Light and Ultrastructural Microscopy Studies on Coreomyces (Laboulbeniomyces - Ascomycota)

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LIGHT AND ULTRASTRUCTURAL MICROSCOPY STUDIES ON COREOMYCES

(LABOULBENIOMYCETES – ASCOMYCOTA)

by

Benjamin Zink

A thesis
submitted in partial fulfillment
of the requirements for the
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Department of Environmental Forest and Biology

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I would first like to thank my professor, advisor, and mentor for the past 6 years, Dr. Alex Weir. I came to ESF pursuing a bachelor’s degree in 2013, eager to learn about the fungal world. Not long after arrival, I heard about a mycology course being offered on campus, by Dr. Weir. Upon taking his class, I was then introduced to the world of electron microscopy through a demo of fungi with the scanning electron microscope. It only made sense to return to graduate school under the guidance of Alex to delve into the world of a very specialized group of fungi, the Laboulbeniomycetes. Utilizing a new state of the art TEM that was recently funded with NSF grants awarded to ESF, the genus Coreomyces was chosen to study in depth. I had a great time with this project and wish Alex, all his future students, and their research, all the best.

I would also like to thank Dr. Susan Anagnost, professor of microscopy and wood studies at ESF. She has been a core individual for guidance in my recent arrival into the world of microscopy, and more specifically electron microscopy. Susan has always been as helpful as possible, and believe she has been a strong influence on the building of my microscopy skill set.

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ABSTRACT

B.C. Zink. Light and Ultrastructural Microscopy Studies on Coreomyces (Laboulbeniomycetes – Ascomycota), 88 pages, 2 tables, 93 figures, 10 appendices, 2020. APA

This study utilized ultramicrotomy, coupled with transmission electron microscopy (TEM) to investigate the ultrastructure of Coreomyces, a member of an unusual group of arthropod associated fungi, Laboulbeniales (Ascomycota). Ultra-thin sections of the fungal cell wall reveal a tri-layered structure, bounded by a double membrane, and lacking perforations. This implies that all nutrition required for development of the fungal thallus is obtained directly from the host via the attachment cell. Ultrastructural details presented for any member of Laboulbeniales include (1) detail of a receptacular septum, revealing the presence of septal pores, (2) sections through the ascospore attachment pad, illustrating a mesh-like fiber network, and (3) the occurrence of a series of invaginations, interpreted as flask-like pores, situated along the outer wall of mature ascospores. Details of the development of the perithecium, asci and ascospores, are also presented, based on serial sectioning through the terminal cell of the thallus.

Keywords: Laboulbeniales, Coreomyces, Ascomycetes, thallus, perithecium, TEM, ultrastructure.

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Introduction and Objectives for Study

Introduction to Laboulbeniomycetes

The Laboulbeniomycetes (Ascomycota) are a peculiar group of fungi that associate with arthropods (mostly insects) in a parasitic fashion (Thaxter, 1896). Historically, their small size and structure made them easily overlooked, or even disregarded, by entomologists (Thaxter, 1896). In fact, 1849 was the first mention of Laboulbeniales to the scientific world, although the study of entomology long preceded this date (Tavares, 1985). Although some studies on these fungi preceded the works of Dr. Roland Thaxter, he is accredited with the most detailed studies, as is evident in: *A Contribution Towards a Monograph of the Laboulbeniaceae: Volumes I-V (1896-1931)*. Dr. Thaxter made such significant contributions to this group, his time in the field is often referred to as “The Thaxter Era” (Benjamin, 1971). Thaxter’s early studies picked up on works by Robin (1853) and his *Histoire Naturelle des Vegetaux Parasites*, where the genus *Laboulbenia* was first mentioned. In more than 40 years of study, Dr. Thaxter described 103 genera, about 1260 species, and 13 varieties of the Laboulbeniales. Perhaps his greatest feat, however, were the 3427-line drawings in 166 plates to accompany the writings (Benjamin, 1971). With such a substantial study, Dr. Thaxter laid a solid framework for future researchers to investigate these fungi. Even today, only about 2000 species are known, so that almost two-thirds of all known species were described by Thaxter.

Although they are non-detrimental to the host, adaptation of a species to not only a host, but to a specific position on that host has long attracted the attention of biologists (Benjamin, 1971). Questions pertaining to these fungi that have yet been studied, or still yet proposed,
include the nature of attachment to the host, the host’s role in nutrition of the fungi, transmission from host-host, and various aspects of the life history of the fungi (Benjamin, 1971).

Molecular Phylogeny and Implications for Study

Goldmann and Weir (2018) were the first to attempt to provide a phylogeny of the Laboulbeniomyces using molecular characters. This study provided a useful starting point to better understand the relationship of these unusual fungi and utilized data from 65 different taxa, including 51 new SSU rDNA sequences (Goldmann and Weir, 2018). Conclusions showed that there was evidence for a strong relationship between Laboulbeniomyces and Sordariomycetes, supporting recent work by Schoch et al (2009). Within the class Laboulbeniomyces nine different clades were distinguished and features of perithecial development, which showed an overall trend towards reduction, appeared to be phylogenetically informative. One distinguished clade, Clade G, included the genera Coreomyces and Chitonomyces, with the former being the genus investigated for this current thesis study. Notes on this clade from the Goldmann and Weir paper (2018) include that these genera are enigmatic and have eluded researchers, particularly as it has been difficult to distinguish thallus development, especially with regard to the perithecial basal cells and outer wall cells in both genera. Goldmann and Weir (2018) suggested that, “Of particular importance in future studies would be detailed anatomical information on the perithecial and antheridial characteristics in species of these genera.”
Objectives and Thesis Summary

The current thesis study *Light and Ultrastructural Studies on Coreomyces (Laboulbeniomycetes - Ascomycota)* attempts to elucidate the fine structure of a key genus of the Laboulbeniomycetes, with a focus on the cellular and sub-cellular levels. The study was conducted on one specific genus of Laboulbeniales, *Coreomyces*, with a few specific questions in mind. Utilization of an instrument that has never been used to study this genus, or many genera of Laboulbeniomycetes overall, will aim to help more easily visualize the ultrastructural detail of these fungi. This instrument is the Transmission Electron Microscope (TEM). The TEM was first developed in 1931 by Max Knoll and Ernst Ruska after successfully building what was first a theoretical device (Bozzola and Russell, 2006). Since then, many improvements to the instrument have been made, and in modern years it has proven to be a successful instrument to use in many different fields of research. The TEM allows for visualization of cells on a level that surpasses the light microscope (Bozzola and Russell, 2006). This makes it key to gaining information that could help with solving questions posed by previous studies, where cellular morphology data is lacking. In fact, Tavares (1985) specifically called for the utilization of electron microscopy in the introduction to her book *Laboulbeniales (Fungi – Ascomycota)*, deeming it “essential.” Although structure pertaining to the genus *Coreomyces* itself is the focus of the study, implications for the fine structure of all Laboulbeniales should be considered. It was not until Thaxter (1896) that the structure of the Laboulbeniales was correctly reported, and more recent investigators have frequently mis-interpreted thallus development in newly reported genera and species (Tavares 1985). Modern TEM, and especially image acquisition,
has advanced dramatically since the few previous studies, and therefore must be considered an extremely powerful instrument to study these minute fungi for the future.

**Overall Purpose of Thesis:** Study a key genus (*Coreomyces*) of the Laboulbeniomycetes (Ascomycota) using light and electron microscopy. These techniques allow for the visualization of the development of the fungus by gaining ultrastructural information. Investigating the terminal cell (perithecium) of the thallus through the technique of ultra-thin serial sectioning, and retrieving anatomical information pertaining to these cells, is a primary objective for study. Further objectives are listed below.

- A secondary objective is to investigate parts of the thallus other than the perithecium with thin sections, to gain fine structure information. Priority areas for consideration include the receptacular, appendiculate cells, the ascospore attachment pad, and details of ascus and ascospore formation.

- A further objective is to interpret the ultrastructural data as to how the fungus performs tasks such as perithecial development and ascospore dispersal. A call for morphological studies came from Shanor (1955), stating at the time of publication only about a dozen of the 1500 described Laboulbeniales species were critically studied through all stages of development.
Literature Review

Corixidae Overview

Before this type of investigation can begin with *Coreomyces*, collection of the infected insect host must be accomplished. An outline of the biology of the host (*Corixidae*) to *Coreomyces* follows. The *Corixidae* are an aquatic family of Hemiptera, usually inhabiting pools, ponds, lakes, and lagoons, and spend the majority of their time on the bottom of these systems (Hungerford, 1948). Feeding strategies of these insects are mostly herbivorous, yet being foragers of the ‘organic ooze’ on the bottom of their habitats, means that animal material cannot be ruled out as some percentage of their overall diet (Hungerford, 1948). This feeding strategy makes Corixids an important part of their ecosystem, being a link in the food chain between organic feeders and the organisms that are predaceous for their food (Applegate and Kieckhefer, 1977). *Corixidae*, like most aquatic Hemiptera, undergo five nymphaal instars in their growth and development, have one to two generations per year, and most species in the genus overwinter as adults (Hungerford, 1948). Males and females can be distinguished from each other by observation of the abdomen, where males have irregular segmentation vs regular segmentation in females (Hungerford, 1948). This can be observed in Figures 1, 4, & 5.

Although Hungerford’s 1948 monograph titled *The Corixidae of the Western Hemisphere* covers this genus extensively, there is no mention of fungal infection by *Coreomyces* in the contents.
Figure 1: “Life History of a Boatman” – Note 13 and 17 being representations of the female and male abdominal sections. (Hungerford, 1948)
Introduction to Thallus Development and Morphology

A main peculiarity of this group of fungi comes from there being a reduced hyphal system, determinate thallus growth, and development patterns that are structurally similar to the Ascomycota (Thaxter, 1896). An overview of a mature specimen will generally contain a receptacle attached at its base by a foot, appendages arising from the receptacle, female reproductive structures arising from the receptacle developing in perithecia, and male reproductive structures that are produced from the receptacle or on the appendages (Shanor, 1955). Shanor (1955) further states that although the overview of a mature individual is relatively simple, there is remarkable variation across the more than 150 genera included in the group.

Thaxter (1896) was the first to describe the development of the ascospores of the Laboulbeniomycetes from initial stages through thallus maturation. Taking into consideration that genera do not all exhibit the same characteristics of development, Thaxter decided upon a more simple form in the genus Stigmatomyces as the overview for the ‘general’ type of development (Thaxter, 1896 pp. 217-234, Pl. I). The terminal region of the septate ascospore was reported to further develop into the antheridial appendage, with the basal end dividing into the foot attachment and female sex organ; the perithecium (Thaxter, 1896). The lower portion of the basal region of the ascospore (foot) is where the ascospore adheres to its host, and further where the receptacle of the fungus develops from (Hulden, 1983). The receptacle grows as a series of determinate cells, varying greatly across the genera of the class (Hulden, 1983). The upper portion of the basal end divides laterally into a cell (α’) that itself further divides, eventually developing into what is referred to as the ‘perithecium proper’ (Thaxter,
During this development, it is observed that from three basal cells (o), four outer wall-cells and four inner parietal cells are formed (Thaxter, 1896). Although division is identical, the two series of cells develop independently, with the outer developing first (Thaxter, 1896). Thaxter (1896) reported that asci bud from ascogenous cells which are then pushed toward the apex of the perithecium, where the ascus walls are degraded. This causes the ascospores to float freely in a cavity of the perithecium, where they are ready for discharge (Thaxter, 1896). A final note by Thaxter pertaining to the development of the perithecium is that although Stigmatomyces can be considered a normal type, it does not represent the development in all cases throughout the Laboulbeniales.

The great variation of the group is not restricted to one specific portion of the thallus. However, for this study the perithecium was of particular interest, as the perithecium is described as almost invariably an outgrowth from the receptacle in the development described above (Weir and Beakes, 1995). Coreomyces, a genus that exhibits perithecium development different than the normal type, will be outlined below.

Coreomyces Background

The genus Coreomyces was first reported and described in 1908 in Volume II of Contribution Towards a Monograph of the Laboulbeniales by Roland Thaxter (Thaxter, 1908). This introduction to the genus uncovered a development of individuals unlike any other reported in the Laboulbeniales, showing an endogenous origin to the perithecium (Thaxter, 1908). Accompanying a written description was a plate with line drawings pertaining to the development of the thallus from young to mature stages (Figure 2). To organize his descriptions, Dr. Thaxter separated the thallus of Coreomyces into three regions: a basal, a
middle, and a distal. The basal region includes the foot attachment as well as the cells basal to the appendiculate cells. The middle region includes the appendiculate cells and their appendages. Perithecial development is contained in the distal region, where the sub basal cell of this region sends two penetrative branches distally that will eventually correspond to the “posterior basal cell” and the “secondary stalk-cell” (Thaxter, 1908). A peculiarity from this type of development is that the true wall-cells are only apparent in the last few tiers at the apex end of the perithecium (Benjamin, 1973). Although stating that further development past these initial stages paralleled an ordinary type of perithecium, Thaxter ultimately decided upon the term “pseudoperithecium” for this structure in its mature state. Conclusions drawn to make this statement seem to have come from Thaxter’s understanding that the perithecium was rather disorganized, and superficially dissimilar to the normal type of perithecium (Thaxter 1908). *Coreomyces* is not mentioned again until Volume V of Thaxter’s *Monograph* (1931), where a note pertaining to the genus states that “There is little to be added to the account of this most curious and anomalous type…”

Tavares (1985) added another note pertaining to *Coreomyces*, with a contradiction to Thaxter’s initial findings. This contradiction comes from the labeling of the perithecium, where it is interpreted by Tavares (1985) as a true perithecium rather than a “pseudoperithecium”. This interpretation comes from the structure being comparable in all respects to the perithecia that develop in other genera, except for their outer wall having its origin preexisting as part of the receptacle (Tavares 1985).

In 2018, Sundberg published what could perhaps be regarded as the most intensive study of the genus *Coreomyces* to date, titled, *Contributions to the understanding of diversity*
and evolution in the genus Coreomyces. This doctoral thesis focused upon the contributions of four respective papers, with various goals in mind. In paper I, Coreomyces was one of four genera used to develop and improve upon existing molecular research (Sundberg, 2018). In this study, it was found that sufficient material for DNA amplification could be acquired from a single thallus. This demonstrates that although often times material is scarce, molecular work can still be performed successfully, and even on an individual level (Sundberg, 2018). Paper II aimed to investigate the degree of host and position specificity in Coreomyces. Previous to this study, there was no molecular work done on the genus Coreomyces (Sundberg, 2018). Findings were in agreement with previous studies where Coreomyces did not show strict specificity to host species (Sundberg, 2018). Furthermore, it was found that none of the four species investigated were restricted to a specific position on the host, although one position was usually preferred (Sundberg, 2018). In paper III, Sundberg and others utilized the methods constructed in paper II to be able to identify four species of Coreomyces, two of which were described as new taxa. Conclusions from identifying different species included recognition of “plasticity” (the variation of morphology with different host positions), making describing new species in this genus a ‘delicate task’ (Sundberg, 2018). Paper IV of Sundberg’s study focused upon the mating type of Coreomyces, and be ability to draw conclusions about homo or heterothallism in the fungus. Expanding upon previously adapted molecular methods, Sundberg was able to draw the conclusion that Coreomyces is a homothallic fungus, or selfing. It is hypothesized that Coreomyces maintained this method of sex due to the advantage of not needing a partner, ensuring that ascospores can be produced regardless (Sundberg, 2018).
Overall, the four papers discussed in Sundberg’s thesis showed much progress was made towards answering many questions pertaining to the genus, but also left some further implications for study, such as exploring the mating types of these fungi in greater depth.

Figure 2: Line drawings of Coreomyces (Thaxter, 1908)
Ascomycetes and Electron Microscopy

Perhaps the most classically studied Ascomycete is *Saccharomyces cerevisiae*, with Lynn and Magee (1970) studying the development of the spore wall during ascospore formation. This yeast holds four ascospores per ascus, and develops ascospores by means of free cell formation (Lynn and Magee, 1970). They observed two-unit membranes that contributed to the inner and outer walls of the ascospores. Furthermore, the inner coat is produced nearly simultaneously with the outer coat. In conclusion, the ascospore wall membranes were hypothesized to develop from the endoplasmic reticulum (Lynn and Magee, 1970).

Historically, the Laboulbeniales have been classified as Ascomycota (Thaxter, 1896). However, there has been some debate if this is the correct grouping (eg Luttrell, 1951). It was not until Hill (1977) performed ultrastructural studies on a member of the Laboulbeniales (*Herpomyces*) that the relationship was solidified between Laboulbeniales and Ascomycetes. Although the Laboulbeniales differ from the majority of Ascomycetes in that there is a lack of a distinct, ramifying hyphal network (Tavares, 1986), it was found that the two parallel each other in spore development, as outlined below in the section *Laboulbeniomycetes and Electron Microscopy (pp. 13)* (Hill, 1977). Before the investigation into Laboulbeniales, Hill (1975) studied the ultrastructure of ascosporogenesis in *Nannizzia gypsea*, a filamentous Ascomycete. During his study, Hill (1975) set out to describe the process commonly referred to as ‘free cell formation’ (Harper, 1899). Utilizing a conventional fixation approach for transmission electron microscopy (TEM), agar bearing cleistothecia were fixed in glutaraldehyde, followed by osmium tetroxide, dehydrated, and then embedded in epoxy resin (Hill, 1975). In conclusion of the study, Hill was able to determine that, “the mechanism of ascosporogenesis in *Nannizzia is
similar to that which occurs in other filamentous ascomycetes.” During investigations into the source of the initial spore membrane, it was found that myelin figures (MF) are the immediate source, rather than endoplasmic reticulum or the nuclear envelope (Hill, 1975).

Furtado and Olive (1970) did an extensive ultrastructural study of ascospore development in *Sordaria fimicola* (*Ascomycota*). This study also utilized conventional fixation for TEM, with sequential glutaraldehyde and osmium tetroxide fixation. During this study, the formation of the outer spore sheath (perispore) was elucidated in detail (Furtado and Olive, 1970). It was found that with adequate wall material, the original investing membrane of the spore detaches, and migrates to the outer wall of the perispore, corresponding to the electron dense line found in electron micrographs (Furtado and Olive, 1970). At maturity, the perispore layer was undulate, and paralleling the gelatinous sheath observed under light microscopy (Furtado and Olive, 1970).

**Laboulbeniomyctetes and Electron Microscopy**

In 1977, Terry Hill investigated the ultrastructure of *Herpomyces*, a common genus in the Laboulbeniales (on cockroaches) in a paper titled, *Ascocarp ultrastructure of Herpomyces sp. (Laboulbeniales) and its phylogenic implications*. Building on previous light microscopy studies (Tavares, 1965 and 1966), Hill was the first to use the TEM to study the ultrastructure of a member of Laboulbeniomyctetes. The goal of the study was to use the ultrastructural information to determine the degree of relationship between the Laboulbeniales and the Ascomycetes versus red algae (Hill, 1977). Utilizing conventional fixation for TEM, Hill was able to acquire micrographs of the ascogenous cells, ascospores, and their maturation through different planes of a mature ascocarp. Due to the TEM having a superior resolution when
compared to previous studies, Hill (1977) provided some conclusions that contradicted previous studies. For example, Benjamin (1973) had previously reported that the ascogogenous cells of the Laboulbeniales float freely in the base of the perithecium with asci growing by budding. However, Hill (1977) was able to demonstrate in his investigation that the ascogogenous cells in *Herpomyces* interacted with both the basal cells as well as with each other, and the division came by centripetal growth not by budding. He was also able to draw the conclusion that, “asci remain attached by common walls and at no time before deliquescence do they become free from their neighbors.” (Hill, 1977). These results also contradict a previous study by Richards and Smith (1955), that reported the release of individual asci. With the aid of ultra-thin microtomy, it was revealed that *Herpomyces* ascospores develop from a process known as free cell formation, which occurs only in the ascomycetes (Harper, 1899). The ascospores of the *Herpomyces* sp. develop free from contact with the plasmalamella of the ascus, leaving unincorporated epiplasm (Hill, 1977). A structured arrangement of two tiers of parietal cells was reported in the neck of the perithecium, with four inner and four outer cells (Hill, 1977). Also reported was a fibrous matrix on the inside of the parietal cells that was theorized to be gelatinous in nature, and to be a possible source of lubrication for ascospore discharge (Hill, 1977). Although ascospores are conspicuously associated in pairs (Benjamin, 1973) Hill reported that during ascospore discharge this pairing is lost and ascospores are released singly due to the small diameter of the ostiole (Hill, 1977). Maturation was also found to be on a gradient, with more mature asci having the tendency to be located more towards the apex of the perithecium (Hill, 1977). With some studies questioning the placement of the Laboulbeniales within the Ascomycetes (eg Luttrell, 1951), Hill (1977) concluded that although their peculiar development
may separate them into a different group, they should not be considered distantly related to the ascomycetes. These conclusions were drawn because *Herpomyces* demonstrated the formation of an ascus vesicle, which has only been reported in euascomycetous fungi (Hill, 1977). Overall, this study was largely impactful and considered to be the first accurate representation of the ultrastructure of the Laboulbeniales.

In 2012, Jessica Gibson investigated various Laboulbeniales using the TEM in her thesis titled, *Ultrastructural observations of the Laboulbeniales*. Gibson had a main focus in the thesis to “develop a protocol applicable to the Laboulbeniales with the primary interests being on sufficient preservation with minimum shrinkage and loss of cellular components...” (Gibson, 2012). Through much trial and error, Gibson was able to find a method of preparation that worked well for the Laboulbeniales. Although some more exotic preparation methods were tested first, Gibson concluded that a more conventional approach would prove to be the best way to preserve the specimens of this group. An example that led to this conclusion was the use of a non-conventional resins such as LR White and LR Gold in Trials 1 and 5. These resins in particular are utilized for immunocytochemistry studies (Newman, 1989) and since there was no primary intention for this type of study, early trials could have benefited from a more conventional approach, as Laboulbeniales material can be thought of as hard to acquire. Sectioning the embedded thalli was also a difficult task, so conclusive results from this study are rather hard to draw. A main positive impact from this study includes laying a foundation for further researchers to have an established starting point at preserving Laboulbeniales. Gibson’s study, along with Terry Hill’s (1977) study, proved to be a good framework for the following investigations into *Coreomyces*, where preservation was achieved with conventional methods.
Materials and Methods

Collection of *Corixidae* and Primary Fixation

In order to observe *Coreomyces*, host insects (*Corixidae*) were collected from a freshwater pond located at Pratt’s Falls County Park, Onondaga County, New York, USA (42°55'51.3"N 75°59'38.3"W). Collection was started in late spring and continued until numbers dropped off in the fall, making the collecting season roughly May through October. For collection, a small sieve was dragged along the bottom edge of the pond at arm’s length, following the perimeter of the pond. *Corixidae* that were collected in the sieve were then transferred to a holding tube with the aid of an insect pooter (aspirator). After collection, the *Corixidae* were transferred to a 20mL scintillation vial and held in pond water. While in the field, using a pipette, pond water was replaced with fixative. The fixative used was prepared 2.5% glutaraldehyde in sodium cacodylate buffer – pH 7.4, (Electron Microscopy Sciences, Hatfield PA). After movement of the *Corixidae* slowed, fresh glutaraldehyde was exchanged into the vial, then left to fix overnight (8-12hr) before scanning the collections the following day.

Scanning *Corixidae* for *Coreomyces* Infections

Fixative was replaced with fresh cacodylate buffer (Electron Microscopy Sciences, Hatfield PA) and rinsed three times, over a period of five minutes per rinse. The collections were then held in fresh buffer, ready to be scanned. Initial scans with a stereoscope indicated presence or absence of *Coreomyces*. Infected individuals were then transferred to a new vial with fresh buffer, and then further prepared for light or electron microscopic studies.
Preparation of Infected Specimens for Light Microscopy Imaging

Infected specimens of *Corixidae* were taken from buffer (post-fixation) and dehydrated through an ethanol series (15-100%), then mounted in glycerol on a glass slide. Specimens were imaged using a Nikon Eclipse E300 microscope, equipped with DIC. Digital images were acquired using a Lumenera Infinity 3 CCD Camera.

Preparation of Infected Specimens for SEM Imaging

Some specimens were collected into 95% ethanol, rather than fixative. These samples were further dehydrated to 100% ethanol, and then dried with tetramethysilane – TMS (Dey et al 1989). Samples were then mounted onto an aluminum stub with carbon paint, and sputter coated with gold/palladium. Imaging was performed using a JEOL JSM IT-100 LV Scanning Electron Microscope operated at 5kV.

Preparation of Infected Specimens for Ultramicrotomy

Infected specimens with *Coreomyces* were dissected in buffer, leaving the infected portion of the insect integument with attached thalli. For this study, infected abdominal sections were analyzed. These abdominal sections went through further preparation for TEM, starting with secondary fixation. This was accomplished by using osmium tetroxide crystals diluted to 1% in dH₂O (Electron Microscopy Sciences, Hatfield PA). This was followed by a five-time dH₂O rinse at five minutes per rinse, followed by a dehydration series through graded ethanol (15-100%). Each exchange was performed for 10 minutes, and three exchanges in 100% EtOH. Samples were then embedded in Epon812 as per manufacturer’s instructions (Electron Microscopy Sciences, Hatfield PA) with the perithecium oriented for both transverse
and longitudinal sectioning. Polymerization was achieved at 60°C for 24 hours. If the thalli were not in the correct orientation after the initial polymerization, the block was trimmed with a rotary tool and re embedded under the same polymerization conditions.

**Ultramicrotomy and TEM Imaging**

Blocks were trimmed to a single thallus, or group of thalli, by hand using a razor blade, and/or utilization of a diamond knife. Sections were then cut with a Diatome diamond knife using a Leica UC6 Ultramicrotome, set at 70nm. Sections retrieved showed a silver color. Quantity of sections cut is digitally counted in the ultramicrotome, and therefore tissue volume could be quantified with ~14 sections equating to 1µm of volume. After confirming initial location of section position with the microscope, specific regions of the thallus could be tracked. Ribbons of serial sections were handled with an eyelash manipulator and separated into fragments of 4-8 sections. Up to two fragments of sections were collected on formvar/carbon coated slot grids (Electron Microscopy Sciences, Hatfield PA), then stained with 2% uranyl acetate (5 minutes) and 2% lead citrate (2 minutes). Sections were rinsed and allowed to dry completely, and then placed in a grid storage box with identification pad to ensure the correct grid was inserted into the microscope during imaging. This study utilized a JEOL JEM 2100F TEM operated at 200kV, equipped with a Gatan OneView 4K CCD camera to capture 4k resolution micrographs with an exposure of 1 second.

**TEM Image Processing**

All TEM micrographs were acquired in full 4096 x 4096 (4K) pixel resolution, and processed with Digital Micrograph (Gatan, USA) and Adobe Photoshop. Any measurements
obtained during analysis were taken in Digital Micrograph, calibrated with the TEM. Images were resized in Photoshop to 8.2 cm\(^2\) with a DPI of 1268, grayscale. Montages were made by overlaying multiple micrographs on a blank canvas, and utilizing opacity changes in Photoshop to align the images.

**Some Notes on Fixation/Processing for TEM**

With there being limited TEM studies on the Laboulbeniales, and with the most important paper dating back some decades (Hill, 1977), fixation would be considered with more current literature, while still recognizing the trials of the past. Fixation, as well as overall processing for TEM, requires the use of harsh chemicals such as aldehydes, osmium tetroxide, and epoxy resins (Bozzola and Russell, 2006). While fixatives retain ultrastructure well, most mycologists do not require this high level of preservation, and therefore collect specimens into ethanol for examination. Furthermore, it is known that ethanol preserves DNA well (Weir and Blackwell, 2001), so studies that involve phylogeny and DNA benefit from ethanol preservation (Goldmann and Weir, 2018). Collecting into ethanol without prior aldehyde fixation will preserve the thalli to an adequate degree for light microscopy studies, however for fine ultrastructural studies a different approach must be considered. Thalli will dehydrate when collected straight into ethanol, causing artifacts on an ultrastructural level. In order to overcome this and to ensure adequate preservation of fine detail, collections of *Corixidae* were killed with glutaraldehyde in the field. This ensured that infected individuals underwent primary fixation prior to their first scan. Rather than scan in ethanol, buffer was the choice. This would allow infected individuals to be continually processed in an efficient workflow for TEM without interruption (see Materials and Methods – Processing Specimens for Ultramicrotomy). Since
the target specimens (thalli) are about the same size as a thick hair, care had to be taken for their orientation while embedding in resin. Samples were first put into flat embedding molds and polymerized with the best attempt at allowing for transverse and longitudinal sectioning. Although polymerization was adequate, most samples moved in the viscous resin, and did not allow for proper sectioning in their first embedment. To solve this issue, excessive epoxy was removed with a Dremel (rotary) tool and small blocks of epoxy containing the thalli were re-embedded with fresh resin, in the correct orientation. During trimming of the blocks, it was found that adhesion of the epoxy resin with the insect was inadequate, and the abdominal segments were falling out of the block. This was probably due to the nature of the exoskeleton of Corixidae, and will probably be true for most Laboulbeniales hosts. For this particular study, where the distal end of the thalli was the main focus, it ended up being an advantage because the thalli themselves were embedding correctly, facedown or across into the epoxy. The removal of the abdominal segment actually made it easy to see and trim the blocks to just one, or a group of thalli. For studies that are interested in the foot attachment/haustoria, a different approach might be considered to aid embedment of the insect itself into the epoxy, rather than just the thalli. Furthermore, if one feels they have a steady enough hand and confident in their embedment skills, thalli may be considered to be detached prior to addition to the embedding wells and embed alone without any part of the insect at all. Sectioning the thalli themselves does not require anything sample specific, but due to their size, block faces usually end up rather small and aid from trimming with a diamond trim tool, if available. This allows for precision down to the micrometer, and allows for sectioning of just one thallus. The thalli themselves are fragile, and often crease and fold during sectioning. Due to this, picking up an
adequate number of sections with ‘extras’ proves a useful tactic, in case there are unseen problems that become evident during imaging. Picking up the sections containing thalli is best done with specialty grids; formvar/carbon coated slot grids. These grids have a 2 x 1 mm wide slot in the middle of a 3mm copper disc that is coated with formvar and carbon. These grids are advantageous because they allow for full examination of the sections without the worry of being blocked by grid bars. Since the section may contain only one single thallus, this one portion of the section is rather valuable, and could prove detrimental if it is continually blocked by grid bars. Furthermore, using grids without any support is rather rough on the sections, and one can usually expect the sections to become slightly damaged via folding or other means during the staining process. This is usually okay for tissue overview studies where the entire section is tissue, but again, with only one potential area of interest in the entire grid, this could lead to a source of information loss during imaging sessions. To eliminate these errors during the section retrieval steps, coated slot grids were used with great success. Grids were stained in the normal fashion of floating section side down on drops of uranyl acetate and lead citrate. Sectioning was done with serial sections in mind, tracking grids and trying not to work with an overwhelming amount of sections during the retrieval steps. This also helps with staining, because large batches of grids tend to have more staining artifacts (precipitate) than smaller batches of 4 or less. For imaging, is not until the sections are under the electron beam during an imaging session that they can be adequately judged for overall preservation on an ultrastructural level. Finally, care should be taken that the fungi are freshly collected into fixative for ultrastructural studies, and that the embedment steps are carefully looked after, as positioning for sectioning is crucial.
Results

Corixidae Collecting and Infection Rates

Collecting *Corixidae* for the study was started in late spring and continued until numbers dropped off in mid fall. Keying *Corixidae* past the family level proves to be a daunting task, even to trained entomologists (Hungerford, 1948). Therefore, the collections were sexed in the 2018 season (Table 2), but identification for this study was not attempted to a species level. *Corixidae* exhibit differences between the sexes in the genitalia as well as the abdomen, where females have regular segments versus irregular segments in the male (Hungerford, 1948). In order to observe the differences between the female and male abdomens, the insects were imaged with SEM (Figures 4 & 5). These images are in line with the sketch drawing as reported in Hungerford 1948 (Figure 1). Infected individuals had a trend to be male in the 2018 collection, although data is lacking to conclusively state that one sex is colonized more often than the other. Furthermore, the abdomen was nearly always was the most obvious and most colonized portion of the insect, agreeing with previous studies that found *Coreomyces* shows some position specificity, with the abdomen as a main locality (Sundberg, 2018). Figure 6 represents an infection that would be considered left ventral when compared with the line drawing given by Sundberg 2018 (Figure 3). Overall totals of *Corixidae* collected, as well as rates of infection for the years of study, are included in Table 1 below.
Table 1: Overview of *Coreomyces* infection rates

<table>
<thead>
<tr>
<th>Year</th>
<th># of non-infected</th>
<th># of infected</th>
<th>Total #</th>
<th>% Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017</td>
<td>580</td>
<td>17</td>
<td>597</td>
<td>2.86</td>
</tr>
<tr>
<td>2018</td>
<td>692</td>
<td>45</td>
<td>737</td>
<td>6.10</td>
</tr>
</tbody>
</table>

Table 2: Overview of Sex-Specific Infection, 2018

<table>
<thead>
<tr>
<th>Year</th>
<th># of females infected</th>
<th># of males infected</th>
<th>Total number of Infections</th>
<th>% of females infected</th>
<th>% of males infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>2018</td>
<td>2</td>
<td>43</td>
<td>45</td>
<td>4.4%</td>
<td>95.6%</td>
</tr>
</tbody>
</table>

Figure 3: Diagram representing the infection positions of *Coreomyces* on its host *Corixidae*. LV = left ventral, RV = right ventral, CV = midventral, LW = left hemelytral margin, and RD = right dorsal (Sundberg 2018)
Figure 4: Female abdominal section showing regular segmentation, ventral view. SEM
Figure 5: Male abdominal section showing irregular segmentation, ventral view. SEM
Figure 6: Overview of corixid abdominally infected with Coreomyces (arrow), ventral view. SEM
Figure 7: Inlay of Figure 6 representing a left ventral infection (Figure 3). SEM
A. Thallus Overview

Figure 8 represents an overview of one mature thallus of *Coreomyces* still attached to an abdominal piece of the integument of its host. This view of the thallus gives some insight to the determinate growth pattern found in the Laboulbeniales (Hulden, 1983). Septations between the cells of the thallus are clearly visualized, typically the thallus is attached by a blackened foot, above this there are three superposed receptacular cells (Cells I, II, and III), a series of small, flattened cells (appendiculate cells) that give rise to branched or unbranched, fertile (intercalary antheridia) or sterile appendages, a fourth receptacular cell (Cell IV), and a single, terminal, perithecium (Figure 8). Note that Cell II also has appendages, but smaller in size when compared to the appendiculate cells themselves (Figure 8). In the chapters that follow, each of these parts of the thallus are investigated in further detail with light microscopy, SEM, and TEM observations. These findings are in parallel with Sundberg 2018, with a line drawing included from that paper below for comparison (Figure 9).
**Figure 8:** Overview of mature *Coreomyces* thallus – DIC microscopy, 100x final magnification. I-IV: Stalk Cell I, II, III, and IV. FA: Foot Attachment. AC: Appendage Cells. A: Appendage. P: Perithecium

**Figure 9:** Line drawing of a young (left) and mature (right) *Coreomyces* thallus (Sundberg, 2018)
While investigating *Coreomyces* with SEM, young stages of thallus development were observed. These stages agree with the line drawings given by Thaxter (1908). Figure 10 is in correspondence with line drawing #9 in Figure 2 from Thaxter, with the receptacular cells becoming individualized and the thallus being terminated by a sterile appendage. Figure 11 represents a stage of development that relates to line drawing #11 in Figure 2, where the thallus has begun to swell, and the terminal appendage has broken off. At this stage of development, the branching of the subbasal cell of the terminal region proliferates distally, however this cannot be visualized by the superficial view from SEM. Figure 12 represents a longitudinal view of the distal end of the young thallus by TEM section. Although the branching of the subbasal cell was not visualized, clear interaction between the septum of these cells was visualized, indicating that these cells act similar to the mature receptacular cells prior to their destruction.

Figure 10: Young stage of *Coreomyces* development. SEM
Figure 11: Young stage of *Coreomyces* development, slightly more mature than Figure 10. SEM

Figure 12: Longitudinal cross section of the distal end of a young thallus. TEM
ii. **Fungal Cell Wall**

Other than the peritheciurn, the fungal cell wall (FCW) was of particular importance for investigation during this study. The fungal cell wall is the outer cell wall of the entire thallus, which can be subdivided into several sections FCW\textsubscript{i, ii, iii} (Figure 13). The fungal cell wall was observed in this study with TEM, through the receptacle cells to the apex end of the perithecium. Part I consisted of the most electron dense area at the very outer edge of the thallus. This extreme outer edge of the thallus is double membranous, and about 30nm wide. The electron dense portion is approximately 150nm wide, and most likely melanin rich, contributing to the color of the fungus. Part II of the fungal cell wall consists of a transition layer between the most electron dense area and the least electron dense layer. In TEM micrographs it is quite literally a “grey area” in color and transition. Part III is the least electron dense, and is about 200-500nm wide. This layer touches the receptacle and appendage cells or in the case at the apex of the perithecium, the outer wall cells of this structure. In some cases, the outer cell wall of the outer wall cell of the perithecium encroaches on this layer of the fungal cell wall (Figure 14). This is the most fluid part of the fungal cell wall in that it follows the invaginating path of the receptacle cell septations. Part I and II are continuous around the entirety of the thallus and do not septate at all from the basal to apex end of the thallus. In all sections examined, the fungal cell wall never appeared to be perforated, was continuous, and deposited in layers that appeared as striations with 30nm spacing in TEM sections (Figure 15).
Figure 13: Outer edge of the perithecium. FCWi: Fungal Cell Wall Layer I. FCWii: Fungal Cell Wall Layer II. FCWiii: Fungal Cell Wall Layer III. C: Cytoplasm. TEM
**Figure 14:** Edge of perithecium showing parietal cell encroaching on FCW$_{iii}$. TEM

**Figure 15:** Striations in the fungal cell wall, 30nm spacing. TEM
B. Basal region of Thallus

i. Foot and Receptacle Cells

In TEM sections all of the receptacle cells (Cells I-IV) resemble each other, and have vacuole-like structures as a large percentage of the overall cross sections. In fixed specimens observed under DIC microscopy, these vacuoles appear as large voids in the cells (Figure 16). Cytoplasm that contains cell organelles such as mitochondria is often the most electron dense area of the cells, and is fluid in its movement through adjacent cell planes (Figures 17 & 18). The vacuoles have differing electron densities as well, with there being a distinction between two types of vacuole constructs, I and II (Figure 18). Often seen in large size is vacuole construct I, which is electron transparent with small fiber like structures scattered throughout its interior (Figure 19). Vacuole construct II is also abundant, more electron dense, and often coated on the circumference with a granular like deposit (Figures 18, 20 & 21). These deposits are variable in size, but a majority can be classified in the size range of 20-50nm (Figures 20 & 21).

After numerous attempts, detailed ultrastructural information on the septum separating adjacent receptacular cells was observed. Theorizing that the interaction might be pore-like, care had to be taken as they would be easily missed in thin sectioning techniques (Figure 22). Figures 23 through 26 represent four serial sections through this septum that divides two receptacular cells, and how it is pore-like in TEM sections. Figure 23 starts in the middle plane of the interaction, which shows that the electron dense portion of the cell wall has become discontinuous. Also observed are Woronin bodies on either side of the pore with small vesicles indicating transport between the cells. Figure 24 is the next consecutive section in the series
and represents a view of diffusion between the two cells as there are multiple vesicles, as well as having the Woronin bodies retracted into the basal end of the pore. Figure 25 continues the series, and although there is an artifact induced during ultramicrotomy, clear ultrastructure can still be visualized. This view starts to show the pore receding back into the receptacular cell, though there is still diffusion of vesicles at this plane. Figure 26 represents the final section in the series. At this plane, the pore does not appear in the cell wall, however there are still some residual vesicles that are potentially diffusing in the ‘Z’ plane towards the pore. Overall, the series represents 350nm of volume in the ‘Z’ direction, the first three are in series, with one section missing between Figure 25 and Figure 26.

Figure 16: Stalk cell showing vacuoles as the majority of its construct. 200x final magnification, DIC microscopy
Figure 17: Cell Overview in the basal region of the perithecium. Area similar to Figure 16. TEM
**Figure 18:** Cell Overview, TEM. C: Cytoplasm Vi: Vacuole construct I. VII: Vacuole construct II. GD: Granular Deposit. M: Mitochondria
**Figure 19:** Vacuole construct I and II. Arrow indicating fibril material in construct I. TEM

**Figure 20:** Granular deposit surrounding vacuole construct II indicated with arrows. TEM

**Figure 21:** High magnification overview of granular deposits. TEM

**Figure 22:** Cell septation overview. TEM
**Figure 23:** Transverse cross section through the middle of a cell septum. WB = Woronin bodies. CW: Cell Wall. TEM

**Figure 24:** Serial section with Figure 23. TEM

**Figure 25:** Serial section with Figure 24, note there is an ultramicrotomy artifact. TEM

**Figure 26:** Final section in series showing no pore. TEM
C. Middle Region of Thallus

i. Appendiculate Cells & Appendages

The flattened appendiculate cells have ultrastructure similar to that of the stalk cells, however in our TEM sections they appeared to be composed of a higher percentage of cytoplasm, resulting in more rigidity when compared with the receptacular cells (Figure 27). This was evident when observing unfixed specimens where these appendiculate cells were usually the best preserved part of the thallus. The appendages of the cells appear to grow from the cell wall in a budding-like fashion. (Figure 28 & 29). The appendages themselves are less electron dense than the rest of the thallus, however they were characterized by an electron dense core (probably relating to their slight amber color under a light microscope) (Figure 30). The appendages are about 2.5um in cross section, with the electron dense core being about 1.5um (Figure 30 & 32). Appendages were also discovered on receptacular cell II (Figure 8). An interesting phenomenon observed around the appendages was the occurrence of a row of circular spherical cells that were approximately 300nm wide when in perfect transverse cross section, and up to 800nm in one direction in oblique sections (Figures 32 & 33).
**Figure 27:** Appendage cells in longitudinal cross section. TEM

**Figure 28:** Initial development of appendage showing budding like growth. TEM

**Figure 29:** Later stage of development compared to Figure 28. TEM
Figure 30: Appendages in longitudinal cross section showing an electron dense core. A = appendage. US = Unknown Spherical Cell. TEM

Figure 31: Spherical cells showing affinity inside an appendage joint. TEM

Figure 32: Spherical cells showing affinity alongside an appendage. TEM

Figure 33: Inlay of Figure 32. TEM
D. Distal Region of Thallus

i. Perithecium

**Figure 34**: Montage of the perithecium of *Coreomyces* in oblique longitudinal cross section, TEM. Bar 5µm

The terminal cell of the thallus is the perithecium, and it is convenient to divide this into three distinct regions (Figure 34). Transverse cross sections were obtained through these different regions, starting with the basal end and working through to the distal end.
The lowermost section is located immediately above Cell IV of the receptacle, and represents the area where perithecial stalk (Cells VI and VII) and basal (m, n, n’) cells are found in other genera of Laboulbeniales. Figure 17 represents a higher magnification view of this basal region of the perithecium, and how it is ultrastructurally similar to the receptacular and appendiculate cells with dense cytoplasm and vacuole construct II comprising a large portion of its contents.

Above the basal region is the middle, and this portion is where the ascogenous cell appears to start division (Figure 35). This initial division seems to occur from the center of the perithecium (Figure 36), with double walled asci developing and surrounding the ascogenous cell. As the inner wall of the ascus develops it encases the surrounding dense cytoplasm (also known as ascoplasm), a pre-requisite for ascospore development (Figure 37). The outer wall of the ascus is thick and continuous, meaning that every ascus is essentially connected to the two adjacent to it. In some of our section’s asci will “share” the outer wall on one side rather than an individual cell wall for each individual ascus (Figure 37). As the ascus cell wall is formed, an electron opaque barrier (about 100nm wide) forms on either side of the outer wall (Figure 38). Observations from developing ascus walls could indicate that cytoplasmic material is being broken down and reallocated as ascus cell wall material. Ascospores develop inside the asci by means of free cell formation (Figure 39) (Harper 1899), with detailed observations of ascospore development included in Section D - ii, Ascospores – Ascospore Overview (pp. 64).

In the middle region of the perithecium, transverse cross sections show asci in different stages of development (Figures 40 – 42). Inside individual asci ascospore maturity is nearly consistent, but different asci in the same plane will have ascospores of different overall
maturity levels indicating that not all asci mature at the same time, or same plane in the perithecium. As the asci develop, the epiplasm is degraded away, leaving only the outer ascus wall and the four mature ascospores (Figure 43). As the sections reach farther towards the apex of the perithecium the asci widen and the ascospores become more spaced out in the asci. Sections in this area of the perithecium correspond to the widest part of the perithecium, before it narrows into distinct outer wall and parietal cells (Figure 44). As the middle region nears towards the distal region of the perithecium, the number of asci in the ‘z’ plane has begun to shrink, and there is evidence that ascus walls start to deliquesce (degrade) resulting in an ‘ascospore cavity’ where the ascospores lie freely within the perithecial venter and neck (Figure 44).

**Figure 35:** Plane in which ascogenous cell starts to individualize and asci begin to develop. AC = ascogenous cell. AS = Ascus. TEM

**Figure 36:** Inlay of Figure 35, showing the ascogenous cell. AC = ascogenous cell. TEM
Figure 37: One maturing ascus. Note that the ascus wall is shared by adjacent cells. AS = Ascus, AW = Ascus wall. TEM

Figure 38: Developed ascus with ascus wall degrading cytoplasm between the wall layers (arrows). TEM

Figure 39: Early stages of free cell formation. AV = Ascus Vesicle, arrow = Enveloping Membrane. TEM

Figure 40: Transverse cross section through asci of differing maturities. TEM
Figure 41: Transverse cross section in a similar plane as Figure 40. TEM
Figure 42: Transverse cross section through a plane in which most asci are mature. TEM
Figure 43: One ascus void of epiplasm containing four mature ascospores. TEM
Figure 44: Plane in which the perithecial cavity starts to form due to ascus deliquescence. TEM
From the previous plane (Figure 44) to the apex of the perithecium is the distal region, and this region exhibits ultrastructure similar to other Laboulbeniales with distinct outer wall cells and inner parietal cells. During the formation of the middle region characterized by the production of asci, neither outer wall cells nor parietal cells are distinct, and the asci appear to be formed against the outer edges of the thallus (Figure 45). The outer wall cells and parietal cells are not apparent, at least as distinct individualized cells, until the distal region of the perithecium, and TEM sections show that the outer wall cells start from one side of the perithecium and develop across a gradient of maturity, with the inner parietal cells developing along a similar gradient, finally encasing the ascospores (Figures 46-49). The transition space between where the outer dense cytoplasm starts to differentiate into outer wall cells and parietal cells and where these cells are in their regular formation has much interaction and development through all planes of the cells. As previously mentioned, the outer wall cells are developed first, followed by the inner parietal cells, making a final arrangement of four outer and four inner cells at maturity (Figure 49). The maturation of both types of cell divide by a membrane that reaches out and grows through the existing cytoplasm, degrading it into an electron opaque barrier on either side of the parietal cell wall (Figure 50 & 51). In sections taken more towards the basal end of outer wall and parietal cell development, these cells cannot quite be distinguished into their 4 + 4 layer, but further divide and interact between themselves and adjacent cells so it looks like there can be 10+ cells in a transverse cross section. Pores can be observed in cross section, an indication that these cells are communicating and sharing cellular components (Figures 52-55). These interactions are not restricted to one cell type, as outer wall cells and parietal cells were observed interacting (Figures 54 & 55). Even at maturity,
both types of cells can subdivide as is evident in the righthand side outer wall cell in Figure 49. These ‘inner divisions’ do not have any different distinction when compared to the walls between the individual cells. Furthermore, as the Z planes become closer to the apex of the perithecium, the parietal cells narrow on the asci, and the overall diameter of the perithecium does not change as dramatically as the inner parietal cells. This begins 40um from the apex of the perithecium, where the outer wall and parietal cells are distinct and arranged (Figure 56). In transverse cross section it is observed that the ascus walls diminish into just one single ‘ascospore cavity’ covering the entire diameter inside the parietal cells. From this plane, and until the apex of the perithecium the parietal cells start to secrete an electron transparent layer thought to be gelatinous in nature, and a lubricant to aid in the dispersal of the ascospores (Figure 57 & 58). As the Z planes begin to near the top 20-30um of the perithecium, the parietal cells continue to narrow on the ascus with a closure of over 5um in diameter in about 10um of height. The overall size of the thallus also begins to shrink at this time, and it starts to take on a more elliptical shape in cross section rather than spherical (Figure 49). Also observed at this plane (30um below apex) is that the parietal cells appear to have more vacuoles in their cytoplasm. About 25um below the apex, the ‘ascospore cavity’ has narrowed to just 10 x 4um and only one ascospore is visible in cross section (Figure 59). As the sections continue towards the apex, the cavity nearly surrounds the entirety of the ascospore, encasing it in the gelatinous secretions previously mentioned (Figure 60). As the perithecium gets ready for ascospore discharge, the inner and outer parietal cells begin to separate and form a cavity at the extreme apex of the perithecium (Figure 61 & 62). Sections taken directly below the apex illustrate that
the outer fungal cell wall begins to rupture, and form an ostiole for ascospore discharge (Figure 63 & 64).

Figure 45: Young ascus developing against the outer edge of the perithecium (arrow). TEM
Figure 46: Start of outer wall cell and inner paretial cell individualization. TEM
Figure 47: Middle point of wall cell and paretial cell individualization. TEM
Figure 48: End of wall cell wall and paretial cell individualization. TEM
**Figure 49:** Plane in perithecium that starts to close down on one spore. TEM

**Figure 50:** Parietal cell septation, indicated with arrows. TEM

**Figure 51:** Parietal cell septation, indicated with arrow. TEM

**Figure 52:** Parietal cell pore interaction. WC = Outer wall cell, WB = Woronin body. TEM
Figure 53: Inlay of Figure 52 showing a high magnification view of the cell interaction. WB = Woronin bodies. TEM

Figure 54: Outer wall cell and parietal cell interaction. WB = Woronin bodies. TEM

Figure 55: Inlay of Figure 54 showing a high magnification view of the cell interaction. WB = Woronin bodies. TEM

Figure 56: Closure of perithecial cavity. TEM
Figure 57: Parietal cell secretion, indicated with arrow. TEM
Figure 58: Parietal cell secretion (arrow) with spore interaction. S = Spore. TEM
Figure 59: Closure of the perithecium to just one spore. TEM
Figure 60: One ascospore fully encased by the parietal cells. PC = Parietal cells, S = Spores. TEM
Figure 61: Oblique section through perithecium in the initial stages of ostiole development. S = Ascospore, PC = Parietal Cell, WC = Outer Wall Cell. TEM

Figure 62: First indication of the Fungal Cell Wall (FCW) rupture, beginning of ostiole. WC = Outer Wall Cell, arrow = FCW rupture. TEM

Figure 63: Oblique cross section showing cell tiers. WC = Outer Wall Cell, PC = Parietal Cell. TEM

Figure 64: Ruptured fungal cell wall, perithecium opening indicating a pathway for spore discharge. WC = Outer Wall Cell, arrow = ostiole. TEM
ii. Ascospores – Ascospore Overview

In keeping with all other known Laboulbeniales, ascospores in *Coreomyces* are elliptical in shape, once septate, taper at each end to a point, with a distinct gelatinous drop at the basal end (Figure 65). These ascospores, when still inside the ascus, appear to be free from contact with the ascus wall (Figures 66 & 68). This is accomplished by the enveloping membrane system, and the process is known as free cell formation, a developmental pattern exclusive to Ascomycota (Harper, 1899). The nucleus encased by the membrane is approximately 1um in size, and not much more electron dense than the surrounding cytoplasm (Figure 67). At this stage, the cytoplasm can be separated into; sporoplasm, or cytoplasm that was surrounded by the investing membrane; and epiplasm, or cytoplasm that remains unbound in the ascus. Under normal conditions, four ascus vesicles are formed (Figure 68), but occasionally atypical development was observed where three normal ascospores formed alongside one with more stunted growth (Figure 69). An ascus with only two, or a single vesicle was also observed, although this appeared to be a very rare condition. As the ascospore matures, the epiplasm directly adjacent to the investing membrane starts to degrade, leaving an electron opaque layer on either side. When the ascus vesicle is fully developed, the sporoplasm starts to become less electron dense when compared with the epiplasm. Shortly after this time, the enveloping membrane system splits, leaving the outer ascospore investing membrane to migrate to the outer portion of the ascospore (Furtado and Olive, 1970). This is the start of the formation of the perispore layer in the ascospore, the electron transparent layer between the two membranes of the ascospore (Figures 69 & 70). The outer wall is very electron dense, while the
wall of a mature ascospore is double membranous, and holds the endospore material which contains cytoplasm and organelles (Figure 71). As the ascospore matures the outer wall develops invaginations that secrete membrane like material through the perispore layer and towards the endospore (Figures 71-74). In transverse section these invaginations appear as two-part flask like structure, with a disc structure positioned on top of a bottom support. The bottom of the two-part structure is 75-90nm wide, with the top part being more variable at 60-100nm at its widest point (Figures 71 – 73). Longitudinal sections reveal how these invaginations appear as vesicle like structures diffusing through the perispore layer (Figure 74).

Figure 75 represents a transverse cross section through one mature ascospore.

![Figure 65: Overview of one ascospore still hanging from the perithecium. AD = Adhesion Drop, arrow = septation. DIC microscopy](image)
**Figure 66:** First stages of ascus vesicle formation. Note that the enveloping membrane does not touch the ascus wall. AV = Ascus Vesicle, arrow = Enveloping Membrane. TEM

**Figure 67:** Inlay of Figure 66 showing the electron dense nucleus enclosed by the enveloping membrane. N = Nucleus, EM = Enveloping Membrane. TEM

**Figure 68:** Ascus Vesicles. TEM

**Figure 69:** Three maturing ascus vesicles with one showing stunted growth. AV = Ascus Vesicle, arrow = stunted vesicle. TEM
Figure 70: Mature ascus still with epiplasm, containing four mature ascospores. $S =$ Ascospores. TEM

Figure 71: Ascospore edge showing invaginating pore depositing vesicle like structures inside the ascospore. $P =$ Pore, Black arrow = vesicle secretion, White arrow = plasmalamella of the ascospore. TEM

Figure 72: Interaction zone between two spores with multiple pores. $P =$ Pore. TEM

Figure 73: Overview of spore edge with vesicle like secretions from the pores. $S =$ Ascospore, $P =$ Pore, arrow = vesicle like secretions. TEM
Figure 74: Longitudinal section through ascospore showing small vesicles (arrows). TEM

Figure 75: Overview of a mature spore still bound by an ascus. G = Golgi apparatus. VE = Vesicles. AW = Ascus wall. Arrow = pore in spore wall. TEM

iii. Ascospores – Ascospore Development in Chronological Order

The following 12 micrograph series (Figures 76-87) represents the gradual maturation of ascospores from the early stages of free cell formation, all the way through to full maturity. Figure 76 represents the stage right before the enveloping membrane system surrounds and encases nuclei and cytoplasm, after the first membrane is deposited, and there are clear ascus vesicles (Figure 77). Cytoplasm directly adjacent to either side of the enveloping membrane is degraded and becomes electron opaque (Figures 78 & 79). This degradation continues as the ascospore develops, and the outer layer matures into the perispore layer (Figures 79 – 82). The ascospore is considered mature when the initial membrane detaches from the inner portion of the ascospore and becomes the electron dense outer cell wall (Figures 80-82). From this point
on, the epiplasm that is left in the ascus is degraded, leaving the ascospores to widen in the ascus (Figures 83 & 84). After the epiplasm is completely degraded (Figures 85 & 86), the mature asci undergo ascus deliquescence and the mature ascospores share a space in what can be referred to as a perithecial cavity before they are discharged (Figure 87).

Figure 76: Developed ascus without ascus vesicles. AP = Ascoplasm, AW = Ascus Wall. TEM
Figure 77: Initial stage of free cell formation showing enveloping membrane system enclosing ascus vesicles. AV = Ascus Vesicle, EM = Enveloping Membrane System, N = Nucleus. TEM
Figure 78: Four ascus vesicles start to degrade the ascoplasms directly adjacent to the outer enveloping membrane. SP = Sporoplasms, AP = Ascoplasms. TEM

Figure 79: Investing membrane is nearly separated from the inner wall. TEM

Figure 80: Investing membrane has migrated to the outer wall, developing the perispore. Overall, the spore is close to maturity. P = Perispore. TEM

Figure 81: Four mature spores in an ascus. TEM
Figure 82: Similar view to Figure 81. Mature ascospores still surrounded by epiplasm. TEM
Figure 83: Epiplasm is beginning to degrade away, and the ascospores start to spread apart. TEM
Figure 84: Epiplasm is nearly completely degraded and the ascospores have migrated away from each other. TEM
Figure 85: Mature spores within an ascus void of any epiplasm. TEM
Figure 86: Ascospores fully matured in an ascus that will soon deliquesce. AW = Ascus Wall. TEM

Figure 87: Ascospores not bound by asci, free floating in the perithecial cavity. TEM

iv. Ascospores – Ascospore Release and Adhesion

Ascospores are apparently released from the apex of the perithecium through the ostiole one at a time (Figure 88). The ascospore is oriented in the perithecium with its basal portion pointing towards the perithecial apex (Figure 88), as this is the end that contains the adhesion drop to help attachment of the ascospore to its host. Figure 89 represents a longitudinal TEM section that reveals how the adhesion drop surrounds the basal end of the tapered ascospore. Figures 90 – 93 represent a transverse view through the adhesion drop, and how the ascospore core tapers until it finally becomes invisible in the section (Figure 92). The drop is composed of a mesh-like fiber network (Figure 93).
**Figure 88:** Ascospore adhesion drop, DIC microscopy

**Figure 89:** Longitudinal section through the adhesion pad showing the protruding basal end. AD = Adhesion drop, SC = Spore cytoplasm. TEM

**Figure 90:** Transverse section through an adhesion drop. SC = Spore cytoplasm. TEM

**Figure 91:** Spore tip close to becoming invisible in adhesion drop cross section, TEM
**Figure 92:** Section through the adhesion drop where the spore tip is invisible. TEM

**Figure 93:** Spore adhesion drop showing a mesh-like structure. TEM
Discussion

Corixidae Infection Rates

The total number of collected individuals of Corixidae over the 2-year sampling period was 1272 and the total number infected with Coreomyces was 62 (4.9%). Roughly equal numbers of corixids were collected in each year of the survey, although the infection rate was much higher during the 2018 season. This higher percentage of infection in 2018 can be attributed to one collection in that year where a ‘bloom’ type of infection was observed. During this collection, 28 of the 45 (62.2%) infected individuals for the 2018 season were collected. Since the ecology of the infection was a minor objective of the thesis, investigations into the ‘bloom’ were not conducted, as this collection gave more than adequate material for ultrastructural study. The overall infection rate compares favorably with other large datasets derived from studies across a range of insect hosts (Jensen et al, 2019 [2.3-3.8%]; Weir and Hammond, 1997[6.1%]) and suggests that, at least in north temperate Corixidae, infection with Coreomyces appears to be quite common. This is somewhat in contrast to previous reports that sufficient material has been the limiting factor for many studies (Shanor, 1955).

Basal region

Fungal Cell Wall

TEM sections of the fungal cell wall of Coreomyces are the first for any member of the Laboulbeniales and show a tri-layered structure bounded by a double-membrane, enclosing an electron dense region that is likely melanin-rich, a transitional zone, and an inner region that is sometimes absent, or hard to see. There were no perforations seen in the continuous fungal cell wall in any of the sections examined. This could suggest that there is likely no diffusion across the outer wall of the thallus, and that the nutrition required by the fungus for normal
growth, development, and reproduction, is obtained directly from the foot cell that adheres to the integument of the host. This foot cell is usually heavily melanized in Laboulbeniales and has been observed in some species (e.g., *Hesperomyces virescens*) to produce an internally ramifying haustorial apparatus (Weir and Beakes, 1996), that likely derives nutrition directly from the host. This study did not focus on the ultrastructure of the host-parasite interface, but our observations on the fungal cell wall provide additional evidence for a major nutritional pathway involving the foot cell. This is an important observation because there has been much debate over the years as to whether members of the Laboulbeniales actually derive nutrition from their host, and should be considered true parasites, with a corresponding negative effect on host fitness, or not.

**Receptacular Cells**

Cells I-IV of the receptacle in TEM sections consist primarily of vacuoles that possibly function as nutrient reservoirs for the relatively large energy demands needed by processes happening at the apex of the fungus (Figs. 6-10). We also observed electron-dense vacuoles in these cells that were often coated on the circumference with a granular-like substance. This type of vacuole is theorized to consist of glycogen (polysaccharide) deposits on the edge of glucose vacuoles. Because corixids are aquatic hosts, this fungus may also be considered to be “aquatic” as well, as it spends the majority of its lifetime submerged and in low oxygen conditions. Perhaps in an attempt to compensate for these low oxygen conditions, *Coreomyces* may have evolved pathways to utilize glycogen, that can be broken down anaerobically. However, investigations using immuno-labeling would have to be done to draw specific conclusions about these statements.
This study is the first to show the ultrastructural detail of the septum separating adjacent receptacular cells, and these were only obtained after numerous sectioning attempts. The septal pores observed are estimated to be around ~700nm wide in diameter. These pores can be easily missed or misinterpreted if serial sections are not obtained and examined. Further investigations across the entirety of one septum would confirm whether or not there is a single, centrally located pore, or a sieve-like arrangement involving multiple small pores. Although small, the observed pore appears to be of a size that would allow cell organelles (except nuclei) to pass from one cell to the next. We also observed electron dense Woronin bodies located close to these septal pores. Woronin bodies are thought to be largely proteinaceous (Markham and Collinge, 1987) and are known only to occur in filamentous ascomycete fungi (including some of their asexual stages). This is the second observation of Woronin bodies in Laboulbeniales, with the first coming by Hill (1977). A main objective Hill’s study was to draw conclusions about Laboulbeniales phylogeny, and observation of Woronin bodies in both studies is a major line of evidence in support of their ascomycetous affinities.

Middle region

The flattened series of appendiculate cells represented the best preserved part of the thallus in unfixed specimens, and they appeared to be composed of a higher percentage of dense cytoplasm compared to the receptacle cells, perhaps resulting in greater rigidity. Appendages arising directly from the outer walls of the appendiculate cells appeared to grow in a budding fashion. One of the most interesting observations from this region was the occurrence of a row of circular spherical cells that were approximately 300nm wide in diameter.
when in perfect transverse cross section, and up to 800nm in one direction in oblique sections (Figures 31-33). Given the habits of the host corixid, feeding in the organic ooze in freshwater systems, these structures could represent a community of bacteria that are associated with (but do not adhere to) the appendages. Another, perhaps less likely, possibility is that these structures might be attributable to spermatia. This seems less likely because we did not observe antheridia in the thalli of Coreomyces that we examined, and these structures appeared to be in an ordered row, rather than haphazardly distributed. This is of particular interest, as when one is familiar with the conventional processing techniques for TEM including the many fluid exchanges, the apparent affinity and order around the appendages without touching them is a peculiar feat. Further work on the distribution, occurrence, and identity of these structures would be very informative.

Distal Region

Perithecium

One of the major foci of this research was to elucidate the morphology and development of the terminal cell of the thallus, the perithecium. Since their first description more than 100 years ago, species of Coreomyces have caused continuous debate regarding the precise nature of the perithecium, and how this develops in an endogenous way inside the upper receptacle cell. In this study we split the perithecium into three distinct regions and equate the lowermost section (about 1/3 of total length of mature perithecium) with the development of perithecial stalk (VI, and VII) and basal (m, n, n’) cells. Ultrastructurally, this region comprises dense cytoplasm and vacuole construct II and appears similar to receptacle
and appendiculate cells. The middle third of the distal region represents an area where asci are continually produced by ascogenous cells. These asci mature in sequence, gradually broaden, and rise (or are pushed) upward in the perithecium. Ascospores develop inside the perithecium by a process called free cell formation. Eventually, the wall of the ascus breaks down which frees the ascospores into a central cavity. At this point, the single septate nature of the ascospores can clearly be seen, with the slightly larger of the two cells oriented upwards in the perithecium. Outer wall cells and parietal cells are not distinct in this region. The upper third of the perithecium is characterized by the formation of distinct outer wall cells and inner parietal cells in a 4+4 arrangement. Observations indicate that the outer wall cells develop first, followed by inner parietal cells. Details of the division of parietal cells were observed, along with some indication of cell proliferation, especially in the sections taken at the basal end of the upper or distal region. The 4+4 arrangement is well known in light microscopy studies of other genera of Laboulbeniales, but continued cell proliferation in this region of the perithecium has not been encountered before. Towards the apex of the perithecium, the ascospore cavity narrows to little more than the width of a single ascospore. In this plane the parietal cells appear to produce an electron transparent layer, thought to be gelatinous in nature. Eventually, the fungal cell wall ruptures, forming an ostiole for ascospore discharge. The perithecial apex in *Coreomyces* seems to lack the terminal apparatus found in some other genera (eg *Hesperomyces* – Weir and Beakes, 1995) that are thought to function in ascospore release. Our sections show a coarse rupture possibly caused by the build-up of internal turgor pressure from the accumulating secretion from the parietal cells.
**Ascospores**

The ascospores of *Coreomyces* are similar to others across the group, but do exhibit structure that has not been reported upon previously. At maturity, the outer wall of the ascospore exhibits invaginations that have been interpreted as pores transporting nutrients into the ascospores. Many different orientations and stages of this pore were observed during the study, and with this it seems that the pore can be sub-divided into a two-part flask; a top portion that buds from the bottom portion after becoming an adequate size. Membrane like material also appears to be able to move through the spore via this pore, as this is often observed near the invaginations (Figures 71 & 73). Upon an extensive literature search, it was found that a similar structure has not been reported before in ascospores. Ornamented spores have been reported, yet these structures appear as outgrowths from the outer spore wall rather than invaginations.

**Ascospore Release and Adhesion**

Many fungi produce adhesive appendages or sheaths to aid with attachment to their substrate (host). This is especially true of marine and freshwater fungi (Jones 2006). This study reveals that *Coreomyces* does indeed have an adhesion pad to aid its attachment to a host corixid. This pad parallels the description given by Hyde and Goh (2003) when defining the six types of freshwater ascospore appendages, with the statement, “1. Release of a drop of mucilage from ascospores.” Ascospores appear to be released from the apex of the perithecium through the ostiole one at a time (Figure 88). It must be assumed to be deposited while submerged in the water, due to the host habitat. The ascospore releases out of the perithecium...
with its basal portion first (Figure 88), as this is the end that contains the adhesion pad. Since this adhesion pad is assumed to be submerged in water, the outer layer that touches the water must be hydrophobic, and have a hydrophilic center. It could be assumed from this observation that the spore adhesion pad is composed of a bi-lipid membrane.

Hypothesis for Ascospore Dispersal

This study of Coreomyces has shown that asci develop sequentially in the perithecium and that, at maturity, the ascus deliquesces and ascospores are released into the perithecial cavity. Towards the apex of the perithecium, the perithecial cavity becomes a narrow channel as a result of parietal cell growth. This channel is just wider than a single ascospore. Eventually, the perithecial apex ruptures, and an ascospore is exuded, hopefully onto its required host. How the timing of this event is controlled is still a mystery, but Goldmann and Weir (2012) showed that in Chitonomyces, ascospore discharge is affected by pressure on the perithecium resulting from precise copulatory positions of the host beetles. An interesting observation paralleling spore discharge due to pressure on the perithecium did come during investigations into Coreomyces, although no video photography of living Corixidae was recorded. Spore discharge was observed in a mature thallus of Coreomyces during light microscope investigations. When cleaning immersion oil from the coverslip, an ascospore was found to be partially discharged through the ostiole after re-examination (Figure 88). Implications from this observation that pressure is a factor in spore release might well be an interesting future research angle.
Conclusions

Once Coreomyces was decided upon as the genus of focus for this study, finding their host corixid was the initial task. Without much effort or guidance on locality, infected individuals were successfully collected during the initial year of study. With this, it seems that infection of Corixidae with thalli of Coreomyces is of common occurrence in the central New York, USA, region. From these infections, detailed ultrastructural studies of the overall thallus (with a focus on perithecial development) in Coreomyces are presented and related to ascospore maturation and dispersal biology. Utilizing a state-of-the-art TEM was a crucial part of this study, as digital 4k resolution micrographs have never been utilized to report detailed ultrastructure of the Laboulbeniales. With help from the TEM, some previous observations were solidified, others found to be misinterpreted, and new observations all together reported to the group Laboulbeniales. TEM studies elucidate the ultrastructure of multiple different portions of the thallus of Coreomyces for the first time, including; the Fungal Cell Wall (FCW), spore adhesion pad, and spore pores. Further compounding on previous studies (Hill, 1977), Woronin bodies in the septal pore were also reported in this study.

The skillset of ultramicrotomy, and more specifically serial sectioning, proved to be extremely useful during this study. With the potential for volume reconstruction, image series from serial sections can elucidate a more three-dimensional visualization of the specimen in question when compared with just a single two-dimensional image. For this study, serial sections were cut and tracked, and therefore was able to elucidate processes that happen in small volumes. For example, the pore in the septations between cells appear to be less than
one micron in width, and therefore equate to less than ten sections of volume. Without serial sections being analyzed, this and other small structures could be continually missed. Overall, serial sectioning helped to elucidate cell interactions, free cell formation, as well as overall perithecium development.

Further pathways of study for Coreomyces include a more detailed approach to the study of the ecology of their host interaction, as well as further ultrastructural studies. Although some conclusions can be made upon things like infection rates and sex of the host, the data set for collecting is ultimately inadequate to have conclusive statistical proof. Although most of the ultrastructure of the thallus was covered in this study, there were parts that were focused upon more than others, such as the perithecium. Further studies on ultrastructure could include the appendages, and the foot attachment. Sundberg (2018) laid a solid framework for the background ecology of Coreomyces, and coupled with ultrastructural data currently presented, further ecological and ultrastructural implications could help the once inscrutable genus become one of the most critically studied genera of the group.

Investigating Coreomyces with the TEM was a study that not only gained insight into the investigation of the Laboulbeniomycetes, but also helped to gain skills necessary to set up efficient TEM studies in the future. Although having had previous experience with the microscope itself, this was the first study that was conducted to this scale. Section tracking, artefact troubleshooting, efficient image collection and data processing, and data organization are examples of many skills that were improved upon during this study.


Appendices

During my investigations with *Coreomyces*, sufficient material for study was not of concern, as I was successful in harvesting the fungus in both years of study. Due to this, many specimens were prepared for TEM, which allowed for the development of a reproducible workflow for processing, sectioning, and imaging. Nearly 200 grids amassing about 1000 individual sections were examined for this study; resulting in over 1000 micrographs being acquired. Realizing that this amount of data is impractical for presenting in a thesis dissertation, I would like to include supplemental micrographs from the study that have not previously been shown in the 93 figures of the body of the thesis. Brief descriptions and figure legends accompany each micrograph.
Appendix 1: This view of a mature ascospore shows clear organelles as well as pore interaction. G = Golgi apparatus, arrow = pore. TEM
Appendix 2: This longitudinal section through two ascospores shows pore interaction along the entire length of the ascospore. TEM
Appendix 3: Longitudinal cross section towards the apex of the perithegium as it gets ready for ascospore discharge. The tips of the parietal cells are pushing through the outer wall cells. PC = Parietal Cell, WC = Outer Wall Cell. TEM
Appendix 4: Transverse cross section through a plane in which the parietal cells have fully enclosed one ascospore and secreted a gelatinous substance to surround it. S = Spore, PC = Parietal Cell. TEM
Appendix 5: Longitudinal cross section through a mature spore showing the spore septation. Vesicles are also visualized in the perispore layer. N = Nucleus. TEM
Appendix 6: Longitudinal cross section through an appendage with developed mitochondria. 
M = Mitochondria
Appendix 7: Oblique section through a portion of the distal region of a Coreomyces thallus. Arrows could indicate cell proliferation (such as outer wall cells) and be an indication of potential perithecial development. Further investigation and interpretation must be accomplished. TEM
Appendix 8: Serial section with Appendix 7, with a section farther towards the core of the thallus in the ‘z’ direction versus that section. Arrows indicate potential outer wall cell proliferation, and the potential that the inner parietal cells are not developed as the topmost portion of this section returns to a more normal ultrastructure when compared to receptacular cells. TEM
Appendix 9: Overview Montage of a mature Coreomyces thallus. TEM
Appendix 10: Overview montage of perithecial closure upon one ascospore. TEM
Resume

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Education:

August 2010 - May 2013  SUNY Finger Lakes Community College (SUNY-FLCC):
                        Canandaigua, NY
                        • Associate of Science (A.S) – Environmental Studies

August 2013 - May 2016  SUNY College of Environmental Science and Forestry (SUNY-ESF):
                        Syracuse, NY
                        • Bachelor of Science (B.S) – Environmental Biology with a
                          minor in Microscopy
                        • 3.388 Cumulative GPA (magna cum laude)

January 2017 - present  SUNY-ESF: Syracuse, NY
                        • Master of Science (M.S) – Environmental Biology with a focus
                          in mycology – Degree in Progress
                        • Thesis relying heavily on TEM work
                        • Title: Ultrastructural studies of a key genera of
                          Laboulbeniomycetes, Coreomyces (Ascomycota)
                        • Defense scheduled April 2020

Awards/Scholarships:

May 2018  Lowe Wilcox and Zabel Scholarship. Amount: $4100. This award
          funded necessary supplies for a TEM focused thesis. It also funded a trip
          to the annual conference held by the Microscopy Society of America
          (MSA), Microscopy and Microanalysis.

May 2019  Lowe Wilcox and Zabel Scholarship. Amount: $1200. This award
          funded the supplies necessary to complete thesis related TEM work.

Skills:

Electron Microscopy  ~3 years of electron microscopy (EM) experience with both transmission
                      and scanning EM. Knowledge of operation, as well as maintenance and
                      upkeep. Completion of a scanning EM course at JEOL headquarters.
                      TEM skills include brightfield imaging, STEM, and EDS analysis.
Electron Microscopy Preparation

~ 2 years of sample preparation experience with a biological focus.
Skills include: Chemical Fixation, Resin Embedding, Ultramicrotomy, Contrast Staining, Critical Point Dryer.
Completion of a short-course titled: Cryo-Preparation of Biological Samples, offered by the Microscopy Society of America

Related Work Experience:

SUNY-ESF
August 2017 – May 2018
Position: Graduate Assistant (GA) for SEM and TEM
Responsibilities: Management of the laboratory sections for the electron microscopy courses at SUNY-ESF. Duties include training students for SEM and TEM operation, maintenance of the instruments and labs, as well as aiding in sample preparation.

August 2018 – May 2019

SUNY-ESF (A&TS)
May – August 2018
Position: Electron Microscopy Technician
Responsibilities: Day to day management of SEM/TEM including sample preparation, imaging, instrument and lab maintenance, and scheduling imaging sessions with outside groups.

May 2019 – February 2020

SUNY-Upstate Medical
June 2019 – December 2019
Position: Lab Research Assistant
Responsibilities: Performing immunogold labeling techniques on cultured cells with the target protein being V-ATPase. Work is performed in the lab of Stephan Wilkens, PhD.

Ichor Therapeutics
Feb 2020 – Present
Position: Laboratory Technician – Biochemistry/Electron Microscopy
Responsibilities: Performing lab tasks related to cell transformation, growth, harvest, protein purification, and protein classification. Further duties include managing an Electron Microscope core facility at SUNY-ESF with a focus on cryo-EM for protein characterization.

References

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