metric Multidimensional Scaling (NMDS) was then conducted on the community and ecological matrices using the vegan package R in order to visualize community assemblage of Laboulbeniales as they relate to ecological factors (Figs. 3.3–5) (Dixon 2003; R core team 2018). Permutational multivariate analysis of variance using distance matrices was carried out using the ‘Adonis2’ function in the vegan package to determine the statistical significance of ecological factors on host parasitization (Appendix, R Code). Species richness plots across five feeding ecology types and seven habitat types are shown in were derived from the meta analysis of Laboulbeniales infection on Heteroptera recorded in the literature and evaluated using ANOVA in the vegan package in R.

All fungal hosts of the new species described in this study were either collected directly in the field by John Polhemus or obtained indirectly via the collection of John Polhemus. Specimens were preserved in 70–80% ethyl alcohol, although some had later been dried, pinned, and placed in museum boxes. All the host insects were identified by John Polhemus. Fungal thalli were removed with a micropin and mounted in glycerin using previously described
methods (Benjamin 1971, 1986, 1993). Terminology and abbreviations used in describing the ascoma (used here for the entire perithecium-bearing thallus) are those used by Tavares (1985). Holotypes of the newly described taxa are deposited in the mycological herbarium at SYRF.

RESULTS

The following families were examined for Laboulbeniales: Anthocoridae, Miridae, Tingidae, Reduviidae, Gerridae, Mesoveliidae, Veliidae, Saldidae, Corixidae, Naucoridae, Nepidae, Notonectidae, Alydidae, Aradidae, Blissidae, Coreidae, Heterogastridae, Lygaeidae, Pyrrhocoridae, Rhopalidae, Termitaphididae, and Thyreocoridae. Of these families, infections were only found on specimens from Mesoveliidae and Veliidae (Gerromorpha), thereby disproving my hypothesis. The overall infection rate was 0.2%, with 17 infected insects out of the 7,728 scanned. The infection rate for Gerromorpha was 1.5%, with 17 infected insects out of the 1,110 scanned.

Species richness plots are shown in Figures 3.1–2. Of the seven habitat types (aquatic, water surface dwelling, riparian, leaf litter, living plants, ground dwelling and tree bark), aquatic, water-surface, and ripraian are shown to have the greatest associated species richness, and habit was statistically significant ($P = 0.013$) when analyzed without outlier (Veliidae). Of the five ecology types (phytophagous, arthropod predation, mycetophagous, mixed, root feeder), the mixed and arthropod predation groups are shown to have the greatest associated species richness. Feeding ecology was not shown to be statistically significant, whether or not the outliers (Veliidae and Corixidae) were included.
NMDS plot for habitat visualized distinct Laboulbeniales species communities for four habitat types (aquatic, riparian, water-surface and living plant), indicating these habitat types may impact species community composition (Fig. 3.3). NMDS plot for feeding ecology showed less distinction between the three feeding ecology types (mixed, arthropod predation, and phytophagous), with mixed being fully discrete from arthropod predation and phytophagous, which overlap considerably (Fig. 3.4). This suggests feeding ecology may have less of an impact on species community composition than habitat. NMDS plot for tendency to aggregate showed overlapping community assemblages, also suggesting that, based upon this data, that tendency to aggregate may have less of an impact on species community composition than habitat (Fig. 3.5). Additionally, while the plot is not included here, NMDS for size as a factor did not reveal discrete community assemblages.

The ‘Adonis2’ function in vegan indicated habitat was the only statistically significant ($P = 0.004$) ecological character for host parasitization by Laboulbeniales fungi (Fig. 3.3). Additional values are presented in R code in Chapter 3 Appendix. The data obtained from scanned insects at AMNH were complementary to the findings of the meta-analysis, as only Laboulbeniales from water-surface dwelling hosts were found to be infected.

Two species of Laboulbeniales were found. *Triceromyces bullatus* was found on two nymphs of the genus *Mesovelia* (Mesoveliidae) (Typification: Turks Caicos, S. Caicos Island; Van-Voast expedition; Coll. EB Hayden R. Giovani; Feb. 11, 1952; AMNH_IZR 00321128). This is the first time that Laboulbeniales infecting nymphs of Heteroptera have been reported. *Prolixandromyces lanceolatus* was found on 15 specimens of *Rhagovelia celebensis* (Veliidae) (Typification: INDONESIA, Celebes, Sulawesi, J & D Polhemus; Paratype; AMNH_IZR 00321129).
Fig. 3.1. Mean (± SE) species richness across seven habitat types derived from meta analysis of Laboulbeniales infection on Heteroptera recorded in literature. When removing outlier (Veliidae), habitat is statistically significant (P = 0.013).

Fig. 3.2. Mean (± SE) species richness across five feeding ecology types derived from meta analysis of Laboulbeniales infection on Heteroptera recorded in literature. These factors were not statistically significant with or without the outliers (Veliidae and Corixidae).
Laboulbeniales have been found on Heteroptera with three different feeding ecologies: arthropod predation, mixed, and phytophagous. Feeding ecology is not a significant factor in determining parasitization.

Fig. 3.3. NMDS plot visualizing community assemblage of Laboulbeniales species and habitat types. Laboulbeniales found on Heteroptera from four different habitat types: aquatic, water surface, riparian, and living plants. Habitat was a significant factor (P = 0.004) in determining parasitization.

Laboulbeniales have been found on Heteroptera with three different feeding ecologies: arthropod predation, mixed, and phytophagous. Feeding ecology is not a significant factor in determining parasitization.

Fig. 3.4. NMDS plot visualizing community assemblage of Laboulbeniales species and feeding ecology. Laboulbeniales have been found on Heteroptera with three different feeding ecologies: arthropod predation, mixed, and phytophagous. Feeding ecology is not a significant factor in determining parasitization.
TAXONOMY

*Laboulbenia cajamarca* Kaishian et A. Weir sp. prov.


*Etymology:* from the Quecha name *Cajamarca*, which is the locality of the type specimens.

Total length 292.5–325 μm from top of foot to tip of perithecial apex. *Ascoma* golden brown. Basal cells I and II forming an elongate, nearly straight stalk; 150–162.5 × 25 μm. Cell I is
approximately half the length of cell II. Cell I 65 × 25 µm; cell II 82.5 × 25 µm; cells III-V are darker gold to brown and adnate to lower posterior end of perithecium, punctate; cell III is slightly elongate 37.5 × 22.5 µm, subtending cell IV which is variable in shape, ranging from softly square to having a concave delineation with cell V; cell IV 32.5 × 25 µm at septation with cell III; cell V is smaller rounder than cells III and IV, approximately 20 × 20 µm with a free and convex upper surface; cell VI is similarly sized to cell V and slightly lighter in color, being more gold than brown, 17.5–25 × 12.5–22.5 µm, variable in shape, ranging from somewhat round to rectangular. Appendage. Insertion cell compressed, opaque lying at or above the middle of the perithecium. Basal cell of outer appendage lacrymiform, 15 × 12.5 µm bearing an appendage with numerous branchlets which extend laterally. The longest observed appendage is 112 µm long. The basal cell of inner appendage is broad and flat, 15 × 7.5 µm. This gives rise to two cells that bear two flask shaped antheridia, 20 × 7.5µm with a darkened basal septa; two rows of darkened basal cells. Inner cell sometimes gives rise to hyphal branch and antheridium. Antheridia are persistent, displaced laterally by development of 7–8 simple branchlets, 75–87.5 µm. Perithecium. Perithecium is 112–130 × 30–47.5 µm, dark golden brown with a small gap beneath apex where the color lightens. The distal half of the perithecium is free. The apex is slightly curved and the outer and inner lip cells are distinct and black. Perithecium curves away from appendage and the inner lip cell extends beyond the outer lip cell, somewhat resembling a bird’s beak.


On upper surface of abdomen of *Paravelia willei* (Drake & Harris), RKB 2847F.

**Notes:** In total, 76 thalli were examined, all occurring on the same host species but different locations on the insect body. This species most closely resembles *Laboulbenia drakei* R.K Benj. (Benjamin, 1967) but can be distinguished based on the following combination of characters: the basal cell of the inner appendage bears two cells in *L. cajamarca* not three as in *L. drakei*; *L. cajamarca* lacks the nearly opaque branch in the outer appendage as is present in *L. drakei*; the size and orientation of cell V, in which cell V in *L. drakei* is about twice as long as it is broad and oriented nearly parallel with the perithecium, whereas cell V in *L. cajamarca* is nearly square; and aligns perpendicularly with the axis of the perithecium.

*Laboulbenia panaoii* Kaishian et A. Weir, sp. prov.

Etymology: from the Quecha name *Panao*, which is the locality of the type specimens.

Total length 300 μm from top of foot to tip of perithecial apex. Basal cell I and II forming elongate, nearly straight stalk 293 × 25 μm at septum; hyaline to pale yellow, and olivaceous at septum; cell II is approximately 25 μm long, being slightly longer than cell I; cells III–V adnate to posterior 2/3 of perithecium, dark olivaceous and well defined, punctate; cell III 40 × 25 μm with widest point at septation with cell IV; cell IV 37.5 × 30 μm, widest at septation with cell III, irregularly shaped with a concave delineation with cell V; cell V 23 × 27.5 μm, rounded; cell VI distinct, 23 × 15–21 μm, approximately square. Appendage. Insertion cell broad, almost opaque, lying above the middle of the perithecium. Outer basal cell rounded to lacrymiform, 15 × 17.5 μm. This gives rise to two primary appendages that then fork into branchlets. The inner basal cell is broadly triangular, giving rise to two subequal cells that bear two antheridia. Antheridia long, flask-like, 30 × 10 μm with darkened septa. Antheridia may be persistent, displaced laterally by development of branchlets. Perithecium. Perithecium is 112.5 × 52.5 μm, dark olivaceous, lightening near tip, becoming hyaline. Upper one third of perithecium is free. Apex is erect and symmetrical.

Other specimens examined: PERU: Huánuco Dept. vic. of Panao, Andes 3000m; 11–13 Sept 1937; F. Woytkowski coll. (leg. J.T. Polhemus, Aug 1973; ex U. Kans.). On femur of middle, left leg of *Paravelia osborniana* (Kirkland), RKB 2848A; PERU: Huánuco Dept. vic. of Panao,
Andes 3000m; 11–13 Sept 1937; F. Woytkowski coll. (leg. J.T. Polhemus, Aug 1973; ex U. Kans.). On femur of rear, left leg of *Paravelia osborniana* (Kirkland), RKB 2848B

*Notes:* In total, 23 thalli were examined. This species resembles *L. cajamarca* but can be distinguished on the basis of the following combination of characters: the tip of the perithecium is evenly blunt and hyaline in *L. panaoii*, and is instead uneven and darkened in *L. Cajamarca*; the basal and suprabasal cells in the appendage are irregular in size, shape, and relative orientation in *L. panaoii*, as compared to *L. cajamarca* in which the basal cells and suprabasal cells are more constant. *Laboulbenia panaoii* can also be compared to *L. rhagoveliae*, but *L. panaoii* differs in that substantially more of the perithecium is free, and the pore is in line with the perithecial axis, and the perithecium is overall about twice as large.

*Laboulbenia lokona* Kaishian et A. Weir, sp. prov.


*Etymology:* Named after the Lokono people who are indigenous to the area from which the specimen was collected.

Total length 137.5–150 μm from top of foot to tip of perithecial apex. *Ascoma.* Cells I and II elongate, slender, much longer than broad, being broadest at the septation. Cell I 45–52.5 × 12.5 μm; hyaline, becoming golden brown near septation with cell II. Cell II 25–37.5 × 12.5 μm at broadest point, oblique septation with cell VI, which is only visible in immature specimens. Cells III–V nearly equal in size, very dark brown, punctate, adnate to posterior three quarters of
perithecium. Upper surface of cell V free and convex. Cell III is much more triangular, while
cells IV and V are similar in size and shape, being softly square. Cells III–V form almost close to
vertical series with distinct demarcation between appendage and perithecium. **Appendage.**
Insertion cell adnate against cells IV and V, at a right angle to main axis of thallus; dark brown,
broad, compressed. Above insertion cell are the two basal cells. The outer cell is larger than the
inner cell, longer than it is broad 10–13 × 8 μm. This cell bears two minute and rounded cells in
a vertical series with dark sepations; the lower cell is larger and longer than the upper cell. The
distal cell bears a finger-like branch that may fork into a branchlet, measuring up to 62.5μm in
length. The inner basal cell is miniscule and flattened, 3.2 × 6.4 μm and irregularly shaped; this
bears a single elongate antheridium that often curves dramatically, growing up to 25 μm long.
Perithecium is 55 × 20 μm, dark brown nearly opaque throughout the posterior ⅔ of perithecial
body, becoming nearly hyaline towards apex, with the perimeter of the lip cells being suffused
with brown. The upper ⅓ of perithecium is free with a dramatic taper. Apex is blunt, with one
hyaline lip cell bearing a minute fork at the tip, and one partly opaque lip cell.

**Other specimens examined:** SURINAME: Brokopondo District; Zanderijsavanne; Sabakoekreek;
base of the femur of middle, left leg of *Oiovelia sp.*; RKB 2867A-2; SURINAME: Brokopondo
District; Zanderijsavanne; Sabakoekreek; in Savanna woodland; 25 July 1969; N. Nesser coll.
(SN040) (leg. J. T. Polhemus, 1973). On the base of the femur of middle, right leg of *Oiovelia
sp.*; RKB 2867B-1; SURINAME: Brokopondo District; Zanderijsavanne; Sabakoekreek; in
base of the femur of middle, right leg of *Oiovelia sp.*; RKB 2867B-2.
Notes: In total, 66 thalli were examined. This species is unique to the other species described here in its overall shape and size: the very minute overall size, the very rounded perithecium-receptacle arrangement, and the singular straight and perpendicular appendage make this species unique even a quick glance. Closer examination will reveal some similarities to *Laboulbenia microveliae* but differ based upon the following characters: the insertion cell in *L. lokona* is always entirely opaque, unlike in *L. microveliae*; the appendages in *L. microveliae* are repeatedly branching, whereas *L. lokona* bears only a single, unbranching appendage. *Laboulbenia oioveliicola* Haelew. & Gorczak (Song et al., 2019) is another species described from this genus. *Laboulbenia lokona* can be easily separated from *L. oioveliicola* based upon the relative size and arrangements of cells III, IV, and V. In *L. lokona*, the cells III and IV appear distinct, subequal and adnate to the perithecium, whereas *L. oioveliicola* are indistinct, forming a III + IV arrangement, which extends distinctly out from the perithecium. Cell V is adnate to the perithecium in *L. lokonae* and entirely free from perithecium in *L. oioveliicola*.

*Laboulbenia weirii* Kaishian, sp. prov.


Etymology: Named after my advisor, Dr. Alex Weir, mycologist and alpha taxonomist of Laboulbeniales.

Total length 350–450 μm from top of foot to tip of perithecial apex.
Ascoma. Basal cell I nearly hyaline, becoming a darker golden nearing septation between cells I and II; cell I 90 × 25 μm straight and elongate; cell II 162–175 × 25μm, about twice the length of cell I, curving with maturity, color dark golden to olivaceous, punctate; cells III–VI may be distinguishable in immature to recently mature individuals, but in most mature individuals the septations between these cells are absent or indistinguishable; cells III and IV start out subequal and mostly square in shape, 20 × 20. Cell V is smaller and softly triangular. Cells III–V are punctate, adnate to the posterior 1/3 of the perithecium; when visible, cell VI is small, elongate, and rectangular, 12.5 × 10 μm. Appendage. Insertion cell broad, opaque, compressed, lying at or below the middle of the perithecium. Outer basal cell of appendage is longer than broad, 17.5 × 10 μm, giving rise to a cell of approximately equal size, which in turn bears branches. The basal cell of the inner appendage is comparable in size to the basal cell of outer appendage, though tending to be slightly shorter in length. This cell subtends two subequal cells. Basal and suprabasal cells have dark septations. Branches are wavy and irregular, and may be up to 125 μm long. The antheridia were not observed. Perithecium. Perithecium dark brown. Overall, perithecium is slender and elongate, 125–137.5 × 37.5 μm at broadest point, tapering gradually to a blunt apex, with the upper three quarters being free. Apex is erect. The inner lip cell (with respect to the appendage) is larger and more pigmented, being almost black except the very tip, while the outer lip cell is smaller and hyaline.

Notes: In total, 24 thalli were examined. *Laboulbenia weirii* differs from the other species described here on the basis of the following characters: the proportion of free perithecial surface; the small size of cell V; the elongate basal and suprabasal cells in the appendage; and the orientation of the receptacle with respect to the perithecium, with the receptacle being nearly parallel in *L. weirii* and nearly perpendicular in *L. cajamarca, L. panaoii*, and *L. lokona*.

**DISCUSSION**

Only one large-scale, systematic approach to Laboulbeniales focused on a particular insect order has been published (Weir and Hammond, 1997). The authors report results for a survey of 80,000 beetles collected from Sulawesi, Indonesia over the course of one year (1985) as part of the Project Wallace Expedition. The 80,000 beetle specimens were scanned for Laboulbeniales infection, and 158 species were infected, totalling 500 infected individuals. In total 0.6% of screened individual beetles were infected. The present study differed from the survey of Coleoptera from Sulawesi in that the specimens examined in the Coleoptera study were all collected from the same general area within the same year, whereas specimens examined in the present study were widely distributed, including localities from the Old and New world, and range from tropical to subtropical environments. In this study, the infection rate of Heteroptera was 0.2%, three times lower than that of the Coleoptera from Sulawesi. However, due to methodological differences between the studies, concrete conclusions about these infection rates are not possible.
Fig. 3.6. A) mature thallus of *Laboulbenia cajamarca*; B) mature perithecium, upper receptacle and appendage of *Laboulbenia panaoii*; C) immature thallus of *Laboulbenia panaoii*; D) mature thallus of *Laboulbenia weirii*; E) immature thallus of *Laboulbenia weirii*; F) mature thallus of *Laboulbenia lokona*; G) immature thallus *Laboulbenia lokona*.
When viewed together, the survey data and meta-analysis reveal a biological basis for the clustering of Laboulbeniales on semi-aquatic or water-surface dwelling Gerromorpha. This may be because a semi-aquatic lifestyle increases the likelihood of contact between various groups of insects from both aquatic and terrestrial environments, increasing fungal transmission. Semi-aquatic insects are also often found in massive aggregations or swarms, often with mixed species or even families (Schuh and Slater, 1995; personal observation), which increases transmission and fungal radiation. Additionally, high moisture environments are thought to be correlated with Laboulbeniales infection (Hammond, 1995; Weir and Hammond, 1997).

As suggested by Tavares (1985), early evolution of Laboulbeniales likely occurred in association with an ancestor of both Blattodea (host to Herpomycetales) and Coleoptera during the Devonian. Arguments, which are laid out by Tavares, can be made for early evolution occurring primarily on either of the insect groups. Following broad diversification of Laboulbeniales on Coleoptera, infection of the Heteroptera occurred independently numerous times, as evidenced by various, distinct lineages found on Heteroptera (Goldmann and Weir, 2018; Kaishian et al., 2020). One relatively isolated fungal lineage on Heteroptera is the genus Coreomyces. Tavares (1985) classified Coreomyces as the lone member of Coreomycetaeae. Molecular phylogenetic work by Goldmann and Weir (2018) places Coreomyces in a relatively isolated group, “Clade F,” along with Chitonomyces. All species within Chitonomyces occur on aquatic Coleoptera, mostly Dytiscidae. Haelewaters (2018) supported placement of Coreomyces with three aquatic genera: Ceratomyces, Chitonomyces, and Zodiomyces, all of which are found on Coleoptera. Together, these findings suggest Coreomyces became established on Corixidae (Nepomorpha) and subsequently diversified, as Coreomyces has never been found on hosts other than Corixidae, and Corixidae have never been reported to host species other than Coreomyces.
Despite the family Corixidae being a well documented host of Coreomyces, no fungi were found on the 719 specimens scanned. This may be due to the fact that all Corixidae scanned for this study were from a single locality in the Bahamas, and island localities tend to have patchy organismal distributions (MacArthur and Wilson, 1967).

Ecological factors used in the NMDS ordination and subsequent hypothesis testing were chosen in order to explore a variety of potential factors that may shape host distribution patterns on Heteroptera. There were some limitations to this due to gaps in insect literature. For instance, indicating aggregating or not aggregating was not possible for many insect families due to insufficient recorded knowledge of certain insect groups. However, the tendency for an insect group to aggregate may predispose the group for parasitization by Laboulbeniales (Weir and Hammond, 1997; Nalepa and Weir, 2007). Predation was considered a potentially relevant ecological factor because predator-prey interactions could facilitate transmission between the two groups. Many predacious groups are currently not known to host Laboulbeniales, but infected families within Gerromorpha and Nepomorpha are predacious. So while predation was not considered a significant factor on its own, it may play a role within the insect-fungal biology of semi-aquatic insects.

Host size range was also considered to be potentially relevant because there appears to be an upper limit of beetle size that host Laboulbeniales, but no apparent lower limit. For example, Ptiliidae is the smallest known beetle family which contains species that range from 0.3–4.0 mm long, and is a recorded host group to Laboulbeniales. Meanwhile, large beetle families (e.g., Histeridae, Scarabaeidae, and Lucanidae) host proportionally fewer to no Laboulbeniales. This analysis did not reveal a significant relationship between host size and Laboulbeniales infection. However, one interesting point is that Belostomatidae (Nepomorpha), the largest group in the
order, ranging up to 110 mm, has never been found to host Laboulbeniales, whereas numerous Laboulbeniales have been found on a variety of Microvelia species (Veliidae: Gerromorpha), which are very small group, ranging from 1.0–6.0 mm. An analysis of all known hosts from across all arthropod groups could be conducted to further investigate this relationship.

Finding Laboulbeniales on nymphs of Heteroptera is noteworthy, as it has never been reported before. Laboulbeniales recorded from immature hosts is exceedingly rare (Benjamin, 1971). Benjamin summarized the following reports of fungi on immature hosts: Laboulbenia hagenii (Thaxter, 1896) was found on immature and adults of Termes (Isoptera). Rickia wasmanii was found on adults, larvae and pupae of Myramica laevinodis (Formicidae) during a laboratory experiment by Baumgartner (1934). Nymphs and adults of cockroach host Blatta orientalis were reported with Herpomyces stylopygae by Richards and Smith (1955a). A larva of a scydmaenid beetle was reported by Lepesme (1945) to be infected with Jeanneliomyces tachyoryctidii.

According to Tavares (1985), Laboulbenia guerinii or a closely related species was found by Hagen (1855) on the nymphs of Macrotermes bellicosus (Smeathman) var. Mossambicus (Hagen) from Mozambique. Rossi and Bergonzo (2008) reported an immature Brazilian beetle, Systena s-littera, infected with Laboulbenia systena. While the host had completed metamorphosis, wings had not yet sclerified. There is one account of Rickia on a larva of an inquiline fly species, Microdon myrmicae (Syrphidae) (Pfliegler et al., 2016). The inquiline fly is postulated to be an alternative host and ecological deadend for the fungus.

As non-holometabolous insects, Heteroptera do not undergo complete metamorphosis like the aforementioned members of Diptera, Coleoptera, and Hymenoptera, but do undergo
ecdysis or molting, which had long been presumed to either remove the fungi, or prevent their establishment entirely (Benjamin, 1971). With evidence that fungal infections can establish, ecdysis may still lower the infection rate of immature hosts. Nymphs in Gerromorpha are frequently found in large aggregates, often in groups of mixed ages and taxa, which would increase the likelihood of community transmission and infection.

Given the relatively high infection rate of the Gerromorpha, future targeted research for new species should focus on this group. Future studies could systematically explore Heteroptera within a given area, like the Sulawesi based study of Coleoptera, in order to see if the infection rate is consistent with the 0.2% found in this study.

This work was made possible by the entomological collections at AMNH, which speaks to the vital and timeless service biological collections serve in modern science. Particularly with mounting pressures of climate change, researchers and funding sources should encourage and engage with research focused on exploring these troves of natural global heritage.

Acknowledgements: The author would like to thank Drs. Ruth Salas and Toby Schuh for graciously accommodating exploration of the entomological collection at AMNH. Your expertise and assistance was essential to this work. Thank you to Claudia Victoroff and Dr. Hyatt Green for your helpful contributions
Table 3.A.1. Results of scanning 7,728 insects at AMNH from 27 out of the 92 known Heteroptera families. In total, 17 infections were found.

<table>
<thead>
<tr>
<th>Infraorder</th>
<th>Insect Family</th>
<th>Total Scanned</th>
<th>No. Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIMICOMORPHA</td>
<td>Anthocoridae</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Miridae</td>
<td>632</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Tingidae</td>
<td>118</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Reduviidae</td>
<td>493</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td><strong>1,293</strong></td>
<td><strong>0</strong></td>
</tr>
<tr>
<td>GERROMORPHA</td>
<td>Gerridae</td>
<td>620</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Mesoveliidae</td>
<td>72</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Veliidae*</td>
<td>554</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td><strong>1110</strong></td>
<td><strong>17</strong></td>
</tr>
<tr>
<td>LEPTOPODOMORPHA</td>
<td>Saldidae</td>
<td>61</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td><strong>61</strong></td>
<td><strong>0</strong></td>
</tr>
<tr>
<td>NEPOMORPHA</td>
<td>Corixidae</td>
<td>719</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Naucoridae</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Nepidae</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Notonectidae</td>
<td>215</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>undet Nepomorpha 1</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>undet Nepomorpha 2</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td><strong>1094</strong></td>
<td><strong>0</strong></td>
</tr>
<tr>
<td>PENTATOMOMORPHA</td>
<td>Alydidae</td>
<td>56</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Aradidae</td>
<td>103</td>
<td>0</td>
</tr>
</tbody>
</table>
### Table 3.A.1. Continued.

<table>
<thead>
<tr>
<th>InsectFamily</th>
<th>Habitat</th>
<th>Feeding Ecology</th>
<th>Size Range (mm)</th>
<th>Gregarious</th>
<th>HostStatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blissidae</td>
<td></td>
<td></td>
<td>550</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Coreidae</td>
<td></td>
<td></td>
<td>98</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Heterogastridae</td>
<td></td>
<td></td>
<td>450</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Lygaeidae</td>
<td></td>
<td></td>
<td>2131</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Pyrrhocoridae</td>
<td></td>
<td></td>
<td>90</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Rhopalidae</td>
<td></td>
<td></td>
<td>174</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Termitaphidae</td>
<td></td>
<td></td>
<td>300</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Thyreocoridae</td>
<td></td>
<td></td>
<td>81</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Undet. Pentatomoidea</td>
<td></td>
<td></td>
<td>9</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Undet. Pentatomoidea 1</td>
<td></td>
<td></td>
<td>38</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Undet. Pentatomoidea 2</td>
<td></td>
<td></td>
<td>8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td><strong>4008</strong></td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

**GRAND TOTAL: 17/7728**

### Table 3.A.2. Ecological data matrix for 29 families with five ecological characters compiled from the literature.

<table>
<thead>
<tr>
<th>InsectFamily</th>
<th>Habitat</th>
<th>Feeding Ecology</th>
<th>Size Range (mm)</th>
<th>Gregarious</th>
<th>HostStatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alydidae</td>
<td>LivingPlants</td>
<td>Phytophagous</td>
<td></td>
<td>Yes</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Anthocoridae</td>
<td>LivingPlants</td>
<td>ArthropodPredation</td>
<td>1.5-4.5</td>
<td>Unknown</td>
<td>Known</td>
</tr>
<tr>
<td>Aradidae</td>
<td>TreeBark</td>
<td>Mycetophagous</td>
<td>3.0-11.0</td>
<td>Yes</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Family</td>
<td>Habitat</td>
<td>FeedingType</td>
<td>Size</td>
<td>Known</td>
<td>Known?</td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------</td>
<td>-----------------</td>
<td>------------</td>
<td>-----------</td>
<td>----------</td>
</tr>
<tr>
<td>Blissidae</td>
<td>Riparian</td>
<td>Phytophagous</td>
<td>3.0-15.0</td>
<td>Yes</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Coreidae</td>
<td>LivingPlants</td>
<td>Phytophagous</td>
<td></td>
<td>Yes</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Corixidae</td>
<td>Aquatic</td>
<td>Phytophagous</td>
<td>1.5-16</td>
<td>Yes</td>
<td>Known</td>
</tr>
<tr>
<td>Cydnidae</td>
<td>LeafLitter</td>
<td>RootFeeder</td>
<td>2.0-20.0</td>
<td>Unknown</td>
<td>Known</td>
</tr>
<tr>
<td>Gerridae</td>
<td>WaterSurface</td>
<td>ArthropodPredation</td>
<td>1.6-36</td>
<td>Yes</td>
<td>Known</td>
</tr>
<tr>
<td>Hebridae</td>
<td>WaterSurface</td>
<td>ArthropodPredation</td>
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<td>Known</td>
</tr>
<tr>
<td>Heterogastridae</td>
<td>LivingPlants</td>
<td>Phytophagous</td>
<td></td>
<td>Yes</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Hydrometridae</td>
<td>Riparian</td>
<td>Mixed</td>
<td>3.0-22.0</td>
<td>Unknown</td>
<td>Known</td>
</tr>
<tr>
<td>Lygaeidae</td>
<td>LivingPlants</td>
<td>Phytophagous</td>
<td>3.0-20.0</td>
<td>Unknown</td>
<td>Known</td>
</tr>
<tr>
<td>Macroveliidae</td>
<td>WaterSurface</td>
<td>ArthropodPredation</td>
<td></td>
<td>Unknown</td>
<td>Known</td>
</tr>
<tr>
<td>Mesoveliidae</td>
<td>WaterSurface</td>
<td>ArthropodPredation</td>
<td>1.2-4</td>
<td>Unknown</td>
<td>Known</td>
</tr>
<tr>
<td>Miridae</td>
<td>LivingPlants</td>
<td>Mixed</td>
<td>1.5-15</td>
<td>Unknown</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Naucriidae</td>
<td>Aquatic</td>
<td>ArthropodPredation</td>
<td>5.0-20.0</td>
<td>Unknown</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Nepidae</td>
<td>Aquatic</td>
<td>ArthropodPredation</td>
<td>15.0-45.0</td>
<td>Unknown</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Notonectidae</td>
<td>Aquatic</td>
<td>ArthropodPredation</td>
<td>5.0-15.0</td>
<td>Yes</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Pentatomidae</td>
<td>LivingPlants</td>
<td>ArthropodPredation</td>
<td>5.0-18.0</td>
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<td>Known</td>
</tr>
<tr>
<td>Plataspidae</td>
<td>LivingPlants</td>
<td>Phytophagous</td>
<td>2.0-20.0</td>
<td>Yes</td>
<td>Known</td>
</tr>
<tr>
<td>Pyrrhocoridae</td>
<td>LivingPlants</td>
<td>Phytophagous</td>
<td>8.0-30.0</td>
<td>Unknown</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Reduviidae</td>
<td>LivingPlants</td>
<td>ArthropodPredation</td>
<td>3.0-40.0</td>
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<td>NotKnown</td>
</tr>
<tr>
<td>Rhopalidae</td>
<td>LivingPlants</td>
<td>Phytophagous</td>
<td>4.0-15.0</td>
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<td>NotKnown</td>
</tr>
<tr>
<td>Rhyparochromidae</td>
<td>LivingPlants</td>
<td>Phytophagous</td>
<td></td>
<td>Yes</td>
<td>Known</td>
</tr>
<tr>
<td>Salididae</td>
<td>Riparian</td>
<td>ArthropodPredation</td>
<td>3.0-7.0</td>
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<td>NotKnown</td>
</tr>
<tr>
<td>Termitaphidae</td>
<td>GroundDwelling</td>
<td>Phytophagous</td>
<td>2.0-3.0</td>
<td>Unknown</td>
<td>NotKnown</td>
</tr>
</tbody>
</table>
Table 4.A.2. continued.

<table>
<thead>
<tr>
<th>Family</th>
<th>Habitat</th>
<th>Feeding Habit</th>
<th>Size Range</th>
<th>Status</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyreocoridae</td>
<td>LivingPlants</td>
<td>Phytophagous</td>
<td>2.5-6.0</td>
<td>Unknown</td>
<td>Known</td>
</tr>
<tr>
<td>Tingidae</td>
<td>LivingPlants</td>
<td>Phytophagous</td>
<td>2.0-8.0</td>
<td>Unknown</td>
<td>NotKnown</td>
</tr>
<tr>
<td>Veliidae</td>
<td>WaterSurface</td>
<td>ArthropodPredation</td>
<td>1.5-9.0</td>
<td>Yes</td>
<td>Known</td>
</tr>
</tbody>
</table>
Figure 3.A.1. Combined morphological and molecular phylogeny of Heteroptera, color coded by habitat and lifestyle (Weirauch et al., 2018). Pink boxes added here to show which groups were sampled in the study from the AMNH collection.
R CODE

names(all)
family<- all[,1]
habitat<- all[,2]
feeding<- all[,3]
gregarious<- all[,5]
matrix<- all[,7:97]
mat_dist<- vegdist(matrix, method = "jaccard")
Matrix_NMDS<- metaMDS(mat_dist, k=2, distance = "jaccard", try = 50, trymax = 50)  
#Generate NMDS. Use which ever matrix you want from above
stressplot(Matrix_NMDS) #Stress plotplot(Matrix_World_Bray) #Plotting roughly
plot(Matrix_NMDS, "sites", method = "bray", main = "Habitat Type and Laboulbeniales Assemblages")
mel_ellip<- ordiellipse(Matrix_NMDS, habitat, col=c("blue", "darkgreen", "purple", "orange"), label=T, cex= 0.75, main = "Habitat NMDS")
legend(locator(1), lwd = 1, col = c("blue", "darkgreen", "purple", "orange"), legend = c("Aquatic", "Living Plants", "Riparian", "Water Surface"), bty = "n", cex = 0.75)
plot(Matrix_NMDS, "sites", method = "bray", main = "Feeding Ecology and Laboulbeniales Assemblages")
mel_ellip<- ordiellipse(Matrix_NMDS, feeding, col = c("red", "purple", "darkblue"), label=T, cex= 0.75)
legend(locator(1), lwd = 1, col = c("red", "purple", "darkblue"), legend = c("Arthropod Predation", "Mixed", "Phytophagus"), bty = "n", cex= 0.75)
plot(Matrix_NMDS, "sites", method = "bray", main = "Gregariousness and Laboulbeniales Assemblages")
mel_ellip<- ordiellipse(Matrix_NMDS, gregarious, col = c("orange", "darkblue"), label=F)
legend(locator(1), lwd = 1, col = c("orange", "darkblue"), legend = c("Unknown", "Gregarious"), bty = "n", cex = 0.75)
## Testing significance with Adonis2 ##

adonis2(comm_pa ~ env$Habitat + env$HostStatus, method = "raup", data = comm ) # significant

# Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

## adonis2(formula = comm_pa ~ env$Habitat + env$HostStatus, data = comm, method = "raup")

Df SumOfSqs R² F Pr(>F)

| env$Habitat | 6     | 3.0335 | 0.26002 | 1.7002  0.004 ** |
| env$HostStatus | 1    | 2.3884 | 0.20473 | 8.0320  0.001 *** |
| Residual       | 21   | 6.2446 | 0.53526 |
| Total          | 28   | 11.6665 | 1.00000 |

######## ANOVA SPECIES RICHNESS ###############

## without outlier(s) #

### without veliidae

env2 <- read.csv("C:\Users\cvict\Downloads\final_env_data_aug27.csv")
comm2 <- read.csv(as.matrix("C:\Users\cvict\Downloads\final_comm_nonames_aug27.csv"))

anova2<- aov(richness ~ env2$Habitat + env2$FeedingEcology + env2$Gregarious, data= comm2)
anova(anova2)

Rich2<- lm(richness~ env2$Habitat, data= comm2)
anova(Rich2)

### without corixids and veliidae

env3 <- read.csv("C:\Users\cvict\Downloads\final_env_data_aug27.csv")
comm3 <- read.csv(as.matrix("C:\Users\cvict\Downloads\final_comm_nonames_aug27.csv"))

anova3<- aov(richness ~ env3$Habitat + env3$FeedingEcology + env3$Gregarious, data= comm3)
anova(anova3)

Rich3<- lm(richness~ env3$Habitat, data= comm3)
anova(Rich3)
ANOVA table with corixids and veliidae

Analysis of Variance Table

Response: richness

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>Pr(&gt;F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>env$Habitat</td>
<td>6</td>
<td>66.488</td>
<td>11.0813</td>
<td>3.8689</td>
<td>0.01284</td>
</tr>
<tr>
<td>env$FeedingEcology</td>
<td>2</td>
<td>7.471</td>
<td>3.7353</td>
<td>1.3041</td>
<td>0.29722</td>
</tr>
<tr>
<td>env$Gregarious</td>
<td>1</td>
<td>4.016</td>
<td>4.0158</td>
<td>1.4021</td>
<td>0.25267</td>
</tr>
<tr>
<td>Residuals</td>
<td>17</td>
<td>48.692</td>
<td>2.8642</td>
<td></td>
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</tr>
</tbody>
</table>

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

>
LITERATURE CITED


CHAPTER 4

Insects and Laboulbeniales (Ascomycota, Fungi) of Lake Eustis & Emeralda Marsh Conservation Area: a pilot case study on urbanization and diversity

Patricia J Kaishian

Department of Environmental and Forest Biology, SUNY College of Environmental Science & Forestry, 241 Illick Hall, 1 Forestry Drive, Syracuse, New York 13210

ABSTRACT

A rapid biodiversity assessment of insects and associated Laboulbeniales fungi was conducted over the course of five nights in August, 2018 at two central Florida lakes: Lake Eustis and the nearby protected and restored National Natural Landmark, Emeralda Marsh Conservation Area (EMCA), which encompasses a portion of Lake Griffin. These locations were selected because Lake Eustis was surveyed for Laboulbeniales in 1897 by mycologist Dr. Roland Thaxter, but has not since been investigated. Because Lake Eustis has been urbanized, with the lake perimeter almost entirely altered by human development, the site offers a look into Laboulbeniales diversity across a 121 year timeline, before and after human development. By surveying Lake Eustis and EMCA, a modern case study comparison of Laboulbeniales and insect diversity between a developed and unrestored system and a protected and restored system is made. A total of 4,022 insects were collected during the rapid assessment. Overall, insect abundance was greater at EMCA, with 3,001 insects collected, compared to 1,021 insects collected from Eustis. Though family level insect richness was comparable between sites, with 55 families present at EMCA and 56 at Eustis, 529 out of 3,001 (17.6%) of the insects collected at EMCA were hosts to parasitic Laboulbeniales fungi whereas only 2 out of 1,021 (0.19%) collected from Eustis were
infected. There were 16 species of Laboulbeniales found at EMCA compared to only one at Eustis. The current number of Laboulbeniales species documented at Eustis was incredibly depaparaute compared to the 27 species recorded by Thaxter in 1897, suggesting the possibility of utilizing Laboulbeniales as indicators of ecosystem health. A figure displaying host-parasite records and a species list of Laboulbeniales fungi is compiled and updated occurrence records for species of *Ceratomyces* and *Hydrophilomyces* are provided.

KEY WORDS: fungi; rapid biodiversity assessment; insects; conservation; environmental health; indicator; Thaxter.
INTRODUCTION
Insect Diversity

Assessing biodiversity of insects and fungi presents challenges. Both groups are enormously diverse and suffer from a paucity of trained taxonomists. Knowledge of insects and fungi can be described as highly uneven, with representative members, often those associated with agriculture, industry or disease, receiving vastly more attention than other groups (Ainsworth et al., 2018; Kim, 1993). In both groups, millions of species remain undescribed (Hawksorth, 2017; Grimaldi and Engel, 2004).

Insect diversity studies have yielded a range of estimates for global and site-specific studies, with a number of researchers trying their hand at different techniques and methods in order to arrive at sound estimates. Insects are so diverse that researchers do not even agree on estimates of currently described species. Grimaldi and Engel (2004) report estimates ranging from 750,000 to 1.4 million (Wilson, 1992; Hammond, 1992, respectively). Based on work by Gaston (1991) and Resh and Carde (2003), Grimaldi and Engel (2004) endorse the estimate of 925,000 for currently named species. While this discrepancy may seem surprising, it is also understandable given the lack of sufficient incentives for researchers to spend time scouring old literature, synonymizing, cataloguing, and producing monographs.

Regarding estimates of living species, both described and undescribed, estimates of insect richness are even more variable. The lowest estimate is about 2 million species (CITE) and the largest is a staggering 30 million tropical insect species (Erwin, 1982). Erwin’s estimate was based on using fogging techniques on tree canopies in neotropical forests, upon which extrapolations were made for total insect diversity. Erwin recorded trees as having unique species of insects in their canopies and used the total tropical tree diversity of approximately
50,000 species to extrapolate. Most researchers now agree this estimate is much too high, largely because the assumption that the insects found in tree canopies would be highly host specific is likely erroneous (Grimaldi and Engel, 2004). Grimaldi and Engel endorse Gaston’s (1991) estimate of about 5 million total living insect species. This estimate was based on surveying collections held by systematists around the world. Despite this method having some potential shortcomings, e.g., collection biases of individual collectors, presence of unexamined or unknown duplicates held across collections, the authors believe it is currently the most accurate estimate of global insect diversity. If this estimation is accepted, then the aforementioned figure of 925,000 named insects would represent 20% of insect diversity.

In a quickly changing climate, there is increasing evidence of mass declines of insects, and therefore a pressing need to monitor insect biodiversity at local and regional scales (Kim and Byrne, 2006). Comparable data has not been recovered for fungi, as fungal conservation is in its early stages (Mueller, 2017). However, there is indication of fungal species richness declines in response to some human disturbances, including but not limited to: nutrient loading, mass tree die-off due to introduced pests and pathogens (Treu et al., 2004), acidification, and habitat loss (Arnolds, 1991). Biodiversity studies usually focus on vertebrate animals and vascular plants while those focused on invertebrates and fungi are rare (Fiesler and Drake, 2016). Despite being ubiquitous and essential components of the biosphere, macro-invertebrates such as insects remain under-served with respect to their risk assessment and conservation status. As of 2006 < 0.1% of described insects had been assessed for inclusion in the Red List maintained by the International Union for the Conservation of Nature (IUCN) (Rodrigues et al., 2006).

Because of the staggering diversity and abundance of insects, there exists feasibility concerns when designing biodiversity studies. Rapid biodiversity assessments of insects are frequently
employed in order to glean broad but manageable data sets that can be, albeit tentatively, extrapolated as a flexible measure of communities and populations (Ward and Larivière, 2004). There is currently a concerted effort by entomologists to establish optimal sampling methods for assessing insect biodiversity by taxa, population, assemblage, community, habitat, and region (Kim, 1993; Brown, 1997; Hughes et al., 2000; Ward and Larivière, 2004). Many scientists agree that establishing protected areas is the most effective way to protect multi-kingdom species diversity, particularly when considering understudied, vulnerable, and uncharismatic groups, which includes many insects and fungi (Hughes et al., 2000).

**Fungal Diversity**

The state of knowledge of fungi is substantially behind that of insects. A widely cited estimate of global fungal diversity is upwards of 1.5 million (Hawksworth, 1991). Mycologists now generally agree this is a conservative estimate, in part because it was based primarily on extrapolations from fungus-plant ratios in temperate regions and did not give due consideration to the hyper-diverse realm of insect-associated fungi, such as the Laboulbeniales, or account for tropical species diversity (Hawksworth, 1991; Hawksworth and Lücking, 2017). The most recent estimate (Hawksworth and Lücking, 2017) of extant fungi is 2.2 to 3.8 million, and the updated fungus-plant ratio for temperate zones is 8:1. Of that, ~135,000 species have been described (Hibbett et al., 2016). With only ~ 6% of the lower estimation being known to science, the remaining task is tremendous. Unlike many plant and animal groups, fungi do not broadly enjoy the benefits of being well studied and clearly understood. New species are most likely to be discovered by investigating relatively understudied habitats and microhabitats, including insect bodies, lichen-dwelling fungi, cryptic species, and through environmental (eDNA) sequencing (Hawksworth and Lücking, 2017).
In addition to fungi being relatively poorly studied, the often sporadic, ephemeral, and unpredictable appearance of fruiting bodies complicates obtaining thorough data on factors such as occurrence and abundance, and has constrained our ability to provide clear objective assessments of fungi overtime. The complex biotic and abiotic forces leading to a species even producing a fruiting body remains unknown in many cases, and likely involves the combination and interactions of degree days, soil temperature, precipitation volume, vegetation patterns, and so forth (Mihail et al., 2007). While some fungi, like some species of morels, can be reliably found in the same place at more or less the same time every year, other species, such as Ionomidotus sp. (personal observation) or Hericium bemedjaense (Jumbam et al., 2019) may be seen once in a given location and then not again for years, if ever. While substantial efforts have recently been made in fungal conservation, this field remains in its early stages (Mueller, 2017). According to the State of the World’s Fungi (Ainsworth et al., 2018), only 56 species of fungi have been evaluated for placement on the IUCN Red List, and 43 of those ended up being included. Comparatively, 25,452 species of plants and 68,054 species of animals have been evaluated. It is therefore imperative that fungi receive increased attention, concern, and action.

**Laboulbeniales**

Laboulbeniales (Ascomycota, Fungi) are microscopic obligate parasites on arthropods, primarily infecting insects. Laboulbeniales are considered the most diverse lineage of insect-associated fungi with ~2,200 described species in 142 genera, but current estimates indicate there are at least 40,000 species awaiting description (Weir and Hammond, 1997). The impact of Laboulbeniales fungi on their insect hosts are not fully understood and basic studies of their biology are still limited. Only a handful of scientists in the world specialize in the study of
Laboulbeniales, and yet, because of the size, diversity, and uniqueness of this lineage, it is undoubtedly a cradle of novel taxonomic and ecological information.

One early and prolific researcher of Laboulbeniales was Dr. Roland Thaxter. During his career at Harvard University between 1891 and 1932, Thaxter described > 1000 species of Laboulbeniales, and made substantial contributions to our understanding of their development and general biology. Most of his life’s work on Laboulbeniales is contained within a five-volume set of his *Contribution towards a Monograph of the Laboulbeniaceae* (1896, 1908, 1924, 1926, 1931), a tremendous contribution to mycology.

**Foundation of Study**

One of Thaxter’s collection sites for Laboulbeniales was in Eustis, a small central Florida city on the east shore of Lake Eustis. Beginning in the early 1800s, colonial Europeans forcefully established Eustis on Seminole land (Preserving Eustis History, n.d.). The city was named after General Abraham Eustis, who was known for his role in wars against the Seminole people (Preserving Eustis History, n.d.). The numerous connected waterways in the region allowed for Eustis to become a hub for steam boats, and the construction of the railroad that connected many Floridian towns in 1880 led to an increase in settlement from < 500 in 1900 to 21,300 in present day Eustis.

According to his travel records, Thaxter was in Eustis during the very early days of the city, from September 25th to October 10th, 1897 (Pfister, 1982), before most of the present urbanization. Because the methods employed by Thaxter during this trip are unpublished and unrecorded, it is unclear precisely how much time was spent collecting or the precise location that he collected from (Pfister, personal communication). Throughout Thaxter’s work, 27 species
and three varieties of Laboulbeniales were recorded and/or described from Eustis, Florida: *Autoicomyces acuminatus*, *Ceratomyces ansatus*, *Ceratomyces camptosporus*, *Ceratomyces cladophorus*, *Ceratomyces confusus*, *Ceratomyces filiformis*, *Ceratomyces floridanus*, *Ceratomyces longicornis*, *Ceratomyces minisculus*, *Ceratomyces mirabilis*, *Chitonomyces affinis*, *Chitonomyces dentiferus*, *Chitonomyces distortus*, *Chitonomyces floridanus*, *Chitonomyces hydropori*, *Chitonomyces lichanophorus*, *Chitonomyces occultus*, *Chitonomyces psittacopsis*, *Chitonomyces uncigerus*, *Hydraeomyces cnemidoti*, *Hydraeomyces halipli*, *Hydrophilomyces reflexus*, *Hydrophilomyces rynchophorus*, *Laboulbenia texana* var. *retusa*, *Laboulbenia texana* var. *rostellata*, *Laboulbenia texana* var. *tibialis*, *Rynchophoromyces elephantinus*, *Teratomyces mirificus*, *Zodiomyces vorticellarius*.

Eustis has changed considerably over the last 100 years with a majority of the lake perimeter being cleared for housing and other human infrastructure (Google Earth, 2018). In addition, since Thaxter’s visit in 1897, no research has been published dedicated to Florida Laboulbeniales, nevermind specifically Eustis.

The overall goal of this study was to return to Eustis in an attempt to re-collect species recorded by Thaxter, which could provide insight into shifts in biodiversity of Laboulbeniales and their associated insects since 1897 (121 years ago). Because Eustis is now impacted, I also sampled Emerald Marsh Conservation Area (EMCA), which includes a portion of Lake Griffin and surrounding habitat, and is located ~ 14 km from the east shore of Lake Eustis. Due to its designation as a National Natural Landmark since 1974 and subsequent and ongoing restoration efforts, EMCA provides a closer approximation of the habitat in which Thaxter collected 121 years ago (Fig. 4.3). Therefore, we decided to sample from both EMCA and Eustis in order to be able to make two biodiversity assessments: one over time (between Eustis in 1897 and Eustis in
2018), and one between habitats (between Eustis and EMCA in 2018). In addition, I was interested in comparing my findings to a lake that has been the focus of a substantial restoration program.

**Hypotheses and Objectives**

The protected and restored site, EMCA, was hypothesized to have greater insect and fungal diversity as compared to Eustis, the unprotected and unrestored site. We further hypothesized EMCA would be more likely to harbor species recorded by Thaxter, as compared to Eustis. These data be a start in understanding how urban development around lake systems may affect biodiversity of insects and their accompanying Laboulbeniales parasites, and to begin to explore if and how Laboulbeniales may serve as a proxy for biodiversity and an indicator for ecosystem health.

**METHODS**

*Site Description*

Lake Eustis and EMCA (including Lake Griffin) are part of the Central Valley Region (Region 7508) (Fig. 4.1). Lakes in this subtropical region are categorized by being large, shallow, and eutrophic (Lake County Water Atlas, Emeralda Marsh, n.d.). Lake Eustis and Lake Griffin are part of the Ocklawaha Chain of Lakes, which includes a total of 10 connected lakes. The headwaters of this chain is Lake Apopka, which is fed by a natural spring and by rain. Lake Griffin is the most downstream of the 10 lakes and Lake Eustis is directly upstream from Lake Griffin. Lake Griffin empties northward into the Ocklawaha River, which ultimately connects to the St. Johns River (St. Johns River Water Management District, Lake Apopka Basin, n.d.).
The surface area of Lake Griffin ~ 38 km$^2$, Lake Eustis is ~ 31 km$^2$. The average depth of Lake Griffin is ~ 2 m and Lake Eustis is ~ 3 m. The bottom of the Lake Griffin is composed of soft organic matter measuring an average of 1.7 m thick (Fulton et al., 2015). Equivalent measurements for Lake Eustis were not available. Over the past ~ 150 years, the Ocklawaha Chain of Lakes have experienced a barrage of human manipulations including, draining, dredging, levying, agricultural conversion, waste dumping, and nutrient loading. In the 1950s, the eastern area of Lake Griffin was levied and drained and converted into agricultural land and muck farms. These farms became an external source of nutrient loading into Lake Griffin (Fulton et al., 2015).

As of 2004, pervious and impervious percentages of the Lake Griffin Basin was 65% and 35% respectively, whereas the basin containing Lake Eustis (Burrell Basin) was 50% pervious/impervious (Fulton et al., 2004). Surface area coverage by emergent and floating-leaved

Figure 4.1. Map of Lake Eustis and Lake Griffin and surrounding area of of Lake County, FL.
vegetation decreased from ~ 50% in the 1940’s to < 2% in the 1970’s (Fulton et al., 2015). Similar data and exact mapping of wetland and vegetation loss is not available for Lake Eustis, however mention is made in an issue of Engineering News and American Railway Journal (1884) of Apopka Drainage Company draining ~ 0.4 km$^2$ between Lake Eustis and Lake Dora for farming. Additionally, > 405 km$^2$ of wetlands and forested uplands were destroyed by human development in Lake County as a whole (Lake County's Comprehensive Plan EAR – Conservation Element, 1997).

Emeralda Marsh was designated as a National Natural Landmark in 1974 (St. Johns River Water Management District, Emeralda Marsh Conservation Area, n.d.). Since at least the early 1980’s, Lake Griffin has been hypereutrophic (Fulton et al., 2015). In the early 1990’s ~ 12 km$^2$ of former muck farms in the EMCA were purchased by the St. Johns River Water Management District (SJRWMD). Over the past 30 years, aquatic and wetland restoration efforts in and around EMCA have focused on revegetation, re-establishing connectivity with Lake Griffin, and reducing phosphorus and pesticide loading (St. Johns River Water Management District, Emeralda Marsh Conservation Area, n.d.; Fulton et al., 2015). Management and restoration projects are ongoing. Most recently, in 2017, a year prior to this study, the remaining levees were breached, reconnecting the area to Lake Griffin (St. Johns River Water Management District, Emeralda Marsh Conservation Area, n.d.).

Restoration activities have been extensive at Lake Griffin but comparable efforts have not been made at Lake Eustis (Fulton et al., 2015). Likely as a result of these activities, total phosphorus (TP), chlorophyll-a, and total nitrogen (TN) have been decreasing with statistical significance in Lake Griffin between 1994 and 2012. Comparatively, Lake Eustis (as well as other lakes in the chain) have not seen significant changes in TP, but did have significant
decreases in chlorophyll-a and TN. Overall, the environmental improvements seen in Lake Eustis were smaller in magnitude than in Lake Griffin. The relatively moderate improvements in Lake Eustis may be due to the upstream restoration efforts at Lake Apopka, whereas Lake Griffin is likely benefiting from the extensive restoration efforts at EMCA in addition to the upstream efforts (Fulton et al., 2015).

**Insect Collection**

A rapid biodiversity assessment was conducted over five days, August 14th–18th, 2018. These dates were chosen to be in the same subtropical season as Thaxter’s visit. Again, while Thaxter’s precise methodology is not known, many of the species recorded from Eustis, e.g., members of *Autoicomyces, Ceratomyces, Hydrophilomyces, Rhynchophoromyces*, and *Zodiomyces* are found on nocturnal aquatic beetles indicating he likely used a light source as part of his collection methods. Insects were therefore collected using an ultraviolet trapping method. This popular entomological collection method was chosen in order to collect a broad range of
taxa (Szentkirályi, 2002; van Wielink and Spijkers, 2013). Given that many insects are nocturnal and given the risk of alligator encounter in and near the perimeter of the lakes, this method was deemed to be both effective and safe. A black light, (2805 DC Light Night Collecting Light, DC, 12 Volt, 15 Watt BL) was set against a white sheet, which was placed approximately 5 m from the water’s edge. Insects were collected via aspirator and transferred to 70% ethanol for storage. Three collectors spent three hours at each of the two sites per night, totaling 90 effort hours of collecting. Equal collection effort was made at both sites and the starting location alternated each night between Eustis or EMCA.

**Fungal Collection**

Insects were scanned for infections of Laboulbeniales under a Nikon stereomicroscope at 20–40x. Presence/absence data were obtained for Laboulbeniales infections of each insect examined. All insect specimens were identified to family level and accessioned into the entomological collections at SUNY College of Environmental Science & Forestry (SUNY-ESF) in Syracuse, New York.

Fungal thalli were removed with a micropin and mounted in glycerin using previously described methods (Benjamin, 1971, 1986, 1993). All fungi were identified to species level (references for IDing). Terminology and abbreviations used in describing the ascoma (used here for the entire perithecium-bearing thallus) are those used by Tavares (1985). Voucher specimens are deposited in the mycological herbarium at SUNY-ESF (herbarium code SYRF).

**Data and Data Analyses**

Abundance, species richness, and species diversity (Simpson’s and Shannon-Weiner, $H'$) were calculated for insects and Laboulbeniales. Comparisons are presented from the two time
periods for collections in Eustis (1897 and 2018) as well as between Eustis and EMCA. As this was a case study, actual statistical comparisons were not made as it would have been pseudoreplication.

**RESULTS**

A total of 4,022 insects were collected during the rapid assessment (Fig. 4.6). Overall insect abundance was greater at EMCA, with 3,001 insects collected, compared to 1,021 insects collected from Eustis (Table 1). Insect richness at the family level was comparable between lake with 55 families present at EMCA and 56 at Eustis. Both diversity indices were comparable between the two sites, with $H' = 0.44$ and $D = 0.09$ at EMCA and $H' = 0.40$ and $D = 0.09$ at Eustis. The different insect assemblages are presented in Figs. 4.6–8. The most noteworthy contrast between the lakes was the relative abundance of the family Hydrophilidae (water scavenger beetles), with 1,923 individuals collected from EMCA and only 13 from Eustis.

At EMCA, parasite prevalence was 17.6%, with 529 out of 3,001 of the insects being host to Laboulbeniales fungi. Comparatively, only ~ 0.19%, or 2 out of 1,021 insects collected from Eustis were host Laboulbeniales. Eleven species of Laboulbeniales were found at EMCA (Table 2): *Ceratomyces filiformis, Ceratomyces longicornis, Ceratomyces mirabilis, Chitonomyces sp., Hesperomyces virescens, Hydrophilomyces gracilis, Hydrophilomyces hamatus, Laboulbenia philonthi, Laboulbenia sp. 1, Laboulbenia sp. 2, Zodiomyces vorticellarius*. Only one species, *Hesperomyces virescens*, was found at Eustis. Infections on insects from EMCA all occurred on the following Coleoptera families: Hydrophilidae, Staphylinidae (rove beetles), Coccinellidae (lady beetles), Carabidae (ground beetles), Tenebrionidae (darkling beetles), and Chrysomelidae (leaf beetles). Infections on the two insects from Eustis were both on Coccinellidae. The vast majority of infected insects, 519 out of 529 in total, were members of the Hydrophilidae with the
remaining ten infections occurring on the other families. Relative insect abundance by order at both sites is shown in Figs. 4.9–10. The dominant insect orders at Eustis were Diptera and Hemiptera, whereas the dominant insect order at EMCA was Coleoptera, host to all detected Laboulbeniales at the sites.

Table 3.1. Species of Laboulbeniales recorded from Eustis in 1897 and 2018 and from EMCA in 2018

<table>
<thead>
<tr>
<th>Species</th>
<th>Eustis 1897</th>
<th>Eustis 2018</th>
<th>EMCA 2018</th>
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</thead>
<tbody>
<tr>
<td><em>Autoicomyces acuminatus</em></td>
<td>X</td>
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<tr>
<td><em>Ceratomyces ansatus</em></td>
<td>X</td>
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<td><em>Ceratomyces camptosporus</em></td>
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<td><em>Ceratomyces cladophorus</em></td>
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<td><em>Ceratomyces confusus</em></td>
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<tr>
<td><em>Ceratomyces filiformis</em></td>
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<td><em>Ceratomyces floridanus</em></td>
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<td><em>Ceratomyces longicornis</em></td>
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<td><em>Ceratomyces minisculus</em></td>
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<td><em>Ceratomyces mirabilis</em></td>
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<td><em>Chitonomyces affinis</em></td>
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<td><em>Chitonomyces dentiferus</em></td>
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<td><em>Chitonomyces distortus</em></td>
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<td><em>Chitonomyces hydropori</em></td>
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<td><em>Chitonomyces lichanophorus</em></td>
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<td><em>Chitonomyces occultus</em></td>
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<td><em>Chitonomyces psittacopsis</em></td>
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<td><em>Chitonomyces uncigerus</em></td>
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<td><em>Chitonomyces sp.</em></td>
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<td><em>Hesperomyces virescens</em></td>
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<td><em>Hydraeomyces cnemidoti</em></td>
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<td><em>Hydraeomyces halipli</em></td>
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<td><em>Hydrophilomyces gracilis</em></td>
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<td><em>Hydrophilomyces hamatus</em></td>
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<td><em>Hydrophilomyces reflexus</em></td>
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<td><em>Laboulbenia texana var. retusa</em></td>
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<td><em>Laboulbenia texana var. rostellata</em></td>
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<td><em>Laboulbenia texana var. tibialis</em></td>
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<tr>
<td><em>Laboulbenia sp. 1</em></td>
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<td><em>Laboulbenia sp. 2</em></td>
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<tr>
<td><em>Rynchophoromyces elephantinus</em></td>
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<tr>
<td><em>Teratomyces mirificus</em></td>
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<tr>
<td><em>Zodiomyces vorticellarius</em></td>
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OCCURRENCE RECORDS

*Ceratomyces* Thaxt.

The genus *Ceratomyces* was established by Thaxter (1892) and currently contains 45 species. Very few publications contain information on the genus *Ceratomyces* since Thaxter’s contributions (Tavares, 1985; Santamaria, 1999; Shen et al., 2009; Bernardi et al., 2014; Goldmann and Weir, 2018). Therefore, the occurrence records for all species of *Ceratomyces* found during this study will be updated with additional occurrence and range extension data published for the first time from the collection of Dr. Richard K. Benjamin, which is currently housed in the mycological herbarium at SUNY-ESF.

*Ceratomyces ansatus* Thaxt.

Specimens examined: EMCA, FL, USA, August 14–18, 2018 on *Tropisternus sp* (Hydrophilidae, Coleoptera) (Fig. 4.4E).

This species has not been formally recorded since its original publication by Thaxter (1908), where he reported the type from Brazil, and a specimen from Eustis, FL. Weir and Rossi (2001) report this species from Santa Cruz Dept., Bolivia. Within the collection of Dr. Richard Benjamin, currently housed at SYRF, there are heretofore unreported specimens of *Ceratomyces ansatus* collected (in chronological order) from: Jackson, IL, USA, 1909 (RKB 436A, RKB 495A); Morelos, Mexico, 1948 (RKB 1956, RKB 1756A); Alachua, FL, USA, 1954 (RKB 1773A); Musuas, Nicaragua, 1945 (RKB 1987); Turrialba, Costa Rica, 1955 (RKB 1990A); San Antonio, El Salvador, 1957 (RKB 3708E); Porto Alegre, Brazil, Date N/A (RKB 1314).
Ceratomyces confusus Thaxt.

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on Tropisternus sp (Hydrophilidae, Coleoptera) (Fig. 4.4B).

This species was first recorded from Tropisternus glaber and T. nimbatus in Milford, CT, and Kittery Point, ME, USA by Thaxter (1896). It was later reported by Thaxter (1908) in Eustis on several different species of Tropisternus. Within the collection of Dr. Richard Benjamin, currently housed at SYRF, there are heretofore unreported specimens of Ceratomyces confusus collected (in chronological order) from: Oaxaca and Michoacán, Mexico, 1948 (RKB 1946A, RKB 1948B); San Diego, CA, USA, 1953 (RKB 1598A, RKB 1704D, RKB 1809C, RKB 2247); Dixie, FL, USA, 1954 (RKB 1759); Los Angeles, CA, USA, 1954 (RKB 1774); Chiapas, Mexico, 1964 (RKB 2304B).

Ceratomyces filiformis Thaxt.

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on Tropisternus sp (Hydrophilidae, Coleoptera) (Fig. 4.4D).

This widespread species was first recorded from Tropisternus glaber and T. nimbatus in Milford, CT, Kittery Point, ME, and Arlington, MA, USA by Thaxter (1896). It was later reported by Thaxter (1908, 1931) in Eustis, FL and in TX, USA, as well as Mexico, Guatemala, Argentina, Brazil, and Chile, on several different species of Tropisternus, as well as on Pleurohomos obscurus from Guatemala. Within the collection of Dr. Richard Benjamin, currently housed at SYRF, there are heretofore unreported specimens of Ceratomyces filiformis collected (in chronological order) from: Trinidad and Tobago, 1913 (RKB 1607C00); Orange, FL, USA, 1945 (RKB 1929C); Jalisco, Chile, 1948 (RKB 1952); Veracruz, Michoacán, and
Morelos, Mexico, 1948 (RKB 1757, RKB 1953A, RKB 1952, RKB 1950, RKB 1948A, RKB 1946B, RKB 1938B); Champaign, IL, USA, 1950 (RKB 1239); Puno Dept., Peru, 1951 (RKB 1943A); Albany, WY, USA, 1951 (RKB 1795); San Diego, CA, 1953 (RKB 1598B, RKB 1704B); Alachua, FL, USA, 1954 (RKB 1753F); Turrialba, Costa Rica, 1955 (RKB 1990C); San Antonio, El Salvador, 1957 (RKB 3708B); Marzan Dept., Honduras, 1957 (RKB 3511G); Chiapas, Mexico, 1964 (RKB 2304C, RKB 2303B); Yellowstone National Park, WY, USA, 1971 (RKB 2810D, RKB 2810E, RKB 2810F).

*Ceratoymyces longicornis* Thaxt.

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on *Tropisternus sp.* (Hydrophilidae, Coleoptera) (Fig. 4.4A).

This species was described from Eustis, FL, USA on *Tropisternus sp.* and has not been reported since (1931) nor outside the original locality.

*Ceratoymyces mirabilis* Thaxt.

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on *Tropisternus sp.* (Hydrophilidae, Coleoptera) (Fig. 4.4C).

This species, considered by Thaxter to be the most common, was first recorded from *Tropisternus glaber* and *T. nimbatus* in Milford, CT, Arlington, MA, and Kittery Point, ME, USA by Thaxter (1896). Later, Thaxter (1931) lists the following places from which this species has been found: New England and FL, USA, Mexico, Guatemala, Costa Rica, Cuba, Trinidad, Cayenne, French Guiana, Rio de Janeiro, Brazil, Amazonas, Argentia, and Chile. All examined species occur on several different species of *Tropisternus,* as well as on *Pleurohomos obscurus*
Figure 4.4. A) *Ceratomyces longicornis*; B) *Ceratomyces confusus*; C) *Ceratomyces mirabilis*; D) *Ceratomyces filiformis* attached to host claw; E) *Ceratomyces ansatus*. 
from Guatemala. Within the collection of Dr. Richard Benjamin, currently housed at SYRF, there are heretofore unreported specimens of *Ceratomyces mirabilis* collected (in chronological order) from: IL, USA, 1907, 1908 (RKB 136A, RKB 136B); Lee, TX, 1908 (RKB 132B, RKB 135B); Trinidad and Tobago, 1913 (RKB 1607B); Puno, Peru, 1918 (RKB 1818A, RKB 1818B); Pima and Santa Cruz, AZ, USA, 1935, 1936 (RKB 3508A, RKB 3937B, RKB 3938A, RKB 3938B); Orange, FL, USA, 1945 (RKB 1929B); San Luis Potosí, Morelos, Oaxaca, Nayarit, Veracruz, Mexico, 1948 (RKB 1954B, RKB 1953B, RKB 1948C, RKB 1947C, RKB 1935, RKB 1936B, RKB 1937A, RKB 1938A RKB 1955); Bernalillo, USA, 1949 (RKB 3934B); Champaign, IL, USA, 1950 (RKB 1238C, RKB 490A, RKB 594B, RKB 594C); Angol, La Araucanía and Coquimbo Dept., Chile, 1950 (RKB 9151B, RKB 1951C, RKB 1934, RKB 1933B, RKB 1932); Chiapas, Mexico, 1950 (RKB 1531); San Diego, CA, USA, 1953, 1954, (RKB 1704C, RKB 1809D, RKB 1810); Putnam, FL, USA, 1954 (RKB 3406); Turrialba, Costa Rica, 1955 (RKB 1989B); San Bernardino, CA, USA, 1956 (RKB 2038C); Santa Barbara, CA, USA, 1957 (RKB 2050); Puebla and Michoacán, Mexico, 1957 (RKB 2079, RKB 2078, RKB 3706C, RKB 3709B); Morazan Dept., Honduras, 1957 (RKB 3511B, RKB 3511C, RKB 3511D, RKB 3511E, RKB 3511F); Loja, Ecuador, 1958 (RKB 2064B, RKB 2064A); Barinas, Venezuela, 1958 (RKB 2256A, RKB 2256B, RKB 2256C, RKB 2256D, RKB 2256E); San Diego, CA, USA, 1960 (RKB 3515A, RKB 3515B, RKB 3515C); Chiapas, Mexico, 1964 (RKB 2303, RKB 2304A); Yellowstone National Park, WY, USA, 1971 (RKB 2810A, RKB 2810B, RKB 2810C); OR, USA, Date N/A (RKB 1958A)
The genus *Hydrophilomyces* was erected by Thaxter (1908) and now contains 17 species. Subsequent contributions to this genus have been made by Picard (1910), Spegazzini (1915), Thaxter (1931), Sarna and Milewska (1977), Majewski (1974, 1983, 1994), Huldén (1983),

*Hydrophilomyces Thaxt.*

The genus *Hydrophilomyces* was erected by Thaxter (1908) and now contains 17 species. Subsequent contributions to this genus have been made by Picard (1910), Spegazzini (1915), Thaxter (1931), Sarna and Milewska (1977), Majewski (1974, 1983, 1994), Huldén (1983),

Figure 4.5. A) *Hydrophilomyces hamatus*; B) a cluster of *Hydrophilomyces gracilis*. 

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136
Tavares (1985), Rossi (1990), Santamaria (2006). Two species of the genus – *H. reflexus* and *H. rhynchophorus* – were originally described from Eustis, FL by Thaxter and were first placed within the genus *Ceratomyces* (1900) before moved to *Hydrophilomyces* in 1908. These species were not re-collected in this study, however, two other members of the genus, *H. gracilis* and *H. hamatus* were collected.

**Hydrophilomyces hamatus Majewski**

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on *Cercyon sp.* (Hydrophilidae, Coleoptera) (Fig. 4.5A).

This species was previously only recorded from the Old World with records from Sierra Leone (Rossi, 1990), British Isles (Weir and Beakes, 1993), and Poland (Majewski, 1994), making this is the first formal report of the species occurring in the New World. Within the collection of Dr. Richard Benjamin, currently housed at SYRF, there are heretofore unreported specimens of *H. hamatus* collected (in chronological order) from: Ocala, FL, USA, 1962 (RKB 2974, RKB 2973); Tavares, FL USA, 1967 (RKB 2968); Thomas, NE, USA, 1967 (RKB 2470, RKB 2469).

**Hydrophilomyces gracilis Majewski**

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on *Cercyon sp.* (Hydrophilidae, Coleoptera) (Fig. 4.5B).

This species was previously only recorded from Poland (Majewski, 1974, 1994), making this the first record in the New World.
DISCUSSION

Despite being relatively understudied compared to other groups of fungi, the Laboulbeniales possess certain qualities that lend themselves towards being a model group for fungal diversity studies in ecosystem health. Weir and Hammond (1997) lay out six features of Laboulbeniales that position them as such. These features are as follows: 1) Laboulbeniales display high host specificity in their association with arthropods, exceeding that of any other group of insect/arthropod-associated fungi; 2) these fungi are reasonably visually detectable ectoparasites and can be found in tact growing on living hosts or preserved in collections; 3) patterns of species richness can be assessed by studying the fruiting bodies (thalli) of these fungi and do not require culturing; 4) due to their obligate association with arthropods, entomological collections serve as a tremendous repository for these collections, allowing the researcher to utilize an alternative source for novel taxa, host lists, or habitat associations; 5) similar to 4, previous systematic and spatial work by entomologists already exist and can be utilized for the study of this group; different studies from different localities or focused on different insect groups can be compared against one another; 6) Thaxterian taxonomic concepts are relatively solid, consistent, and comprehensive, making this group particularly accessible and systematically reliable.

Furthermore, as discussed above, some fungi, particularly those that form fleshy sporocarps, may only fruit during extremely narrow and/or sporadic windows of time. Because Laboulbeniales lack a known asexual or vegetative stage, and possess spores that are only briefly viable in the environment (if at all) (De Kesel, 1996a), the presence of their fruiting body in the environment is more reliable and consistent indictor of species presence than many other groups of fungi. The combination of all these factors uniquely positions the Laboulbeniales as a focal group for fungal diversity studies.
Previous studies have begun to explore the potential of Laboulbeniales as indicators. Sugiura et al. (2010) conducted a study of Carabidae beetles and their associated species of Laboulbenia across different habitats in central Japan — a riverside, secondary forest and farmland and the microhabitats therein — to quantify insect-fungal interactions at the host assemblage level. This study found that 14/156 or 8.97% of Carabidae collected at the riverside site were infected with Laboulbenia, individuals; 2/214 or 0.93% in the forest and 0.161 or 0% at the farmland habitat, building evidence that the host habitat partly impacts the prevalence of Laboulbeniales. However, collection of insects in this study took place during December, and while Laboulbeniales are present on hosts overwinter, seasonal impacts of Laboulbeniales richness and abundance are not fully understood and may vary. This study expanded upon studies by Anderson and Skorping (1991) and De Kesel (1996b), which both demonstrated, via field work and experimentation respectively, that host microhabitat impacts occurrence of Laboulbeniales and the presence of host taxa alone does not guarantee presence (or detection) of fungal counterparts.

In our study, the contrast of the abundance of Hydrophilid,ae, more specifically the subfamily Hydrophilinae, between sites is of particular note, given that Hydrophilinae are host to most of the fungal infections recorded at EMCA. Because Hydrophilinae are nocturnal and attracted to light (Thorp and Rogers, 2015), it is possible that ambient artificial light from the town of Eustis diminished the relative attraction of the UV light, contributing to the difference in Hydrophilid,ae abundance between sites. However, given the well established and devastating effect of artificial light at night on many nocturnal insects, it is not unreasonable to posit that artificial light introduced with urban development coupled with outright habitat destruction around the perimeter of Lake Eustis and surrounding wetlands disrupted normal biological activities of the
Hydrophilinae population, leading to population decline (Owens et al., 2019). The loss of emergent and floating-leaved vegetation may also play a role in the insect population declines. Such vegetation was relatively abundant at EMCA collection site (as it had been purposefully re-introduced (Fulton et al., 2015)) compared to Eustis where no such efforts have been reported. If the insect population declined, then the associated fungal population may have also declined. At EMCA, the infection rate of the Hydrophilidae population was 1 in 5, but 0 of the 13 Hydrophilinae collected from Eustis were infected. This would perhaps suggest insect population dwindled to levels no longer facilitative of social transmission of Laboulbeniales, which is thought to be the primary mode of transmission for this group (De Kesel, 1993; Weir and Beakes, 1995). This raises the question: is there a quantifiable threshold at which insect population decline leads to extirpation of Laboulbeniales species?

Future studies should seek to address the above question and build upon and replicate the methodology of this study in numerous habitats in order to garner evidence for the utility of Laboulbeniales as indicators of ecosystem health. When considering Laboulbeniales, collection of insects offers a two-for-one assessment of insects and fungi, providing a more textured, multi-kingdom understanding of biodiversity at a given site. In this study, similar richness of insect families was found at both sites, while the presence of fungi was markedly different, with a 17.6% infection rate at EMCA and a 0.19% infection rate at Eustis. In this case, without attention paid to the fungal dimension, incorrect conclusions may be drawn about the biodiversity of these two habitats. A multi-kingdom assessment consolidates resources and effort, which increases the feasibility of conducting biodiversity monitoring. Making such work more feasible is attractive given the mounting pressure of climate change and the related impacts on biodiversity. These findings also highlight that specialist organisms, such as the highly host
specific and obligately associated members of the Laboulbeniales, may be of particular risk for population decline, range restriction, loss of genetic diversity, extirpation, and total extinction in a changing climate (Thomas, 2000; Warren et al., 2001). For such organisms, establishing protected areas and carrying out focused monitoring protocols is of great importance (Chape, 2005).

Fig. 4.6. A logarithmic scale graph showing insect family richness abundance and infection from EMCA.
Fig. 4.7. Insect family richness, abundance, and infection at Lake Eustis.
Fig. 4.8. Insect family richness, abundance and infection at EMCA and Lake Eustis. Note that both of the two Coccinellidae individulas collected at Lake Eustis were infected, thus appearing red and not blue.
Figure 4.9. Relative abundance of insects by order from Eustis. Hemiptera and Diptera were the two dominant orders at Eustis.

Figure 4.10. Relative abundance of insects by order from EMCA. The dominant order at EMCA was Coleoptera, hosting all detected species of Laboulbeniales.
Table 4.A.1. Insect order, family, count and infection count at Eustis.

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## Table 4.A.2. Insect order, family, count, and infection count at EMCA.

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Acknowledgements:

Thank you to my field assistants Emma Kaishian and Will Stallings. Thank you to my undergraduate research assistant Aaliyah Jason. Thank you to the Edna Bailly Sussman Foundaiton for funding this work. Thank you to Drs Alex Weir, Melissa Fierke and Tom Horton for reviewing and editing the work.

LITERATURE CITED


CHAPTER 5
The Science Underground: Mycology as a Queer Discipline

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Hasmik Djoulakian

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We are human only in contact and conviviality with what is not human.

-David Abrams, Becoming Animal
Abstract
This article explores the science of mycology through a queer theory framework with the intention of situating the state of the field in a historical and social context. With the understanding that everything is in community with fungi, we look to the biology and ecology of these organisms for transformative inspiration and a deep-time sense of belonging. Our scientific understanding of mushrooms and other fungi has been shaped and indeed impeded by mycophobia, a condition of fear and revulsion that we compare here to queerphobia. In this work, we argue that mycology relies upon queer methodologies for knowledge acquisition given both the nonbinary, cryptic, and subversive biological nature of fungi as well as a hegemonic, Western, cultural rendering of fungi as perverse and unworthy of formal investigation. We further argue that the queer methodologies of mycology that developed in response to these conditions have enhanced rather than hindered our knowledge of fungi. Because our ultimate quest as scientists is the pursuit of truth, as best as we can determine, we suggest that scientists would do well to meaningfully reconcile with the inescapable and oftentimes queer reality of bias and subjectivity.

Keywords: fungi, queer, kinship; mycophobia; symbiosis
INTRODUCTION

Clustered in supergroup Opisthokonta, fungi, animals, and amoebae share a more recent common ancestor than with plants or bacteria. The vegetated environment that enabled the transition of animals to land and evolution of amphibians, reptiles, birds, then mammals, was bound to symbiotic fungi known as mycorrhizae. Over 90% of plants form these associations, and myceliated landscapes sustain cascades of nested biological systems, from which every evolutionary layer of our human biology is indistinguishable, arising and persisting in conviviality with fungi or fungal-bound organisms. As terraforming bodies, fungal transindividualism is our collective ecological history. Fungi are engaged in continual processes of renewal, interfacing with death, creating life through decomposition, nutrient re-allocation, and the spectrum of symbiosis. Fungi can remediate environments by digesting fossil fuels and converting them into fungal sugars. Fungi can accumulate heavy metals and radioactive materials, and a fungus has even been found to metabolize ionizing Cesium-137 in the reactors of Chernobyl. Both single cellular forms and filamentous, hyphal networks of fungi can be found in almost any conceivable niche: of, on, within, and for human and nonhuman bodies.

Despite this, complex social histories have influenced outcomes and trajectories of mycology, rendering it a marginalized science. Kingdom Fungi has been persistently maligned, feared, and misunderstood, and these cultural forces have directly sabotaged scientific understanding of this group for hundreds of years. In Western Europe and in the United States particularly, children are typically raised to fear all mushrooms, which are unilaterally viewed as poisonous, diseased, and degenerate. Although science, in its ideal form, should be an equal opportunity investigative methodological tool, we know that the history of modern science has been disproportionately written by white, often Christian, men from Western Europe, excluding
other voices. Consequently, dominant cultural lenses—heteronormativity, racism, sexism, ableism, and binaries inherent to them—have influenced scientific understandings.

Tiokasin Ghosthorse, a member of the Cheyenne River Lakota Nation of South Dakota and scholar at Yale University’s School of Divinity, Ecology & Forestry, explores relational and egalitarian thinking processes familiar to the Lakota people, as compared to rational, hierarchical thinking processes within Western cultures. Ghosthorse says that, for Lakota people, language is inherently relational and all things are bound together. Ghosthorse (2019) writes,

> The rational mind is the human living within the hierarchy of a box that seeks to capture it through its own narcissistic addiction to the anthropocentrism of a society or a people who hold themselves up as somehow more grandiose than others in that box of conscience. It deadens the intuitive or non-dogmatic life. This is where the separation begins — with a concept and a word that doesn’t exist within many intuitive languages, such as Lakota. That word is domination. (paras. 10-11)

By invoking rationality over intuition to defend a viewpoint, a person makes the assertion that the intuitive—often feminized—lens is neither legitimate nor legible, with no footing in any discursive cultural space. Often with derision, it is written out of the conversation. Such disregard for intuition is part of the pathway toward domination that Ghosthorse describes. Dualistic thinking about life and non-life, male and female, and other categories Ghosthorse alludes to, permeate mycophobic discourse.

Western scientific thinking seeks security through objective assessment, but objectivity is meaningless when it comes to gender and queerness because attempts to determine the bounds of queerness are already exercises of power. Donna Haraway (1988) discusses falsity of objectivity in her essay on situated knowledges, which describes the partial perspective any one person is able to have depending on sociopolitical factors influencing their gaze; these partial perspectives form a patchwork of messy, layered knowledge that inches toward some measure of shifting
objectivity. This encourages rethinking scientific endeavors not as observations of subjects, but as interactions with them. Similarly, fungi defy objectivity and standardization. Specifically, sporadic, ephemeral, and unpredictable appearances of fruiting bodies complicates mycologists’ ability to obtain thorough population data. The complex biotic and abiotic forces that lead to a species producing a fruiting body remain unknown in many cases, and likely involves the combination and interactions of degree days, soil temperature, precipitation volume, vegetation patterns, and so forth (Mihail et al., 2007). While some fungi, like some species of *Morchella* (the morels), can be reliably found in the same place during the same, small seasonal window every year, other species, such as *Ionomidotus sp.* (personal observation) or *Hericium bembedjaense* (Jumbam et al, 2019) may be seen once in a given location and then not again for years, if ever. But is its mycelium still present in that spot? We often do not know. If so, does that count as being present? Then there is the issue of quantification of an individual. If you find a scattered grouping of mushrooms growing around a tree, are they one genetic individual? If so, do you quantify them as one mushroom? Or do you count the number of mushrooms, reporting them as individuals? There is not a clear and universally applied answer to these questions. This lack of conformity to quantifiable boxes has put many fungi at a greater risk of extinction. Their biological realities are not given necessary accommodations in our current conservation assessment framework, whose attempts to standardize data diminish many essential properties of fungi. Interrogating our dualistic, mycophobic view of fungi—and our often pathologizing attempts to understand them—can help make Science more accountable.

Mycology is a science that, by its very nature, challenges paradigms and deconstructs norms. Mycology disrupts our mostly binary conception of plants versus animals, two sex mating systems, and discrete organismal structure, calling upon non-normative, multi-modal
methodologies for knowledge acquisition. Mycelium is the web-like network of fungal cells that extends apically through substrate, performing sex, seeking nutrients, building multispecies and multikingdom symbioses. This essay seeks to remediate our relationship with fungi and all organisms—thereby queerness—by collapsing and myceliating the emotional space between human and non-human. In order to do this, we explore dogma of institutional [capital ‘S’] Science, as well as the biology, history, and methodologies of mycology through a queer theory framework, as seen by a queer mycologist and a feminist educator.

**History of “Queer”**

Historically, “queer” was used pejoratively to describe non-heteronormative behaviors. People now self-identify as queer, to describe their existence outside heteronormativity. In the US, “queer” was reclaimed and gained popular usage during AIDS political activism of the 1980s and 1990s. “We’re Here! We’re Queer! Get Used to It!” was the rallying cry of Queer Nation and ACT UP, which sought to unify subgroups not quite captured by the terms “Gay” and “Lesbian.” The word “nation” suggested a coalition of queer people, bound together in their non-heteronormativity, redefining what it meant and what it looked like to have a sense of belonging rooted in shared identity and struggle (Chen, 2012, pp. 61–63). Whether people identify as queer to describe their homosexuality, gender-nonconformity, or transgender identity, “queer” is fluid, invoking a spirit of community and a history of defiance (Butler, 1990; Sedgwick, 1990; Clare, 2015)

Queer theory explores constructed dichotomy of “normative” and “deviant” sexuality and systems and frameworks that interact with sexuality, including race, nationality, dis/ability. This field grew from Feminist and Gay and Lesbian studies, which focus on challenging “essential” qualities of women and femininity, as well as the normativity of heterosexuality, which becomes
posed as the unspoken, unnamed standard and expectation for romantic and sexual relationships. While this essay interrogates the relationship between mycology and queerness by defining “queer” as non-heteronormative identities and expressions, there is value in thinking more broadly of “queer” as referring to identities, bodies, and behaviors pushed to the margins of Western, hegemonic, heteronormative life.

Queer theory has drawn from a number of philosophers and theorists including poststructuralists and postmodernists such as Jacques Derrida. One of Derrida’s contributions to semiotics is the concept of “deconstruction,” which seeks to “deconstruct” logocentrism, the idea that there are inherent, stable truths, calling attention to the importance of language in the formation of our framework of truths. Derrida (1981) writes,

....by means of this double play, marked in certain decisive places by an erasure which allows what it obliterates to be read, violently inscribing within the text that which attempted to govern it from without, I try to respect as rigorously as possible the internal, regulated play of philosophemes or epistimemes by making them slide--without mistreating them--to the point of their nonpertinence, their exhaustion, their closure. (p. 6)

Probing the limits of socially ingrained concepts is an exercise of deconstruction and reshapess systems of power. A project of queer theory is deconstruction of heteronormative concepts, such as a family as a procreative unit, which exposes contingencies, obsolescence, and fallacies in these norms, reinforcing the viability of alternative structures and spaces. By challenging protected social groups and their associated dogmas, queer theory seeks to make plastic seemingly stable notions of fact, knowledge, knowledge acquisition, and Science, whose otherwise reified, institutionalized standing maintains status quo. Queer ecology is an intervention specifically targeted against institutionalization of heteronormative modes of scientific thought, unraveling abounding queerness.
History of Science

People all over the world have systematically documented their surroundings and interactions with non-human organisms, abiotic factors, and ecologies for millenia. By way of reproducible knowledge acquisition, mass observational patterns and longitudinal documentation, many systems of knowing have long existed. Despite this, some forms of knowing and conceptions of truth have been given priority over others. In Western Europe, women had historically been keepers of ecological knowledge, but their voices were excluded from formal participation in science, their knowledge often dismissed as “folk tales,” “witchcraft,” or “old wives’ tales,” meant to indicate that their knowledge was irrational—sometimes, unnatural, evil, otherworldly—with no basis in reality, because women’s knowledge was fundamentally threatening to hegemonic institutions of knowledge-creation (Barstow, 1995). Val Plumwood (1993) writes, “Feminine ‘closeness to nature’ has hardly been a compliment,” (p. 19) and instead an assertion that women did not possess rational, intellectual, and positively human capacities of liberal, modern, Western men, who channeled “untamed” nature into reputable, reified Scientific Knowledge. The culture of institutional Science has been disproportionately shaped by a small subset of people, and the consequences can be limiting to discovery and dangerous for posterity.

With the spread of institutional Science, Christianity continued to spread, in part through colonialism. It is well-known and documented that Christianity strongly influenced scientists. Scientists loyal to the Church, such as Descartes, Euler, and Newton, often were loyal to the Church in their supposedly objective pursuits of knowledge. Large-scale agriculture also interacted with science and Christianity, with scientific discoveries enabling new manipulations of land and crops, and with Christian domestic and marital structure organized in connection
with agriculture in what can be termed *agro-heterosexuality*. Rachel Stein (2010) states, “...Christian thinkers compared human sexual actions to planting a field and only those activities that corresponded to “seeding,” or procreation, were accepted as natural; other activities impeding or ignoring reproduction, whether performed with members of the same or opposite sex were forbidden as against nature (Bullough and Bullough, 1977, p. 286). Bodies that perform forbidden actions themselves become marked as unnatural, offensive, defiant — and forbidden. This is also where queer sexuality meets queer ecology, including the effect of fungi on agriculture and agricultural metaphors.

Anna Tsing (2012) explores the relationship between emergence of intensive agriculture as the standard of modernity and progress, and solidification of fungi as the enemy of those ideas of modernity and progress. The relationship is tinged with irony: when agriculture disrupts and strips bare natural ecologies, the resulting monocultures are increasingly vulnerable to pests and pathogens, some of which are fungal. Tsing (2012) writes, “The emergence of vast fields of grain offered fungal plant parasites a field day — and a reputation as the enemy of civilisation and, later, progress” (p. 147). This notion of “progress” implies forward movement, productivity, growth, and improvement, concepts which queerphobic discourse suggests become hindered through a lack of heterosexual procreation. Science is not inherently a capitalistic endeavor, but discourse of progress can strap capitalistic notions of productivity to scientific spaces and pursuits. Tsing (2015) explores this idea with the word *scalability*: the capitalistic drive of perpetually scaling up, making research questions apply to greater and greater scales without changing the research question, which is apparent with industrial agriculture. Tsing even refers to mushroom forests, sites of boundless “indeterminate encounters” with no prescribed measures of productivity or success, as “anti-plantations” (p. 51). It is through
interplay of these factors—dominant heteronormative structure and capitalist drive—that disdain for fungi crystalized within Western Europe, and later within post-colonial USA.

Tsing (2012) writes, “Biological and social diversity huddle defensively in neglected margins…most everywhere, a negative correlation exists between diversity and intensity of capital investment and state control!” (p. 151). As cereal farming under capitalism intensified throughout Eurasia, families were expected to give a portion of their yields to elites. Mushrooms grew wild on untended margins of these farms, and were incorporated into people’s diets “under the table,” providing a form of nourishment beyond the reach of the state. Under large-scale agriculture, crops are organized through “capitalist logics of commodification,” within which “things are torn from their lifeworlds to become objects of exchange” (Tsing, 2012, p. 158). These wild-growing mushrooms demonstrate non-extractive, non-capitalist entanglements with surrounding ecosystem lifeworlds. One type of truth is that humans are different from fungi due to our evolutionary history and genetics. Another type of truth is that we are similar because we both respond to the intensity of capital investment and state control in a similar way. We die.

In science, a tool can look tantalizingly like a truth, until the tool is deconstructed into its foundational elements. As scientists, we use tools and methods to access knowledge, and we standardize our work to create a common language. This is of tremendous power and value, as it allows for a language for globalized participation in discovery. With all potential knowledge being infinite, science is collective incrementalism, each discovery leading to infinity minus one. Replication and standardization are hallmarks of science for good reason. The difference between “pseudoscience” and alternative ways of knowing should be clarified: purporting to be science and failing to abide by the scientific method is fundamentally different from openly and
explicitly operating outside the confines of the scientific method, and it is essential that they be regarded differently. “Pseudoscience” can be destructive and dishonest, and is rightfully criticized. Trouble arises, however, when replication and standardization are conflated with knowledge itself. Some will argue knowledge cannot exist absent these structures, and while science requires these structures, it is critical to emphasize the difference between tools and truth. The strength of science can also be its weakness: linear, logical atomization of information excavates clean and standardized data, but in our infinitely complicated universe, such expectations for cleanliness seem improbable; to choose, at times, messiness is to see more fully.

Taxonomy, the naming, describing and classifying of organisms, is a vital and often undervalued tool for communication in organismal science. Taxonomy is often undervalued because it is considered simple, observational, “basic research.” Within our system of categorization, species concepts for fungi can be ambiguous, shifting depending on the objective of the investigator. While a phylogenetic or biological species concept might be true in the context of phylogenetics and evolution, the ability to strictly define a species is not a requisite for accessing the truth of all organismal relationships. For example, a yeast (a single-celled fungus) could hardly be more morphologically and genetically distinct from a human body, and yet, there is a suite of yeasts found within human bodies (the mycobiome) upon which we depend for basic bodily functions (Seed, 2015). These species are critically interdependent but this understanding cannot be derived from species concepts. Scientists have found that common morphologies are not always an indicator of relatedness; that populations within a species are sometimes on the verge of speciation; that data organization by way of DNA is data organization for the sake of
data continuity. Things are fluid, scientific inquiry happens in a snapshot of deep-time, and this is also a truth.

The construction and imposition of units in taxonomy and biological surveys mimics individualized concepts of self, specifically the reified notion of self coveted historically through Western standards of white, masculine, self-sufficient personhood. Discrete units of self diminish the relational and interdependent nature of humanity and deny the labor of others in accounting of one’s success and viability. Declaring units of fungi feels forced and coercive because their fluid, interderminant biology clashes radically with such abrupt and linear constructions. Clean taxonomic and population units deny the messiness of fungal biology—almost always embedded, connected, and dynamic—forcing scientists to question our own objectives in this pursuit. Failure to reassess objectives in science can leave one clinging to tools at the expense of better answers.

From some perspectives, science during the Age of Enlightenment stressed difference over similarity. Carl Linnaeus’s work on taxonomy specifically was organized around the pursuit of an inherent quality that differentiated, rather than bound, organisms into their own discrete taxonomic units (Wilchins, 2004, p. 119). But queer differences in fungi went misread by Linnaeus, and many mycologists agree that Linneaus’s treatment of fungi did more of a disservice to the group than good. Linnaeus called lichens “rustici pauperrimi” or “poorest peasants” of vegetation (Plitt, 1919). Fungi were given the mycophobic title of “lower plants,” which directly references perception of fungi as inferior and, hinting at teleological notions held at that time, less evolved. Based largely on nutritional modes, with fungi being heterotrophic and plants autotrophic, fungi were properly established as their own kingdom within the five kingdom system proposed by Whittaker in 1969. Even after this, however, mycology was placed
under the purview of Plant and/or Forest Pathology. Currently, if an institution has a mycology dedicated lab at all, and few do, it will most likely be placed in a department or sub-department titled Plant/Forest pathology. Scientific understanding of fungi has therefore been constrained by confirmation bias and social forces; fungi are approached through a pathologizing framework as something to be fought, controlled, and eliminated. No one should doubt the importance of these perspectives—after all, many fungi are pathogens, parasites, and disease agents of agriculturally and economically important crops—but the vast majority of fungi are not limited to those roles, if they fill them at all.

**Mycophobia**

Fungi are not plants, nor are they animals, and this binary conception is how many people today are inclined to interface with the natural world. Fungi are seen as poisonous, agents of disease, degenerate, deadly, freaky, gross, and weird—language historically leveled against both queer and disabled people—and as having no positive interrelationships with their environment(s). Mycology is rarely taught to undergraduate students, nevermind sufficiently addressed in primary and secondary schools. Examples of dramatic and disdainful characterizations of mushrooms are abundant in many facets of Western European culture. In *Magical Mushrooms, Mischievous Molds*, George Hudler (1998, p. 73) explores socially important fungi throughout history and presents examples of these characterizations, such as the following poem by Emily Dickinson (1896):

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Had nature any outcast face
Could she a son condemn
Had nature an Iscariot
That mushroom – it is him.
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Dickinson compares mushrooms in her poem to Judas Iscariot, the disciple who betrayed Jesus Christ to the chief priests, leading to his crucifixion. She suggests there is a sanctity to nature that is defiled and betrayed by mushrooms, as if they were not only an aberration of nature, but also an active, malicious threat to a holy place. A later section of the poem refers to mushrooms as the “Elf of Plants,” echoing the Linnaean title of “lower plants.”

The following is an excerpt from Sir Arthur Conan Doyle’s *Sir Nigel* (1906):

A sickly autumn shone upon the land. Wet and rotten leaves reeked and festered under a foul haze. The fields were spotted with monstrous fungi of a size and colour never matched before – scarlet and mauve and liver and black – it was as though the sick earth had burst into foul pustules. Mildew and lichen mottled the walls and with that filthy crop, death sprang also from watersoaked earth.

Doyle intentionally uses language grounded in ableist and queerphobic sentiment to create a picture in which, instead of being integral to forest health, mushrooms are seen as dirty, foreign, and frightening markers of death. For instance, his ignorance—and resulting insecurity and hostility—about the critical ability of fungi to decompose organic matter and help maintain the nutrient cycle necessary for life leads him to depict fungi as “foul pustules,” suggesting illness and a visceral sense of wrong.

**Mycophobia and Queerphobia**

*Mycology is queer at the organismal level.* Fungi are nonbinary: they are neither plants nor animals, but possess a mixture of qualities common to both groups, upending the prevailing binary concept of nature. It is rare for a fungus to have only two biological sexes, and some
fungi, such as *Schizophyllum commune*, have as many as 23,000 mating types. When two compatible fungi meet, their mycelia will fuse into one body, sexually recombine, then remain somatically as one as “they” continue to live, grow, and explore in their environment. Members of phylum Glomeromycota are only known to be asexual. Fungi in order Laboulbeniales sometimes have distinct bodies for male and female reproductive structures (dioecious), or both may be found in the same body (monoecious), and sometimes monoecious and dioecious bodies co-occur. *Mycology has queer investigators*. Of respondents to the Mycological Society of America 2018 survey, 12% of mycologists identified as LGBTQ, which is 3–4 times the national reported average (Haelewaters, personal communication, 2019). One will quickly notice that mycological spaces have dispensed with certain expectations of formality, and even academic mycological conferences have a comforting, casual air of an odd family reunion. *Mycology is queer methodologically*. Mycologists use sensing, intuition, experience, and storytelling, with experts operating outside of institutional affiliation more often than with other organismal fields. For many mycologists, our relationships with fungi stir powerful emotions tied deeply to our core. We sometimes cry or burst into song when we find these special and beautiful beings.

Organisms, bodies, and behaviors that are difficult to neatly categorize stir insecurity and a lack of control within the viewer, who responds with fear, revulsion, and hatred. What becomes difficult to discern or dominate through heteropatriarchal systems of oppression and dogmatic scientific understandings becomes a threat to institutions that codify bodies of knowledge. Queer bodies are seen as less “fit” and “functional,” which calls on ableist language in similar ways as mycophobic language does. This includes the relegation of mycology to subfields of “pathology” within ecosystem sciences, coloring institutionalized understanding of the many lives of fungi.
Whatever poisonous and destructive qualities exist among a small subset of fungi have become mapped onto the character of fungi as a whole. This is indicative of the devaluation of fungi, as the same could not be said of kingdom Plantae, even though there exist subsets of poisonous and destructive plants. Plants and other organisms classified as belonging to “higher” kingdoms are recognized as possessing a greater degree of social capital (Bourdieu, 1986)—for example, they are more likely seen as helpful, valuable, and capable of performing some service—that allows them to encompass an abundance of attributes in people’s imaginations. In contrast, fungi and other “lower” kingdoms, such as Archaea and bacteria, do not occupy this elevated space in people’s imaginations. In general, people who belong to marginalized groups experience a flattening of their person into a static state that suggests behaviors by individual members of a respective group can be generalized onto the supposedly singularly definable quality of the group as a whole; this is one of the ways mycophobia and queerphobia operate using similar logics. It is worthwhile to wrestle with the apparent tensions when marginalization is referred to as “dehumanization,” because while language that compares humans to nonhuman animals and other life forms is meant to strip those humans of empathizable qualities, leaving them vulnerable to “justifiable” violence and harm, it is crucial to resist human/nonhuman binaries and hierarchies that are part and parcel of violent, oppressive systems. Plumwood (1993) writes about this tension: “Thus, for example, behind the view that there is something insulting or degrading about linking women and nature stands an unstated set of assumptions about the inferior status of the non-human world” (p. 26).

Well-intentioned scientists have utilized queerphobic rhetoric in depictions of environmental hazards. Whether or not this usage is unwitting, it stems from deep-seated, subconscious knowledge that sensationalizing and heteronormative language spurs public
condemnation. Giovanna Di Chiro (2010) writes about the concept of “eco(hetero)normativity” in regards to ableist, queerphobic, and heterosexist language—such as “sexual abnormalities,” including “male-to-female gender shifts and intersex conditions” among fish and other animals—environmentalists and popular media may use to rouse concern about environmental pollution and toxicity (pp. 201-202). Such language moves beyond the need conduct science, inform readers, and demand healthy environments — it sensationalizes depictions of queer and disabled bodies as tragic aberrations from a supposedly (hetero)normative order to life that must be upheld and protected. Analysis around the harmfulness of this language must be extended to discourse around fungi. Mycophobic language paints fungi almost as unnatural toxins themselves – a force whose spectre of invasiveness is constructed through a similar mobilization of queer-fear. This depiction of fungi sustains legitimized and “respectable” bodies of scientific thought and methodologies of research.

Even when researchers are not actively queerphobic it is impossible to act fully outside hegemonic discursive norms. It is only once this tension is named and a person recognizes that their perceptions of reality are culturally mediated that they can perform methodologically thoughtful research. Articulating the way this cultural entrapment functions, Monique Wittig (1992) writes, “There is nothing abstract about the power that sciences and theories have to act materially and actually upon our bodies and our minds, even if the discourse that produces it is abstract. It is one of the forms of domination, its very expression, as Marx said. I would say, rather, one of its exercises” (pp. 53–54). In a general sense, people struggle to imagine the enormous breadth of fungal life because fungal bodies exist well outside gender binaries, heterosexuality, and other exercises of power that create, as Wittig says, false “absolute meaning[s]” of gender, sexuality, nature, and health.
In her writings on feminism, Sara Ahmed (2017) discusses the idea that being a lesbian/queer means inhabiting a body that feels constantly questioned. Ahmed states, “Sometimes, whether or not you are asked a question, you feel questionable …. You feel like a question mark; you feel marked by questions” (pp. 120-121). Mycology is marked by questions by those on the outside: Why would you possibly study those things? Will you get sick or poisoned? What kind of job can you get with that degree? This further evokes parallels to “coming out.” Queer people are often met with confusion, fear, and even disgust and abuse if they reveal their orientation or identity. Even in more mycophillic countries where foraging for mushrooms is more likely a shared cultural pastime, scientific passion and research focused on this kingdom of life is often seen as whimsical or confusing, or even disturbing.

Despite this real danger, a slippery space often exists between revulsion and commodification. Simon Estok (2009) introduces the term “queer ecocriticism” on the pretext that “the commodification of nature and sexual minorities are similar, each depending on a large consumer base that seeks a vicarious experience, rather than the thing itself” (pp. 213–216). People may desire a superficial, extractive experience while maintaining distance from the queer subject. Within mycology, there are examples of how people engage with fungi opportunistically, but fail to regard them as the essential, deeply ecological beings they are. Many people have had their lives saved by Penicillin, without realizing that the medicine is derived from a fungus, *Penicillium*. Only a small handful of mushrooms that can be cultivated for culinary purposes, such as the Portabello (*Agaricus bisporus*), have made their way into the realm of acceptability. They are accepted for two reasons: 1. They can be controlled and they exhibit predictable behaviors that can fall neatly under domination of a cultivator, and 2. They serve a direct purpose to humans, in this case as a food source. Fungi that fit into only one of
those categories, such as wild “choice” edible mushrooms, are sometimes deemed acceptable. There is an increasing popularity in dining on wild mushrooms, but in the US, these gourmet mushrooms are often sold at a high price, tying them to wealthy and often white clientele, who are often gatekeepers of acceptability within dominant culture.

The consumer of expensive “wild” mushrooms may feel adventurous, daring, knowledgeable, and perhaps even superior to those who cannot afford those mushrooms. The excitement felt from exclusive access to a queer(ed) other is a hallmark of commodity culture. bell hooks (1992) writes about commodification and objectification of Black bodies through an analysis of white (often male) fascination with and desire for sexual contact with dark Others – “the consumption of the dark Other,” as hooks puts it (p. 30). hooks explains that this fetishization of the body of the Other helps white people to feel like transgressive and bold Subjects, while Black people remain in the position of “primitive” Objects. While hooks’s argument is about white cultural imperialism and not queerness, her argument about the thrill white people feel when they come into close contact – a type of consumption – with an “unknowable” (and in some sense, queer) Other can be extended to queerness as well. Consumers of expensive “wild” and “rare” mushrooms also assert their power over mushrooms, queer symbols and representations of death, and the seemingly mysterious origins they sprang from. Among people who forage for mushrooms they will later sell, however, exist more complicated social, cultural, and economic relationships with mushrooms. Tsing (2015) describes these as “pericapitalist” spaces, or sites of “salvage accumulation,” in that they “take advantage of value produced without capitalist control” and both are, and are not, capitalist interactions with mushrooms (in Tsing’s research, specifically matsutake, a highly-coveted and costly mushroom) (p. 83). Pericipatialist spaces allow enactment of people’s own sense of
freedom, often tied up in their cultural legacies, that connect them with mushrooms. Even when mushrooms are not so costly as to fall along these economic pathways, they still are a site of social convergence, bringing together academic and nonacademic scientists and an assortment of people who feel their freedom is theirs to reclaim. Mushrooms offer witness to human vulnerability.

Stacy Alaimo (2008) identifies nature as a critical ideological junction in feminist thought: “the contradictory, ubiquitous, and historically varied meanings of “nature” have made it a crucial site for various feminist social struggles, including feminist anarchism, socialism, birth control, racial equality, and lesbianism” (p. 229). Alaimo asserts that, although the wedge driven between nature and body is understandable as a reaction to the intentionally demeaning historical association of women and nature, this “flight from nature” is “one of the most unfortunate legacies of poststructuralist and postmodern feminism.” Calling for inhabitation of “trans-corporeality,” Alaimo seeks to reestablish a feminist intimacy between bodies and nature by emphasizing material engagements of space and time. In this trans-corporeal, space-time partnership of beings, human and nonhuman bodies exert their own intermingled agency and life-making power, leading Alaimo to ask, “How is it possible to understand agency without a subject, actions without actors? How can we rethink matter as activity rather than passive substance?” (p. 245). These questions strike at the heart of feminist efforts to de-naturalize the pervasive grip of dualistic thinking. Fungi, with their ability to decompose and transform matter across space-time, enabling reconstitution of bodies into new materialities, are crucial agents of non-capitalist, anti-dualistic interchange.

Plumwood (1993) writes that mutually reinforcing dualisms, such as culture vs. nature; male vs. female; reason vs. emotion (nature); subject vs. object; and self vs. other, result from “a
certain kind of denied dependency on a subordinated other” (p. 41). Plumwood goes on to argue that “virtually the whole set of dualisms can be mobilised for this purpose of inferiorising the sphere of nature and those human-beings who may be counted as part of nature” (p. 47). Human experience of the world becomes categorized, understood, and curtailed by the alienation and domination these dualisms naturalize and sustain. Queer people are simultaneously compared to animals, but also characterized as unnatural, which itself utilizes the false divide between nature and culture. Similarly, fungi are regarded as wild and unruly elements of nature with no connection to humans, and simultaneously unevolved or somehow representatives of another, more evil realm.

Haraway (2007) offers an intervention into binaristic discourse that alienates humans from the natural world; such discourse also ensnares our fungal kin. Haraway communicates the inextricable dynamicism and co-constitutive relationship between nature and culture through her term “naturecultures,” which says that any invocation of nature has a cultural context, and similarly, cultures do not exist apart from nature (p. 25). Haraway argues that companion species—organisms whose communal and corporeal existence and identity demand interdependence—arise from naturecultures. She writes, “I am not a posthumanist; I am who I become with companion species, who and which make a mess out of categories in the making of kin and kind. Queer messmates in mortal play, indeed” (p. 19). Messmates occupy space together; they witness one another’s intimate moments, and their day-to-day lives depend on synchronous interplay with one another. This includes humans and the fungi we coexist with on a daily basis: from yeast cells mingling in our guts and nutrifying our foods, to symbiotic fungi in the forests that sustain our global environment.
When language is limited to a binary conception of gender, those who do not fit squarely in those constructs are often thought to not exist, and explicitly written out of existence through their exclusion. Fungi are often unnamed — only about 120,000 of an estimated 2.2 to 3.8 million fungi have been named and described (Hawksworth and Lücking, 2017). While the call to name all currently undescribed fungi will be a step in the right direction, and is in fact the pursuit of one of the authors of this paper, taxonomy alone cannot cure this problem, because it does not break from the need to delineate the bounds between fungi and humans which, to borrow from Derrida, is a hierarchical regime. Derrida (1981) argues,

To deconstruct the opposition, first of all, is to overturn the hierarchy at a given moment. To overlook this phase of overturning is to forget the conflictual and subordinating structure of opposition. Therefore one might proceed too quickly to a neutralization that in practice would leave the previous field untouched, leaving one no hold on the previous opposition, thereby preventing any means of intervening in the field effectively…. The necessity of this phase is structural; it is the necessity of an interminable analysis: the hierarchy of dual oppositions always reestablishes itself.... That being said-and on the other hand-to remain in this phase is still to operate on the terrain of and from within the deconstructed system. By means of this double, and precisely stratified, dislodged and dislodging, writing, we must also mark the interval between inversion, which brings low what was high, and the irruptive emergence of a new "concept," a concept that can no longer be, and never could be, included in the previous regime (p. 42)

Extending this linguistic idea to the material, fungal realm, we argue that naming all fungi would be an oppositional act, but such an engagement would leave us wrought in the same oppositional tension of our current framework. Rather, we must phase shift into a newly imagined, but simultaneously ancient, non-hierarchical space in which human-nonhuman (fungal) oppositions are deconstructed.

Robin Wall Kimmerer (2007) reflects on Western scientific discourse’s need to mark the bounds of existing knowledge. She explains that the term Puhpowee in Potawatomi means the
force which causes mushrooms to push up from the earth overnight. A member of the
Potawatomi Nation and an academically trained scientist, she writes,

As a biologist, I was stunned that such a word existed. In all its technical vocabulary, Western science has no such term, no words to hold this mystery. You’d think that biologists, of all people, would have words for life. But in scientific language our terminology is used to define the boundaries of our knowing. What lies beyond our grasp remains unnamed...The makers of this word understood a world of being, full of unseen energies that animate everything. (p. 49)

This inability—or refusal—to engage with language that reveals the limitations of our perception curtails our engagement with fungal life (and as a parallel, with queerness). But as Kimmerer points out, this disconnect is inherent to the English language and its naturalized assumptions of animacy or lack thereof; Science then institutionalizes these assumptions. It is not impossible, however, for English language speakers to recognize flaws and fissures in language, even if words to meaningfully bridge them do not exist.

Religious ideology and discourse influences understandings of animacy and relations between beings. Some biblical scholars believe that “dominion” was a mistranslation, and the original text used a word more closely synonymous with “stewardship.” It is through this translation that some Christians felt empowered to dominate and exploit Earth (Weldon, 2016). Riki Anne Wilchins (2004) writes about this drive for dominion: “We want to have, as the Bible says, ‘the Word made flesh,’ something we can have dominion over (p. 46).” The remarkable ability of fungi to subvert human domination and defy expectations has bound fungi with other supernatural forces often feared by Christians, including witches, devils, and the demonically possessed. Whether the context has to do with religion or any other institution, culture and language cyclically reinforce discourse. Wilchins writes,
Discourse is a set of rules for producing knowledge that determines what kinds of intelligible statements can be circulated within a given economy of thought. For example, in the discourse on gender, you can only say meaningful things about two kinds of bodies that will make sense. References to third genders will always sound fanciful, nonsensical, or just ridiculous. Discourse is the "cookie cutter"...The social truths we have about gender have to do not with the body, but with the cutter. (p. 73)

In this analogy, science and methodology are the cutter, and the dough is all that can be learned. The cutter is shaped to accommodate biological and methodological realities of non-fungal organisms. This cleaves off entire sections of what we can know about fungi so that they keep shape with other organisms. Biological discourse has limited our framework of possibility for fungal biology because this discourse was formed in the context of mycophobia. Mycology is on the margins, where biological discourse has been abruptly cut by the cookie cutter; on the boundaries of discourse that prioritizes and enforces normalcy of other organisms.

Psilocybin within psychedelic mushrooms was posited to have evolved to defend mushroom bodies against mycophagous insects (Reynolds et al., 2018). However, a subsequent study disputed this, finding evidence for a mutualistic role of psilocybin between insects and fungi (Awan et al., preprint). This same compound facilitates the birth of alternative epistemologies in human minds by connecting and energizing regions of the brain that have atrophied atop our sterile, individualized, and isolated position in the self-declared hierarchy of Western philosophy. Much like a mycorrhizal network, neurotransmitters flow like carbon, nitrogen, and phosphorus, connecting and reconnecting regions of our brains, reminding us of our deep-time home, the sentience of Earth, and interlocking biotic systems of We. Psychedelic mushrooms take people to a vulnerable site of deconstruction, where what was once considered to have social or cultural significance suddenly feels obsolete and what was once seen as static and inert is suddenly pulsing and humming with animacy. It is not a coincidence that the
relational discipline of ecology (from Greek oikos ‘house’) gained public traction 1970s, in concert with psychedelic, anti-war, civil rights, and environmental movements — a greater recognition of the interdependence of all beings was taking shape.

The experience of marginalization lends a critical vantage point and potential for subversive examination. Patricia Hill Collins (2000) discusses using alternative epistemologies, which are entirely new frameworks of thought, as fundamentally subversive challenges to dominant “knowledge claims” (pp. 266-271). Mycological history is replete with numerous alternative epistemologies, such as the concept of mutualism—a humbling concept often seen as an insult to the primacy of humans—which has historically gained and lost traction, mirroring trends in the zeitgeist. Original hypotheses on mutualisms of mycorrhizal networks put forward by A.B. Frank in the late 19th century were considered revolutionary when introduced and were therefore highly contested. Around the same time, the biology of lichens was being fiercely debated, with mycologists such as De Bary and Swchendener first probing into notions of symbiosis between fungi and algae (Plitt, 1919). A century and a half later, these ideas are only just becoming well-integrated into scientific literature and lexicon, and remain poorly understood and under-recognized outside of mycology (Trappe, 2005). Scientists are typically quite conservative when treating alternative epistemologies; there is extreme pressure to “just follow the data.” And yet, according to philosopher of science Thomas Kuhn (1962), this is what scientific revolution looks like: embracing what was marginal and elucidating new epistemologies.

Queer Pedagogy & Methodology

As Amy E. Winans (2006) states, queer pedagogy “entails decentering dominant cultural assumptions, exploring facets of the geography of normalization, and interrogating the self and
the implications of affiliation” (p. 6). In an attempt to challenge dominant logics and cultural assumptions, queer pedagogy asks: What has been normalized, why, and how can knowledge be produced differently by taking into account the function of power?

For most mycologists, mycological education was fostered outside the traditional classroom. There is often a reliance on individuals who were/are autodidactic, pursuing knowledge of this subject apart from dominant education systems. Many mycologists remember their first mycological experience very clearly; usually, it was by way of a charismatic teacher who brought to focus this queer world. Upon realizing that there was actually this other world, where the rules did not quite apply, many mycologists felt at once safe and awakened. Mycology speaks to the personal, sexual, and/or political lives of its often marginalized investigators.

Mycological Societies are also an example of alternative sites of knowledge. They de-center institutions as the sole source of knowledge, and challenge hierarchies through a structure that is member-supported, low-cost, and often provides free, public educational experiences. This type of structure is central to feminist methodology, which insists that learning and thinking are never confined to the classroom, and in fact are richer when experienced in community with others, out in the world. Anarchist organizers, designers, architects, and others look to nature, including fungi, for inspiration through a process called biomimicry, a term popularized by Janine Beynus’s 1997 book Biomimicry: Innovation Inspired by Nature. Examples of this include an architecture group based in Brooklyn, NY called Terreform Open Network Ecology that looks to nature to design sustainable architecture and a group called Fungi for the People that brings people together for educational workshops. Artists are even
increasingly looking to fungi for inspiration. Whatever the group, those who learn and borrow from fungal biology are compelled by its dynamic, mutualistic, transformative possibilities.

Despite this burgeoning, eclectic appreciation for fungi, funding is typically hard to come by for mycologists because the field is still largely in the phase of “basic research.” In an academic environment mired in capitalistic notions of progress, application, patents, and “impact,” funding basic research is difficult because it lacks a certain thrill. Discovery for discovery’s sake is a hard sell to a person who compulsively asks the claustrophobic, capitalistic question: but what's the point? Mycological societies have flourished because they allow people to explore in peace, for the sake of exploration. The simple state of curiosity is the only requisite for joining such societies.

While many classical American naturalists such as Henry David Thoreau, Aldo Leopold, and Rachel Carson were not tuned into mycology, many of today’s mycologists drew early inspiration from their patient, holistic, and arguably sacred and dutiful art of observation. This art, this deep, daily, ritualistic communion with patterns, phenology, and nonhuman beings gives many of us a sense of purpose. Naturalism is beautiful because it is not inherently directed, there are no required hypotheses, practitioners need not have an academic affiliation. In fields such as mycology, as well as facets of organismal biology and ecology, lineages of classical naturalism have persisted, holding the practice of simple observation close to our hearts. These naturalists are the observers, the ones whose “indeterminate encounters,” as Tsing (2015, p. 50) says, without a script or fixed set of goals, create mutually transformative relationships. By forging messy, shifting, anarchist human and nonhuman kinships, these encounters demonstrate the creative and productive energy possible outside capitalist bounds. Such non-extractive,
transformative mingling of beings requires communication that listens, not demands, and research that seeks to observe and understand, not suture any sites of ambiguity.

In the age of climate change, it is becoming increasingly apparent that the tiny pin pricks of data recorded by observers have incrementally built enormous bodies of knowledge. Museums containing vast collections of organisms that most would call too insignificant to know, have been diligently preserved, catalogued, and curated by observers. We can use these treasure troves to understand how life once was, before such destruction, and to reimagine our future. The project now is to help break the obsession with progress by de-emphasizing scalability, challenging research frameworks, and supporting the world’s observers. Science prioritizes quantitative data, but disciplines adjacent to or entirely outside of Science understand that qualitative data are also informative and sometimes carry greater explanatory power in certain contexts. Jack Halberstram (1998) posits that queer methodology is a multi-modal “scavenger methodology,” which points to a process of searching and excavating that does not come easily at first glance. Mycologists might prefer the term “forager methodology,” as mycology is similarly built on a mix of quantitative and qualitative data which includes sensing, intuition, oral histories, and literally foraging. Stacey Waite (2015) encourages embracing messiness as necessary and fruitful:

This essay invites readers to think about and experience logics that contradict, tenses that shift, genres that mix, futures that are messier than what the present moment seems to allow. Further, it asks scholars of composition and teachers of writing to become scavengers and to make seemingly disconnected worlds collide. (p. 52)

Mycological surveys are difficult to do in the quantified, restricted ways that botanists use. Mycologists use and publish methods such as “timed wander” to survey a site (Victoroff, 2020). Extremely standardized transects and plot-based work of botany often feel unnecessarily
limiting to mycologists, who are often guided to find fungi by a combination of intuition, sensing, and an intimate knowledge of the landscape and macro/micro habitats that foster mushroom growth.

Smell and taste are important methodological tools in identifying fungi; the communal, instinctual, cultural, and emotional reverberations of these senses potentiate more personal, nuanced research. People working in groups to identify fungi may often ask one another, “What does this smell (or taste) like to you?” and form inexact, but deeply provocative, systems of organization based on these senses. Even this invitation to smell carries a queer intimacy. The affective attachment mycologists have with fungi is apparent at mycological summits and conferences when someone asks, for example, what *Inocybe* smells like, and people respond with descriptors such as “spermatic” or “swimming pools!” There is indeed a queer and joyous sense of community when a group of people collectively comes up with a verbal smellscape of increasingly silly descriptions of ejaculate smell to describe a fungus. For those who have smelled *Inocybe*, the descriptions spark memories of those embodied and ephemeral moments, layered with dimensions of others’ descriptions; for those who have not smelled *Inocybe*, listening to others share their impressions can sketch a full, patchwork, and memorable picture.

Initiating newcomers into tasting mushrooms for experiential identification is a vulnerable and exciting moment. Some mushrooms, such as members of genus *Russula*, are difficult to identify to species level without incorporating taste because they are morphologically similar. However, some species are edible and taste mildly “mushroomy,” and others will cause gastronomic distress, such as *Russula emetica* (from Greek *emetikos* meaning emetic or vomit-inducing), and will have a spicy or acrid taste. Few people know that all mushrooms are safe to touch (unlike plants) and fewer know and viscerally believe that all mushrooms are also safe to
put in your mouth, so long as you spit them out! In our classrooms, we sometimes ask a brave volunteer to come forward and take a small nibble of two similar-looking *Russula* mushrooms and taste for difference. An intimate space is created as students gather around to witness their classmates’ reaction as curious tastes roll over their tongues.

With some of these methodologies, there is risk of losing the ability to cleanly replicate, but the benefit is that there is a richer understanding of the lifeworlds of mycoflora in a given area. Forgoing straightforwardly replicable methodologies opens a multitude of pathways, perspectives, and points of entry for mycologists who appreciate that notions of objectivity constrict ability to understand fungi. This creates friction with some of the foundational tenets of traditional Science, but mycology stretches and redefines the idea of replicability, because fungal growth does not lend itself to clean replicability. Mycological methodologies rely on a greater sense of intimacy between mycologists and both fungal organisms and the landscape, which speaks to Ahmed’s (2017) reflections about intuition: “A gut has its own intelligence. A feminist gut might sense something is amiss” (p. 27). A mycologist’s feminist gut demands that intuition, a feminized mode of knowing, be trusted. Fungi, in turn, demand the same of mycologists, and all those who seek to make kin with fungi. Maybe not coincidentally, human guts are home to an assemblage of microbes, fungi and bacteria, that communicate with and impact our brains.

**Conservation**

The hologenome concept asks, what is the relevance of an isolated human genome? Bodies are, in fact, communities, and there are more fungal and bacterial cells in the human body than there are human cells. As Haraway (2007) puts it, “To be one is always to become with many” (p. 4). We become with, because there is no *us* without others; even words such as “us” and “others” are misleading, as our bodies are mutually constitutive entanglements. Recently,
scientific investigations have begun to attach quantitative data to interdependencies that have been recognized outside of Western culture for millenia. The notion that humans exist and function independent of other beings stems from a fear of our vulnerable dependencies and often indecipherable, queer entanglements, and that, as Myra Hird (2004) says, “the penultimate embodiment of queer may be bodies themselves” (p. 87). By embracing this queerness, we chip away at fallacious, capitalist myths of the neoliberal, individually agential Western subject.

Belief in human exceptionalism has devastating consequences for humans and all life. Impacts of mycophobia are immediately, materially apparent. According to State of the World’s Fungi, only 56 species of fungi have been evaluated for placement on the International Union for Conservation of Nature (IUCN) Red List, with 43 species ending up listed. Comparatively, 25,452 species of plants and 68,054 species of animals have been evaluated (Ainsworth et al., 2018). This list aims to be the world’s most comprehensive inventory of species and their risks of extinction, and yet a kingdom with over one million species has only 43 species listed. This poor representation is not due to some miraculous ability of fungi to move unscathed through the Anthropocene and the world’s sixth great mass extinction event. Rather, it is because there is a failure to achieve quantification based standards proving a species is endangered. This is largely because the ephemeral nature of many mushrooms and fungi makes their documentation patchy. The relative lack of mycologists compared to botanists and zoologists also means there is less data available to argue for protected status. This lack of protected status means there is less funding available for their protection, therefore fewer studies are conducted, and fewer mycologists are paid to do necessary work to generate more data. Holding fungi to biological determinations of more normative groups—say trees or birds—is to deny their basic biology, which puts them at risk for
extinction. The solution is not to find out how to force fungi into the normative box. The capitalistic drum that beats for extinction will not slow its tempo for the painstaking assessment of fungi, no matter how diligently mycologists work. The solution is to recognize that fungal data should not have to match that of trees or birds. Mycologists’ experience, intuition, and sensing should be given priority in establishing whether or not a fungus is threatened or endangered. Conservationists should also be deferential to modes and practices of Traditional Ecological Knowledge, which has a rich history of profound, intimate knowledge of Earth. Radical circumstances such as mass extinctions demand radical solutions, and fungi demand to be seen in their messy totality. We must disentangle science from capitalism and Western hegemony. We must trust and support observers, turning to our deep-time microbial gut to draw strength in our advocacy for our interdependent communities. To deny the value of these systems of knowing is to shore up colonialist and queerphobic mindsets.

Conclusion

Mycology is queer insofar as it is marginal, subordinate, contested, ridiculed, but more critically, mycology is queer insofar as it is disruptive, collective, transformative, revolutionary. Fungi show us cooperative, alternative, promiscuous, entangled, interdependent, more-than-individuated, and more-than-human modes of living worth studying, imitating, learning from, and which queerness in humans has often shared. Just as fungi are capable of reclaiming land, bodies, and nutrients, so too can humans reclaim our relationship with fungi as siblings. Much like mycorrhizae, humans can forge mutually beneficial relationships with fungi. We can steward their land, and care for their bodies, much like how they have persistently continued to steward our land and care for our bodies. We can remediate poisoned relationships by challenging the paradigm in which they have been demonized and dispossessed, much like
how they can remediate waters and lands poisoned by capitalist greed. Moreover, we can apply lessons learned from fungal biology to our human organization and forge stronger networks of interdependence and mutual aid. Science can be and has been instrumental in challenging dangerous, exploitative notions of hierarchy, but it has also been employed at the service of those hierarchies. Through a thorough and honest recognition of the limits of human investigative reasoning and methods we can become better and more ethical scientists. Through challenging assertions of objectivity and purity, we push ourselves into the unknown, both with optimism and a critical outlook. It is past time that humans turn to the fungi to which we are bound, step into our mutual totality, and create space and futures for our wild ways of being.

Notes

1. Gloria Anzaldúa and Cherrie Moraga write about US Third World feminisms in the anthology *This Bridge Called My Back*.

2. For further writings on embodied feminist epistemologies, Audre Lorde’s essay “Uses of the Erotic” discusses power that comes from the erotic, which involves a vulnerable, spiritual, and bodily relationship with our surroundings and beings within them.
LITERATURE CITED


Doyle AC. 1906. Sir Nigel. Tauchnitz.


APPENDIX

NSF-style Proposal: Laboulbeniales as indicators of environmental health at lake systems across continental United States

Project Summary

In a quickly changing climate, there is increasing evidence of mass declines of insects, and therefore a pressing need to monitor insect biodiversity at local and regional scales (Kim and Byrne, 2006). Comparable data has not been recovered for fungi, as fungal conservation is in its early stages (Mueller, 2017). However, there is indication of fungal species richness declines in response to some human disturbances, including but not limited to: nutrient loading, mass tree die-off due to introduced pathogens, acidification, and habitat loss (Arnolds, 1991; Treu et al, 2004). Biodiversity studies usually focus on vertebrate animals and vascular plants while those focused on invertebrates and fungi are rare (Fiesler and Drake, 2016). Despite being ubiquitous and essential components of the biosphere, macro-invertebrates such as insects remain underserved with respect to their risk assessment and conservation status. As of 2006 < 0.1% of described insects had been assessed for inclusion in the Red List maintained by the International Union for the Conservation of Nature (IUCN) (Rodrigues et al, 2006). Because of the staggering diversity and abundance of insects and fungi, there exists feasibility concerns when designing biodiversity studies. Rapid biodiversity assessments of insects and fungi are frequently employed in order to glean broad but manageable data sets that can be, albeit tentatively, extrapolated as a flexible measure of communities and populations (Ward and Larivière, 2004).

A preliminary pilot study was conducted with the objective of beginning to build evidence for the use of Laboulbeniales as indicators of environmental health. Laboulbeniales (Ascomycota, Fungi) are microscopic obligate parasites on arthropods, primarily infecting insects (Fig. 1–2). Laboulbeniales are considered the most diverse lineage of insect-associated fungi with ~ 2,200 described species in 142 genera, but current estimates indicate there are at least 40,000 species awaiting description (Weir and Hammond, 1997). By comparing insect and associated fungal biodiversity between a protected and restored site and a developed and unrestored site, we aimed to explore whether Laboulbeniales in particular were correlative with either habitat types, hypothesizing that we would find greater fungal richness and abundance at the protected and restored site. The study focused on two lakes in a central Florida lake system: Emerald Marsh Conservation Area (EMCA) which encompasses a portion of Lake Griffin and Lake Eustis. While both lakes have been substantially altered by human development, EMCA has been the focus of extensive restoration efforts for several decades and Lake Eustis has not. Results showed that EMCA had strikingly higher abundance and richness of Laboulbeniales and higher insect abundance than Lake Eustis, while overall insect richness was comparable.

The study is therefore proposed to be expanded and replicated first across Florida and subsequently across the continental United States. The first year will focus on four lake couplets in central FL. Each couplet will be composed of two lakes determined by the EPA to be “good” and “poor” with respect overall health. Each couplet will be sampled monthly with an array of
insect trapping methods starting in May and going through September. Using the EPA ratings, we will test the hypothesis of the preliminary study, while expanding community data collection and potentially revealing seasonal patterns of insect and fungal biology. The second and third years of this study will take the hypothesis outside of FL, testing lake couplets across the nine ecozones as identified by the EPA in the continental United States. This phase of sampling will involve streamlined methodologies as determined by the results of the FL sampling year. If our hypothesis is correct, Laboulbeniales can be used as sensitive indicators of environmental health.

**Project Description**

**Background:** Assessing biodiversity of insects and fungi presents challenges. Both groups are enormously diverse and suffer from a paucity of trained taxonomists. Knowledge of insects and fungi can be described as highly uneven, with representative members, often those associated with agriculture, industry or disease, receiving vastly more attention than other groups (Ainsworth et al., 2018; Kim, 1993). In both groups, millions of species remain undescribed (Hawksworth and Lücking, 2017; Grimaldi and Engel, 2004). Insect diversity studies have yielded a range of estimates for global and site-specific studies, with a number of researchers trying their hand at different techniques and methods in order to arrive at sound estimates. Insects are so diverse that researchers disagree on estimates of currently described species. Grimaldi and Engel (2004) report estimates ranging from 750,000 to 1.4 million (Wilson, 1992; Hammond, 1992, respectively). Based on work by Gaston (1991) and Resh and Carde (2003), Grimaldi and Engel (2004) endorse the estimate of 925,000 for currently named species. While this discrepancy may seem surprising, it is also understandable given the lack of sufficient incentives for researchers to spend time scouring old literature, synonymizing, cataloguing, and producing monographs.

Regarding estimates of living species, both described and undescribed, estimates of insect richness are even more variable. The lowest estimate is about 2 million species and the largest is a staggering 30 million tropical insect species (Erwin 1982). Erwin’s estimate was based on using fogging techniques on tree canopies in neotropical forests, upon which extrapolations were made for total insect diversity. Erwin recorded trees as having unique species of insects in their canopies and used the total tropical tree diversity of approximately 50,000 species to extrapolate. Most researchers now agree this estimate is much too high, largely because the assumption that the insects found in tree canopies would be highly host specific is likely erroneous (Grimaldi and Engel, 2004). Grimaldi and Engel endorse Gaston’s (1991) estimate of about 5 million total living insect species. This estimate was based on surveying collections held by systematists around the world. Despite this method having some potential shortcomings, e.g., collection biases of individual collectors, presence of unexamined or unknown duplicates held across collections, the authors believe it is currently the most accurate estimate of global insect diversity. If this estimation is accepted, then the aforementioned figure of 925,000 named insects would represent nearly 20% of insect diversity.

The state of knowledge of fungal biodiversity is substantially behind that of insects. A widely cited estimate of global fungal diversity is upwards of 1.5 million (Hawksworth, 1991).
Mycologists including Hawksworth generally agree this is a conservative estimate, in part because it was based primarily on extrapolations from fungus-plant ratios in temperate regions and did not give due consideration to the hyper-diverse realm of insect-associated fungi, such as the Laboulbeniales, or account for tropical species diversity (Hawksworth, 1991, Hawksworth and Lücking 2017). The most recent estimate (Hawksworth and Lücking, 2017) of extant fungi is 2.2 to 3.8 million, and the updated fungus-plant ratio for temperate zones is 8:1. Of that, ~135,000 species have been described (Hibbett et al., 2016). With only ~6% of the lower estimation being known to science, the remaining task is tremendous. Unlike many plant and animal groups, fungi do not broadly enjoy the benefits of being well studied and clearly understood. New species are most likely to be discovered by investigating currently understudied habitats and microhabitats, including insect bodies, lichen-dwelling fungi, cryptic species, and through environmental (eDNA) sequencing (Hawksworth and Lücking, 2017).

In addition to fungi being relatively poorly studied, the often sporadic, ephemeral, and unpredictable appearance of fruiting bodies complicates obtaining thorough population data, and has constrained our ability to provide clear objective assessments of fungi overtime. The complex biotic and abiotic forces leading to a species even producing a fruiting body remains unknown in many cases. While some fungi, like some species of morels, can be reliably found in the same place at more or less the same time every year, other species, such as Ionomidotus sp. (personal observation) or Hericium bembedjaense (Jumbam et al., 2019) may be seen once in a given location and then not again for years, if ever. While substantial efforts have recently been made in fungal conservation, this field remains in its early stages (Mueller, 2017). According to the State of the World’s Fungi (Ainsworth et al., 2018), only 56 species of fungi have been evaluated for placement on IUCN Red List, and 43 of those species were included. Comparatively, 25,452 species of plants and 68,054 species of animals have been respectively being included. It is therefore imperative that fungi receive increased attention, concern, and action.

Previous studies have begun to explore the potential of Laboulbeniales as indicators. Sugiura et al. (2010) conducted a study of Carabidae beetles and their associated species of Laboulbenia across different habitats in central Japan — a riverside, secondary forest and farmland and the microhabitats therein — to quantify insect-fungal interactions at the host assemblage level. This study found that 14/156 or 8.97% of Carabidae collected at the riverside site were infected with Laboulbenia, individuals; 2/214 or 0.93% in the forest and 0.161 or 0% at the farmland habitat, building evidence that the host habitat partly impacts the prevalence of Laboulbeniales. However, collection of insects in this study took place during December, and while Laboulbeniales are present on hosts overwinter, seasonal impacts of Laboulbeniales richness and abundance are not fully understood and may vary. This study expanded upon studies by Anderson and Skorping (1991) and De Kesel (1996b), which both demonstrated, Fig. 2. Hydrophilomyces gracilis attached to host, Cercyon sp.
via field work and experimentation respectively, that host microhabitat impacts occurrence of Laboulbeniales and the presence of host taxa alone does not guarantee presence (or detection) of fungal counterparts.

**Preliminary Study:** Sampling was conducted at two sites, Lake Eustis and Emeralda Marsh Conservation Area, which encompasses a portion of Lake Griffin. These lakes are part of the Central Valley Region (Region 7508) and are part of the Ocklawaha Chain of Lakes, which includes a total of 10 connected lakes. The headwaters of this chain is Lake Apopka, which is fed by a natural spring and by rain. Lake Griffin is the most downstream of the 10 lakes and Lake Eustis is directly upstream from Lake Griffin. Lake Griffin empties northward into the Ocklawaha River, which ultimately connects to the St. Johns River (St. Johns River Water Management District, Lake Apopka Basin, n.d.). Emeralda Marsh was designated as a National Natural Landmark in 1974 (St. Johns River Water Management District, Emeralda Marsh Conservation Area, n.d.). Over the past 30 years, aquatic and wetland restoration efforts in and around EMCA have focused on revegetation, reestablishing connectivity with Lake Griffin, and reducing phosphorus and pesticide loading (St. Johns River Water Management District, Emeralda Marsh Conservation Area, n.d.; Fulton et al., 2015). Restoration activities have been extensive at Lake Griffin but comparable efforts have not been made at Lake Eustis (Fulton et al., 2015). These sites were chosen for the preliminary study because Eustis was a site visited by mycologist and expert on Laboulbeniales, Dr. Roland Thaxter in 1897. In addition to investigating the potential for Laboulbeniales to be used as indicators of environmental health, the preliminary study was an attempt to re-collect species recorded by Thaxter, which could provide insight into the shifts in biodiversity of Laboulbeniales and their associated insects since 1897 (over 121 years ago). The results of which are presented and discussed by Kaishian (2020).

A rapid biodiversity assessment was conducted over five days, August 14th–18th, 2018. Insects were collected using a black light, (2805 DC Light Night Collecting Light, DC, 12 Volt, 15 Watt BL), which was set against a white sheet and was placed approximately 5 m from the water’s edge. This popular entomological collection method was chosen in order to collect a broad range of taxa (Szentkirályi, 2002; van Wielink and Spijkers, 2013). Insects were collected via aspirator and transferred to 70% ethanol for storage. Three collectors spent three hours at each of the two sites per night, totaling 90 effort hours of collecting. Equal collection effort was made at both sites and the starting location alternated each night between Eustis or EMCA. Insects were scanned for infections of Laboulbeniales under a Nikon stereomicroscope at 20–40×. Presence/absence data were obtained for Laboulbeniales infections of each insect examined. All insect specimens were identified to family level and accessioned into the entomological collections at SUNY College of Environmental Science & Forestry (SUNY-ESF) in Syracuse, New York. Fungal thalli were removed with a micropin and mounted in glycerin using previously described methods (Benjamin, 1971, 1986, 1993). All fungi were identified to species level (references for IDing). Terminology and abbreviations used in describing the ascoma (used here for the entire peritheciun-bearing thallus) are those used by Tavares (1985). Voucher specimens are deposited in the mycological herbarium at SUNY-ESF (herbarium code SYRF).

A total of 4,022 insects were collected during the rapid assessment. Overall insect abundance was greater at EMCA, with 3,001 insects collected, compared to 1,021 insects collected from Eustis (Fig. A.3). Insect richness at the family level was comparable between lakes with 54 families present at EMCA and 53 at Eustis. Both the Simpson’s (D) and Shannon-Weiner (H’)...
The most noteworthy contrast between the lakes was relative abundance of the family Hydrophilidae, with 1,923 individuals collected from EMCA and only 13 from Eustis. Abundance, species richness, and species diversity (Simpson’s and Shannon-Weiner) were calculated for insects and Laboulbeniales. Comparisons are presented from the two time periods for collections in Eustis (1897 and 2018) as well as between Eustis and EMCA. To avoid pseudoreplication, this was considered a case study and actual statistical comparisons were not made. In this study, similar richness of insect families was found at both sites, while the presence of fungi was markedly different, with a 20% infection rate at EMCA and a 0.1% infection rate at Eustis. These findings also highlight that specialist organisms, such as the highly host specific and obligately associated members of the Laboulbeniales, may be of particular risk for population decline, range restriction, loss of genetic diversity, extirpation, and total extinction due to habitat destruction (Thomas, 2000; Warren et al, 2001).
The contrast of the abundance of Hydrophilidae (water scavenger beetles), more specifically the subfamily Hydrophilinae, between sites is of particular note, given that Hydrophilinae are host to most of the fungal infections recorded at EMCA. Because Hydrophilinae are nocturnal and attracted to light (Thorp and Rogers, 2015), it is possible that ambient artificial light from the town of Eustis diminished the relative attraction of the UV light, contributing to the difference in Hydrophilidae abundance between sites. However, given the well established and devastating effect of artificial light at night on many nocturnal insects, it is reasonable to consider that artificial light introduced with urban development, coupled with outright habitat destruction around the perimeter of Lake Eustis and surrounding wetlands potentially disrupted normal biological activities of hydrophids, leading to population decline (Owens et al, 2019). Loss of emergent and floating-leaved vegetation may also play a role in the insect population declines. Such vegetation was relatively abundant at EMCA collection site (as it had been purposefully re-introduced (Fulton et al., 2015)) compared to Eustis where no such efforts have been reported. With this possible insect population decline, the fungal population may have also declined. At EMCA, the infection rate of the Hydrophilidae population was 1 in 5, but 0 of the 13 Hydrophilinae collected from Eustis were infected. This would perhaps suggest the insect population has dwindled to levels that no longer facilitated social transmission of Laboulbeniales, which is thought to be the primary mode of transmission for this group (De Kesel, 1993, Weir and Beakes, 1995).

Proposed Research Plan

Objective: Explore if and how Laboulbeniales may serve as a proxy for biodiversity and an indicator for ecosystem health.

When considering Laboulbeniales, collection of insects offers a two-for-one assessment of insects and fungi, providing a more textured, multi-kingdom understanding of biodiversity at a given site. Without attention paid to the fungal dimension, incorrect conclusions may be drawn about the biodiversity of these two habitats. A multi-kingdom assessment consolidates resources and effort, which increases the feasibility of conducting biodiversity monitoring. Making such work more feasible is attractive given the mounting pressure of climate change and the related impacts on biodiversity. The above findings suggest it is worthwhile to develop a more intensive investigation. In order to confidently assess the results and merit of the core question of our preliminary study – can Laboulbeniales be used effectively as indicators of environmental health – expansion and replication of the preliminary methodology is required.

First, one essential question that remains unanswered from the preliminary study is: are the differential Laboulbeniales richness and abundance results found between EMCA and Eustis an actual response to the difference in lake system health between the sites, or due to some other effect, such as microclimatic phenomena, specific pollution exposure, etc. Thus, replication is necessary to establish whether or not there is statistical significance between restored and disturbed/unrestored sites generally. Similarly, another unanswered question is: is the phenomenon of finding pronounced differences in Laboulbeniales abundance and richness between site types specific to a given habitat or ecozone? Is this phenomenon restricted to these two lakes, to central Florida, or to a broad range of habitats and regions? In order to answer this
question, the first year will be dedicated to extensively sampling lake couplets throughout Central Florida. Three lake systems in addition to EMCA and Eustis will be assessed for insect and associated Laboulbeniales richness and abundance, and compared. Sampling will begin in May and run through the end of September (Table 1). Sampling across the arc of peak insect activity will reveal if there is a seasonal effect on Laboulbeniales richness and abundance. While these fungi are present on their host year round, social transmission is impacted by insect behavior, and seasonal shifts in abundance may be detectable. Determining peak season for detectable Laboulbeniales richness and abundance may be useful in selecting optimal sampling timing in future studies. Each couplet will be visited for one week (seven days) per month. All data per site will be summated, but different sampling days will be recorded separately so daily and seasonal results can be later examined independently.

Table 1. Sampling schedule for first year of project.

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<th>Week 1</th>
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<tr>
<td>May</td>
<td>Couplet A</td>
<td>Couplet B</td>
<td>Couplet C</td>
<td>Couplet D</td>
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<td>June</td>
<td>B</td>
<td>C</td>
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<td>July</td>
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<tr>
<td>August</td>
<td>D</td>
<td>A</td>
<td>B</td>
<td>C</td>
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<tr>
<td>September</td>
<td>A</td>
<td>B</td>
<td>C</td>
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In addition to this replication program, the methodology will be expanded to include a variety of trapping methods along with the ultraviolet light traps used in the preliminary study. Hammond (1990) reports results of a beetle survey from a study in a 5 km² patch of land in northern Sulawesi, an island in Indonesia. As part of this study, 1.7 million Coleoptera were examined over the course of one year (1985) Of the 1.7 million beetles, 80,000, representing the full range of morphotypes, were scanned for infection of Laboulbeniales (Weir and Hammond, 1997). Part of this study examined different trapping methods that have been employed for insect collection, as well as used to the end of collecting Laboulbeniales. These methods include: flight intercept traps, pitfall traps, Malaise traps, and ultraviolet light traps. Based on this study, the unbaited pitfall traps and the flight intercept traps were determined to be the most effective methods for obtaining groups of beetles that host infections. Of course, these data are focused solely on Coleoptera and does not deal with other orders. But because the majority of infections occur on Coleoptera, and within Coleoptera most infections (~50%) occur on Staphylinidae and Carabidae, this knowledge is highly functional (Weir and Hammond, 1997). This study also indicated different traps have biases towards different groups of insects. More specifically, the authors found beetles with a habit of living on or amongst dung, fungal sporocarps, leaf litter, and carrion were more likely obtained via light intercept and pitfall traps. Malaise traps were more likely to trap insects associated with wood and other fungi, and light traps were biased towards attracting riparian and aquatic insects. The study reported that beetles associated with wood or living plants tend to have disproportionately few infections, so methods biased towards
collecting those groups would tend to yield fewer infected individuals than methods biased towards beetles associated with dung and decaying matter. Therefore, incorporating a suite of trapping methods into our study design will facilitate optimal sampling towards the goal of sampling the most accurate representation of insect-Laboulbeniales assemblages. All traps will be run concurrently at each site. Care will be taken to set the traps as consistently at each site, particularly maintaining a consistent trap dimensions, make and models, and directionality with respect to water and amongst comparable amounts of vegetation and canopy coverage (Lamarre et al, 2012). Insects collected from each trapping method will be summated for each site, but will also be recorded separately so the varying trapping methods be examined independently. The remaining months of the first year will be dedicated to data processing. Because five days of sampling at EMCA and Eustis generated ~4,000 insects, 140 days of sampling could generate ~112,000 insects for processing, although this number could be substantially higher with the addition of multiple trapping methods. This is an enormous amount of data which will take no less than the remainder of the year (8 months) to process with a team of researchers. Processing the data includes: scanning insects for infections with Laboulbeniales, removal of Laboulbeniales, preparation of permanent microscope slides, and insect identification, accessioning of specimens to collections of affiliated institutions and data analysis. In order to achieve this, collaboration with an entomological lab with taxonomic expertise will be essential. Workers will be trained on scanning insects for Laboulbeniales and the course sorting of insects to the order level, later to be passed on to collaborating entomologists. Major host groups will be identified.

The above work will reveal the answer to the question: do Laboulbeniales serve as an effective indicator of environmental health in lake systems in central Florida? While our preliminary results from EMCA and Eustis suggest that the answer to this question will be “yes,” further replication will need to be conducted in order to assess whether this result will remain constant in a variety of habitats, localities, and ecozones beyond central Florida. The next question we plan to address in this study is: can Laboulbeniales be used effectively as an indicator of environmental health across a variety of ecozones? In order to answer this question, we will sample lake couples from the nine ecoregions identified by the EPA: Northern Appalachian, Southern Appalachian, Coastal Plains (using year 1 data from FL), Southern Plains, Temperate Plains, Upper Midwest, Northern Plains, Xeric and Western Mountains (Fig. 4). Based on data obtained by the Environmental Protection Agency (EPA) National Lake Assessment: A Collaborative Survey of the Nation’s Lakes (NLA) eight replicate pairs of lakes will be selected.
(USEPA, 2009). For every lake surveyed, the NLA identified a “reference lake,” which is defined as “a lake (either natural or man-made) with attributes (such as biological or water quality) that come as close as practical to those expected in a natural state, i.e., least-disturbed lake environment.” The NLA scored the overall condition of the lakes as compared to each associated reference lake based upon a compilation of biological, chemical, recreational, and physical indicators. “Good” denotes a close comparison to the reference lake, “fair” denotes a minor negative difference between the two lakes, and “poor” indicating a total negative condition as compared to the reference lake. For this study, “good” and “poor,” naturally occurring (as opposed to man-made) lakes will be chosen in couples to serve as replicate couples per region.

This sampling program will be conducted over the next two years. Results from the previous year of sampling in Florida will inform the sampling design for the other ecozones. For example, if 100% of the couples sampled in FL show a difference in Laboulbeniales richness and abundance with Laboulbenilaes being richer and more abundant at the healthier or “good” site, than that would suggest that sampling 1 lake per ecozone may be sufficient to record this effect in other ecozones. However, if the results are statistically significant overall but not recorded at 100% of the couples, then this would suggest that multiple lake couples would need to be sampled to ensure proper record of this effect. Confounding ecological factors (such as extreme weather events or anomalous conditions) for outlying data would need to be considered for such instances.

Results from the previous year will also reveal a potentially streamlined methodological approach for broader ecozone sampling. For example, if certain traps consistently yield higher rates of infection (as per Weir and Hammond (1997)) then we will focus on employing those traps. Similarly, the FL results may help clarify the duration of sampling time needed to acquire representative results. If the daily, monthly, and total results are roughly proportionate across all sites, (i.e. if ~ 100 infections are recorded daily per site, and ~ 700 per week, and ~ 3,500 infections are recorded in total over the five weeks) than, logically, a daily or weekly sampling regimen would be sufficiently informative. Similarly, major host groups identified from the previous year will be targeted so as to reduce the number of insects killed unnecessarily.

With these numerous possible scenarios to be considered after the first year of sampling, flexibility will be required in developing the sampling plan for the following two years. A tentative sampling plan can be made with the following assumptions:

1. Because our preliminary results suggest a strong effect of habitat on Laboulbeniales richness and abundance, we expect that the expanded FL sampling regimen will yield statistically significant results.
2. We expect the results to be significant, but not found at 100% of the sites, thus there will be a need to sample multiple sites.
3. We expect that the number of infections found per site will remain in relative proportion as the data are summated (day : week : season). Therefore, we expect that a timescale of approximately one week will be sufficient to detect salient results per lake couplet.
4. Because ~ 80% of known Laboulbeniales occur on Coleoptera, we expect that Laboulbeniales diversity could be represented by targeting Coleoptera in our collection methods and data analysis, particularly widely distributed and commonly parasitized groups such as Carabidae, Dytiscidae, Hydrophilidae, and Staphylinidae. To that end,
unbaited pitfall traps, flight intercept traps, and light traps may prove to be the most effective traps for collecting Carabidae, Staphylinidae and Dytiscidae and Hydrophilidae respectively and sufficiently representing the population of Laboulbenilaes at each site.

Table 2. Sampling schedule for second and third years of project.

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<tr>
<td>Southern Midwest</td>
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<td></td>
<td>Xeric</td>
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<tr>
<td>Temperate Plains</td>
<td></td>
<td>X</td>
<td></td>
<td>Western Mountains</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern Appalachia</td>
<td></td>
<td>X</td>
<td></td>
<td>Northern Plains</td>
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<td></td>
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<td>X</td>
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<tr>
<td>Southern Appalachia</td>
<td></td>
<td></td>
<td>X</td>
<td>Southern Plains</td>
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<td>X</td>
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As such, year two will be dedicated towards sampling in the four remaining ecozones east of the Mississippi including: Northern Appalachia, Southern Appalachia, Temperate Plains and Southern Midwest. Year three will sample from the four ecozones to the west including: Northern Plains, Southern Plains, Xeric and Western Mountains. Each year, one month will be dedicated to each ecozone, starting in June and running through September (Table 2). The northern most regions will be sampled later in the summer to align with peak insect activity. Three lake couplets will be sampled per ecozone. Each couplet will be sampled for one week (seven days).

Data Analysis

Two Student’s t-tests will be used as statistical tests to compare the means of two groups – “good” vs. “poor” lakes in each region and in total. Two T-tests will be used to determine whether or not there is a significant difference in insect and fungal diversity between “good” lakes and “poor” lakes using the measurements from Simpson’s and Shannon’s index (described below). Another separate t-test will be performed on the abundance data. In this scenario, the null hypothesis is: there is no difference in the insect and fungal diversity and abundance (respectively) between the two lake types (“poor” and “good”). In the event of outlying data, such as a massive, disproportionate population of *Tropisternus sp.* and associated fungal infections of *Ceratomyces sp.*, t-tests that both included this data, and that did not will be performed, and statistical and ecological arguments for both scenarios will be explored and discussed.

Insect and fungal abundance, species richness, and diversity (Simpson’s Diversity Index and Shannon-Weiner Index). In this Simpson’s index, species diversity increases along with richness and evenness. A diversity value (D) is given between 0 and 1, where 1 represents no
diversity and 0 represents infinite diversity. The Shannon Index is also a measure for diversity, but is more sensitive to species richness and gives more weight to rare species than Simpson’s Index. With this index, a diversity value (H’) is given. Like Simpson’s the Shannon index can be normalized to be expressed as a value between 0 and 1, with lower values representing higher diversity and higher values representing lower diversity. Because there is variation in how these two indices interact with the data, running these indices in parallel is common practice in ecological studies. We anticipate that the insect and fungal diversity will be closer to 0 than 1 at both sites, with the “good” sites having a higher index than the “poor” sites with both indices.

Elucidating community composition will be crucial to aid our understanding of system ecologies. Different assemblages of insects may correlate to different lake systems and with different assemblages of Laboulbeniales. Texturing our biodiversity indices with community analyses will facilitate a more powerful understanding of the ecological forces at play and will make for a scientifically robust indicator program. Therefore, Multidimensional Scaling (NMDS) will be conducted on community and ecological matrices assembled from the literature and from newly generated data using the R package vegan (Dixon 2003; R core team 2018) in order to visualize community assemblage of Laboulbeniales as they relate to ecological factors and host biology. For example, of the insects infected, what shared biological traits may they possess and are these traits significant in determining parasitization? In order to assess whether certain ecological and/or biological traits are of statistical significance, permutational multivariate analysis of variance will be carried out using the ‘Adonis2’ function in the vegan package.

Expected Outcomes

Financial pressures often force scientists and governing bodies to keep biological monitoring efforts low cost. According to Nimis et al (2002), this has led to the development of poorly studied indicator organisms that oversimplify highly complex biological realities, misinforming biological monitoring efforts. Nimis et al argue that it is necessary to link broader baseline data of a given system with indicator data, so as to ensure that an indicator is an effective proxy for that system. By focusing on two kingdoms, this project will collect a large swath of data, all of which will be databased in an open source format for future research. Furthermore, the obligate and highly host specific parasitic nature of Laboulbeniales makes for concentrated ecological information because Laboulbeniales inherently represent the biology of multiple kingdoms, which can be defined as a low cost/high information value system (Hunsacker, 1993). Based upon findings from the EPA’s long-running Environmental Monitoring and Assessment Program (EMAP), Hunsacker (1993) discusses characteristics that make for an effective indicator. The characteristics are summarized as follows: 1) potential to be applied to geographically broad areas; 2) reflect changes in both space and time; 3) can be clearly tied to an endpoint or goal; 4) can be monitored cost-effectively; 5) responsive to stimuli or stressor(s) of relevance for management; 6) can be standardized methodologically; 7) low measurement error; 8) available historical data and/or the potential for database development.

Laboulbeniales have the potential to meet the aforementioned specifications for effective biodiversity indicators, which could render them pivotal to effective biological monitoring programs. Because the results from our preliminary study were stark, we anticipate that we will find a statistically significant difference between Laboulbeniales and insect richness and abundance between lake systems as we carry out our enhanced methodological and replication
regimen across central FL. The preliminary data are in fact so pronounced, that we would need to record an opposite effect at the same magnitude to statistically offset these results. This seems unlikely given that the data from the preliminary work are reasonably explainable in the context of habitat destruction vs habitat protection and restoration. As we broaden our study to include replications across the continental United States, we believe that it is plausible that Laboulbeniales will emerge as a sensitive indicator of environmental health.

We anticipate finding new species of both Laboulbeniales and possibly of insects, as well as new species records for North America and at the more specific lake localities. We expect to collect species of Laboulbeniales that have not been collected since Dr. Thaxter over 100 years ago. As discussed above, presence of host taxa alone does not guarantee presence of fungal counterparts. Therefore, we expect Laboulbeniales will serve as a more sensitive biodiversity and environmental health indicator than insects. With these anticipated results, more sensitive biodiversity monitoring programs could be developed to track restoration efforts. Efficacy of various restoration and conservations programs could be compared, with higher fungal diversity indicating more effective programs.

**Broader Impacts**

**Urgency.** The earth and its inhabitants are in the throws of a 6th mass extinction event, making biological conservation of the utmost importance. As we become increasingly aware of the decline of the world’s insect populations due to climate change, it is increasingly apparent that the Laboulbeniales and other insect associated fungi will be negatively impacted. Understanding the dynamics of fungal diversity and abundance is essential to practicing informed and effective conservation measures, but comprehensive fungal inventories are infamously challenging due to the ephemeral nature of many of the more well known mushroom species. Exploration of the use of Laboulbeniales fungi as a biodiversity and environmental health indicators could provide insight to broader fungal diversity patterns and habitat quality. Because these fungi are present year round with their hosts, they may prove to be uniquely useful as indicators of multi-kingdom biodiversity and environmental health.

**Science Communication.** There is a dire need to communicate to the public the state of biodiversity in our current and changing climate and this needs to be achieved through a variety of tactics and forms of media. Findings of this work will be interpreted through interdisciplinary projects focused on effective and ingenuous science communication. Simultaneous to producing the fundamental science, collaboration with journalists, theorists, artists, and politicians to relay our data in creative ways will serve to heighten public engagement, a crucial component of designing effective conservation policy. In particular, I am interested in facilitating collaboration between Traditional Ecological Knowledge (TEK) from indigenous naturalists in my professional network to highlight various ways of knowing our natural world. This could be accomplished through a lecture series on TEK in conjunction with academic science. As a scientist, I have prioritized building a multi-talented network of dynamic thinkers and I plan to mobilize that network into action around climate change.
Diversity, Equity, Inclusion. Scientific spaces are typically not representative of Black, Indigenous, or Latinx people (Bernard and Cooperdock, 2018). Addressing racial inequality is not simply a matter of increasing representation, but doing the work to foster truly equitable, safe, and empowering spaces for Black, Indigenous and Latinx people in particular. Racial discrimination remains a central issue for inclusion, safety, retention, and success (both short- and longterm) of the aforementioned groups. It is therefore imperative that research groups reflect critically on the potentially racist practices of their group. In the early formation of this project, all Principal Investigators and collaborators will refer to and codify the work of Chaudhary and Berhe (2020), Ten simple rules for building an anti-racist lab. The ten rules are summarized below, but should be read thoroughly by everyone involved in the project.

1. Lead informed discussions about anti-racism in your lab regularly
2. Address racism in your lab and field safety guidelines
3. Publish papers and write grants with BIPOC colleagues
4. Evaluate your lab’s mentoring practices
5. Amplify voices of BIPOC scientists in your field
6. Support POC in their efforts to organize
7. Intentionally recruit BIPOC students and staff
8. Adopt a dynamic research agenda
9. Advocate for racially diverse leadership in science
10. Hold the powerful accountable and don’t expect gratitude

LITERATURE CITED


CURRICULUM VITAE

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Education

Wheaton College, Norton, MA, 2009–2013
B.A. Biology, Concentration: Environmental Studies

SUNY-ESF, Syracuse, NY, 2015–2020
Doctoral Candidate, Department of Environmental & Forest Biology
Concentration: Forest Pathology & Mycology
Student of Dr. Alex Weir

Employment & Work Experience

Instructor of Mycology, SUNY-ESF, July 2016 – Present
Teaching mycology section of Ecological Monitoring and Biodiversity Assessment (EFB 202) at Cranberry Lake Biological Station; designed and executed the field course Fungi of Bolivia (EFB 500) in collaboration with Latin American universities, conducted in the Amazon and Andean regions of Bolivia (January 2017).

Project Manager & Digital Archivist, The Microfungi Collections Consortium, August 2015 –2018
Digitizing ESF’s herbarium entire collection of microfungi as part of a multi-institutional project, funded by the National Science Foundation; managing a dozen undergraduate students for the project.

National Geographic Explorer Grant, Researcher, December 2018
Member of a biological research expedition crew to the Ecuadorian Andes to conduct a multi-kingdom biodiversity inventory of a rainforest threatened by an international mining company.

General Ecology Lab Coordinator, SUNY-ESF, August 2017 – January 2018
Managing the curriculum and equipment for 10 lab sections; preparing the Teaching Assistants for the weekly lab material.

Graduate Teaching Assistant, SUNY-ESF, January 2017 – Present
Teaching laboratory component of Cell Biology & Genetics and Mycology.

Herbarium Technician, SUNY-ESF, August 2015 – August 2017
Assessing and remounting botanical collections damaged in flood.

Educator, Onondaga Nation and Clark Reservation State Park Nature Center, September 2016 – Present
Guiding mycological walks for children and adults.
Guest Lecturer, SUNY-ESF, September 2016, February 2020
Lecturing on fungal diversity for the course Diversity of Life (EFB 210)

Wilderness Guide and Naturalist, Gear to Go Outfitters, Brooklyn, NY, 2013-2014
Guiding both adults and children through outdoor excursions across New York state; teaching clients the how to ID local flora and fauna; designing and teaching outdoor naturalist field courses for 3rd grade students.

Researcher, Project Amazonas, Peruvian Amazon, 2013
Surveying macrofungi in the forest surrounding Madre Selva Biological Station in the Peruvian Amazon in myco-blitz fashion; collecting ethnographic information regarding the culinary, medicinal, spiritual uses of fungi in the local communities; assembling a reference guide for fungi of the station.

Biology Advisor, Terreform ONE & Genspace Community Biology Lab, 2013
Advised the architecture and design firm, Terreform ONE, on the incorporation of biological materials into art piece “Bio City Map of 11 Billion.” Worked in a lab at Genspace culturing & sub-culturing bioluminescent E. coli used in the art piece. This project was the recipient of the Architizer A+ Awards, 2014 and was featured in museums around the world and a TED talk. http://www.terreform.org/projects_urbanity_bio_city_map.html

Certified Naturalist, Cornell Cooperative Extension, 2009
Proficient at identifying local flora and fauna for the purpose of educating the public and promoting environmental conservation; identifying and eradicating invasive species; monitoring current environmental trends and issues.

Publications & Presentations


Presenter at New Moon Mycology Summit - https://www.newmoonmycologysummit.org; Thurman, NY. August 2019. Contact: Olga Tzogas (newmoonmycologysummit@gmail.com)


Presenter at The Charles Horton Peck Foray - [http://www.plantpath.cornell.edu/CUPpages/Peck/index.html](http://www.plantpath.cornell.edu/CUPpages/Peck/index.html) Newcomb, New York. September 2017. Contact: Alex Weir ([aweir@esf.edu](mailto:aweir@esf.edu))

Presenter at Northeast Mycological Federation - [www.nemf.org](http://www.nemf.org); Stratton, Vermont. July 2017. Contact: Gary Lincoff ([gary@noahsquark.com](mailto:gary@noahsquark.com))

Presenter at SFSU Sierra Nevada Field Campus - [http://www.sfsu.edu/~sierra/](http://www.sfsu.edu/~sierra/); Spring Fungi of the Sierra Nevada (BIOL 315). Bassetts, California. June 2016. Contact: Dr. Dennis Desjardin ([ded@sfsu.edu](mailto:ded@sfsu.edu))

Presenter at Central New York Mycological Society - [www.cnyms.org](http://www.cnyms.org); Syracuse, New York. April 2016. Contact: Dr. Tom Horton ([thorton@esf.edu](mailto:thorton@esf.edu))

Current Research

Taxonomic contributions to Laboulbeniales on Neotropical Heteroptera; published (as above) and in progress.

Laboulbeniales of the Heteroptera: a systematic approach to the collections at the American Museum of Natural History; in progress.

Laboulbeniales (Ascomycota, Fungi) of Lake Eustis & Emeralda Marsh, FL: a pilot study on urbanization and diversity.

Laboulbeniales of Ecuador; in progress.

Fungi of Bolivia - expeditions to the Andean and Amazonian regions of Bolivia to document broad fungal biodiversity with collections deposited in local herbaria; published presentations.

Grants, Fellowships & Awards

**Josiah L. Lowe – Hugh E. Wilcox Graduate Fellowship, 2019**
SUNY-ESF Department of Environmental & Forest Biology scholarship for graduate students in plant physiology, mycology, and plant pathology; $3,000

**Excellence in Teaching Award, 2018**
SUNY-ESF Gradate Student Association award for a graduate student recognized for their excellence in teaching.

**Edna Bailey Sussman Foundation, 2018**
Recipient of national foundation award that sponsors research and internships that apply hard science to solving existing environmental problems; $6,000
Josiah L. Lowe – Hugh E. Wilcox Graduate Fellowship, 2018
SUNY-ESF Department of Environmental & Forest Biology scholarship for graduate students in plant physiology, mycology, and plant pathology; $5,000

Award for Best Poster -- Congreso Latinoamericano de Micologia (CLAM) IX, 2017
New species of Prolixandromyces (Laboulbeniales) from South America

Charles Lathrop Pack Memorial – Graduate Student Travel Award, 2017
SUNY-ESF Graduate Student award to attend the IX Latin American Mycological Congress, in Lima, Peru; $500

Josiah L. Lowe – Hugh E. Wilcox Graduate Fellowship, 2016
SUNY-ESF Department of Environmental & Forest Biology scholarship for graduate students in plant physiology, mycology, and plant pathology; $18,500

Josiah L. Lowe – Hugh E. Wilcox Graduate Fellowship, 2015
SUNY-ESF Department of Environmental & Forest Biology scholarship for graduate students in plant physiology, mycology, and plant pathology; $5,000

Wheaton College Traditional Chinese Medicine Grant Recipient, Yunnan, China, 2011
Studied TCM at Yunnan Provincial Hospital of Traditional Chinese Medicine, Kunming, China; apprenticed doctors in acupuncture, massage therapy, herbalism; investigated the scientific and philosophical differences between Western and Chinese medical systems; $3,000.

Professional Service

Peer Reviewer, 2020
Sydowia: http://www.sydowia.at/
Mycokeys: https://mycokeys.pensoft.net/