MUSHROOM-INSECT INTERACTIONS

THE ECOLOGY AND NETWORK STRUCTURE OF MUSHROOM-INSECT INTERACTIONS FROM TWO FOREST REGIONS IN SOUTHERN ONTARIO

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Lay abstract

Mushrooms and insects are both highly important organisms within ecosystems around the world. Mushrooms play key roles in breaking down organic matter within forests and contributing to plant health, while insects are important decomposers and pollinators. This project involved collecting over 1,000 mushrooms from two regions in Southern Ontario and identifying the insect species found living within these mushrooms in order to examine patterns in the interactions. To our knowledge, this is the first time a survey of mushroom-insect associations has been conducted in Canada. The collected mushrooms were inhabited by a diverse range of insect species, which included mostly flies and beetles, and each of the main sampling locations had their own unique mushroom and insect communities. Some insect species displayed preference in their choice of mushroom host. Overall, this study contributes greatly to our current understanding of mushroom and insect diversity in this part of Canada.

Abstract

Mushrooms and insects are both integral components of ecosystems worldwide. Interactions between these two groups of organisms are particularly interesting to study due to the transient nature of mushroom fruiting bodies, which form for a short period of time when environmental conditions are optimal to allow the fungus to distribute its spores. Despite this unpredictability in where and when mushrooms will grow, a variety of insect species use mushrooms as a food source and a substrate on which to lay their eggs. Interactions between these two groups of organisms have been documented extensively in Europe. However, little is known about the diversity of insects that associate with mushrooms in Canada. In this study, 1,017 mushrooms were collected from forests in Hamilton and the Tillsonburg, Ontario regions between fall 2018 and fall 2019. Mushrooms and their associated insects were identified through DNA barcoding using sequences of the nuclear ITS region and the mitochondrial CO1 gene for the mushrooms and insects, respectively. In total, more than 100 insect species from at least 35 families and five orders were identified from the approximately 200 mushroom species collected. While some insect species displayed evidence of specificity in their choice of mushroom host, the larger network of associations was moderately generalized and many insect species inhabited mushrooms from multiple families and orders. This study highlights the incredible diversity of organisms that rely on mushrooms for survival and contributes to our overall understanding of mushroom-insect associations in this region of Southern Ontario.

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I would like to thank my supervisor, Dr. Jianping Xu, for providing me with the opportunity to gain my very first research experience as a fourth-year undergraduate student in his lab three years ago. It was through this experience that I not only realized I am passionate about ecology and evolution, but that mushrooms are fascinating organisms. I am grateful for the support and guidance that I received throughout my master's project. I learned a lot and gained many valuable skills that I will apply in the future as I start my Ph.D. studies. Thank you as well to Dr. Chad Harvey and Dr. Brian Golding for providing feedback during my committee meetings and to Dr. Susan Dudley for serving on the defence committee.

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Declaration of Academic Achievement

I (Sarah Sandor) conducted the majority of the work associated with this thesis including the mushroom and insect sample collection, lab work, data analysis and writing of the thesis. My supervisor, Dr. Jianping Xu, helped with the design of the study, assisted with some of the mushroom sample collection and provided guidance about the data analysis. Two McMaster undergraduate volunteers (Annamaria Dobrin and Jonathan Ma) and a visiting scientist to our lab (Li Ya) assisted with some of the lab work. Dr. Yang-Yang Cui, from the Kunming Institute of Botany, Chinese Academy of Sciences, China, assisted with some of the mushroom identifications.

1. Introduction

Fungi and insects are both integral components of ecosystems worldwide. As decomposers, fungi play a key role in breaking down organic matter and cycling nutrients. Many plant and animal species also rely on interactions with fungi for survival. Insects contribute equally important ecosystem services as decomposers, pollinators and a food source for many other animals. Given the ecological significance of both fungi and insects, mycologists and entomologists have long been fascinated by interactions between these two groups of organisms.

1.1. Classic examples of fungus-insect interactions

Fungi-insect interactions range from mutualism to parasitism[1] and some interactions are particularly well-studied. For example, fungus-farming termites form a mutualistic association with *Termitomyces* mushrooms, which they cultivate for food. The termites grow mushrooms within specialized structures of their nest and the fungus, in return, is able to propagate and thrive[2]. Ants and ambrosia beetles both engage in similar mutualistic interactions with other fungal species and the larvae of these insects rely on consuming the fungi in order to complete their lifecycle[3]. These mutualistic interactions evolved independently in termites, ants and beetles around 40-60 million years ago[3]. Insects also serve as a vector for many fungal species by distributing their spores. Yeasts, for example, emit volatile organic compounds to attract flies, which will consume the single-celled fungi. As the flies migrate to new locations, they excrete the fungal spores, allowing

the yeasts to colonize new locations[4,5]. The plant endophyte *Epichloe typhina* also relies on insects for propagation. The symbiont fly species *Phorbia phrenione* lays its eggs in the fungus, which is a source of food for the larvae. As the fly travels between grass stalks, it transports the spermatia of the fungus along with it, facilitating mating between individuals of the opposite mating type that are growing on different stalks[6]. These are just a few examples of mutualistic interactions that have been documented between fungi and insects. The interactions follow a similar pattern: insects consume fungi and in the process, allow the fungus to propagate and thrive.

Some fungi are parasites and more than 1,000 fungal species are known to infect insects[7]. For example, fungi from the genus *Ophiocordyceps* are common insect parasites that can infect caterpillars (e.g. the fungus *Ophiocordyceps sinensis*) and carpenter ants (*Ophiocordyceps unilateralis*). Upon infection, these fungi alter the behaviour of their insect hosts and cause them to move either out of the soil or into vegetation. The fruiting body of the fungus bursts out of the head or body of the insects, which allows the fungus to distribute their spores while killing the insect in the process[7]. There are also many pathogenic molds, such as species from the well-studied *Beauveria* genus that infect over 700 different insect species around the world[8].

1.2. Mushrooms: A brief overview

Mushrooms are reproductive structures that are produced by some groups of fungi as a way to distribute their spores. The bulk of the fungus actually exists beneath the soil as a network of mycelia, which are thin, thread-like structures[9]. Broadly, there are two main types of mushrooms: saprophytic mushrooms, which feed on and break down dead organic

matter, and ectomycorrhizal mushrooms, which form a symbiotic relationship with many plant species by intertwining their mycelia with the roots of the plant hosts[9]. The plants provide these ectomycorrhizal fungi with carbohydrates while the mycelia help the plant absorb nutrients and minerals from the soil[9]. Mushrooms are short-lived, with many lasting for less than a week, and these fruiting bodies will only form when environmental conditions are optimal[9]. Despite the unpredictability and the ephemeral properties of mushrooms, many intricate and fascinating interactions have been documented between mushrooms and insects.

1.3. Interesting mushroom-insect associations

Orchid species, such as *Gastrodia pubilabiata,* rely on the association between *Mycena* mushrooms and flies to propagate. The pollen and nectar produced by these flowers are not consumed by *Drosophila* pollinators and *Drosophila* larvae cannot survive on the tissue of this plant. However, these flies are highly attracted to decaying *Mycena* mushrooms, which are often found in close proximity to these orchids, as they provide nutrients to the flower. When flies visit the mushrooms to oviposit, they will occasionally lay their eggs on the orchid instead and as they fly to new locations, they will help pollinate the flower[10].

Insects are also affected by the compounds produced by some mushrooms. For example, mushroom species from several families produce psilocybin as a secondary metabolite. Many of these mushrooms colonize dead wood in environments where insects are abundant. It has been suggested that psilocybin production is ecologically beneficial to the mushroom because once consumed by any insects, it may inhibit further feeding[11]. As

another example, honeybees are known to feed on the mycelia of certain mushroom species. Extracts from polypore mushrooms contain compounds that have anti-viral properties against deformed wing virus and Lake Sinai virus, two viruses that are widely infecting honeybees across North America. It is therefore possible that honeybees consume some mushrooms as a form of self-medication[12]. Overall, these three examples highlight the diversity of mushroom-insect associations that have previously been studied.

1.4. Mushroom-insect associations more generally

Flies (order Diptera) are common inhabitants of mushrooms. Globally, there are more than 4,500 known species of fungus gnats (superfamily Sciaroidea)[13]. Other fly species are commonly found in association with mushrooms as well[14]. Most flies use fungi as a source of food[14,15], a place to breed[16] and a substrate in which to lay their eggs[17,18]. Adult flies will typically oviposit within the stipe or cap of the mushroom[19] and the larvae will feed on the mushroom tissue. However, some mycologists have found larvae within mushrooms on the first day that the full fruiting body appears, suggesting that flies may lay their eggs within developing mushrooms before they are mature[15]. Due to the short-lived nature of many mushroom species, flies that complete the larval stage within mushrooms generally have short lifecycles[13], while some larvae will leave the fruiting body and pupate in the soil nearby[20,21]. Mushrooms are generally not seriously harmed by the insects that feed and live inside them, since the fruiting bodies are short-lived anyway. In addition, when flies migrate to new locations, this allows the mushroom to distribute its spores[14].

Dozens of surveys over the past 70-80 years have recorded interactions between thousands of mushroom and Diptera species from across Europe[14,22-24]. Interactions

between mushrooms and fruit flies (genus *Drosophila*) were also the focus of multiple studies conducted in the late 20th century in New York, USA, that investigated topics such as larval competition within fruiting bodies[15,16], host preference[17], as well as niche breadth[18] and adaptation[25] of several *Drosophila* species.

Flies are not the only insects that readily use mushrooms as a resource. Beetles (order Coleoptera) commonly feed on and inhabit many mushroom species[26] and disperse the spores of these mushrooms[3,27]. Some bracket fungi increase their release of volatile organic compounds during sporulation[28]. These compounds attract different beetle species to the mushrooms and influence the structure of beetle communities that are inhabiting these fungi and the surrounding logs[29,30].

Studies examining broad patterns of mushroom-insect associations in other parts of the world outside of Europe, such as North America, are lacking[13]. In addition, many of these previous surveys were primarily observational and only recently have researchers begun conducting additional analyses to quantify properties of these interaction networks (e.g. [24,31]).

1.5. Methodology of previous studies

The most common method used to study mushroom-insect interactions is through rearing experiments. Mushrooms are identified based on morphology, then placed into covered containers that are kept under controlled conditions to prevent the mushrooms from decaying. Over the course of several weeks, insects that emerge from the mushrooms (i.e. mature insects that are already inside the fruiting body or insects that develop from larvae) are collected and identified, usually based on morphology as well[14,22]. Although rearing

methods are frequently used, there are several disadvantages to this approach. First, not all larvae will necessarily develop into mature insects under these conditions[32], so not all insects may be documented. In addition, relying on morphological identification requires extensive knowledge and experience with identifying a wide range of insects, potentially across multiple orders. Not only would this be difficult, it is also highly time-consuming.

1.6. New approaches

Other methods for examining mushroom-insect interactions have been explored. For example, in 2019, Schmid et al. investigated the feasibility of using time-lapse cameras to take pictures of mushrooms every five seconds to document insect activity. While the photos were useful for examining patterns in insect abundance on different mushroom species and at different times of day, the photos generally did not have high enough resolution to allow for the identification of insect species[33]. DNA barcoding is another approach. Barcoding eliminates the need to be able to identify taxa based on morphology and the organisms do not need to be in a specific developmental stage to be identified. In addition, obtaining sequence data from collected specimens can allow for downstream analyses of factors, such as taxonomic similarity, that may be influencing the structure of communities.

The nuclear internal transcribed spacer (ITS) region of the ribosomal RNA gene cluster was proposed as a barcoding marker for fungi in 2012[34]. While protein-coding genes, such as *RPB1*, have historically been used to infer the phylogenetic relationships of fungi, the ITS region was ultimately chosen for its higher amplification success rate and overall well-defined barcoding gap across a range of fungal lineages[34]. However, some species

display higher levels of intraspecific variation than interspecific variation (i.e. display a weak barcoding gap), making it difficult to use this marker to accurately identify all fungal species[35].

The mitochondrial cytochrome oxidase 1 (CO1) gene was proposed as the animal barcoding locus in 2003 and has since become well-established as a barcoding marker, showing high success in identifying various animal species[36,37], including insects[38,39]. Some studies have challenged the utility of CO1 for barcoding. Large intraspecific variation within some groups of insects, such as Diptera species, that exceeds the standard 2% sequence divergence cut-off, makes it challenging to accurately identify some insect species[39,40]. However, CO1 is the accepted barcode for animals that has widely been used by the International Barcode of Life Consortium initiative to document the diversity of organisms on earth.

DNA barcoding as a method to identify larvae inhabiting mushrooms was first proposed in 2012 and a preliminary survey demonstrated its potential[32]. Since then, at least two studies have employed metabarcoding approaches as a way to study mushroom-insect interactions[41,42].

1.7. Network analyses

Interactions between mushrooms and insects can be represented as a bipartite, or "two-mode", network. These networks involve two groups of organisms that interact with each other, but not with other members of the same group. Other common types of ecological bipartite networks include plants and pollinators, parasites and hosts and predators and prey[43]. Network analyses involve quantifying the interactions that occur

between species. Various properties of the network can be calculated, such as the degree of specialization (i.e. how specialized the interactions are within the network), the degree of specialization each species displays in its interactions and the extent to which the network can be divided into subgroups (modules) of interacting species that associate with each other more frequently than species in other groups[43]. These analyses can provide insight into how ecological networks are structured.

1.8. Overview of the current study

Given the limited knowledge of mushroom-insect interactions in Canada, the overall goal of this project was to examine these associations within mushrooms collected from forests in two regions of Southern Ontario. There were two main objectives for this project. The first was to conduct a broad survey of wild mushrooms, collecting as many species as possible, and to identify the mushrooms and their associated insects through DNA barcoding. This part was primarily observational and involved evaluating the degree of similarity in the mushroom and insect communities between sampling locations. The second objective was to quantify the interactions and look for patterns in the associations, guided by the following research questions: 1) To what extent do insects display evidence of preference or specificity in their choice of mushroom host? and 2) What ecological or evolutionary factors might explain any patterns observed between interacting species? Preliminary network analyses were conducted to examine the degree of specialization and modularity within the mushroom-insect network.

Mushrooms were collected from forests in Hamilton (Cootes Paradise and the Dundas Valley Conservation Area) and three private properties in the Tillsonburg, Ontario

region. The sampling locations within Hamilton were chosen because of their proximity to McMaster, which allowed for frequent sampling. Forests in the Tillsonburg area were chosen because during fall 2018 when the project started, mushrooms were highly abundant on all three properties and were therefore surveyed to increase the overall sample size of fruiting bodies that were examined. In addition, two of the three properties have different ecological characteristics than both forests in Hamilton: one property is dominated by pine trees and the other contains provincially significant wetlands. Environmental factors can greatly influence which mushroom species grow in certain areas. Thus, mushrooms were surveyed from different locations and across three collection seasons (fall 2018, summer 2019 and fall 2019) to maximize the diversity of mushrooms species collected, document as many interactions as possible and allow for spatial and temporal comparisons of the interactions.

Cootes Paradise, one of the main sampling locations in Hamilton, is an ecologically important bioreserve within Ontario and as such, the plant and animal diversities that inhabit this region have been carefully documented[44,45]. However, little information is available about the mushroom diversity from this forest. For example, a brief statement in the September 2018 Cootes Paradise Management Plan notes that foraging of mushrooms and other plants occurs in Cootes Paradise and overharvesting could negatively impact the environment[45]. However, while the diversity of flora and fauna are both discussed in detail, no additional information is provided about mushrooms within this forest. This is potentially the first survey to carefully document the mushroom community within Cootes Paradise.

Examining species interactions is an important area within ecological research. Species do not simply exist in isolation within the environment and are instead interacting with a range of different organisms in intricate networks[46]. These interactions shape ecosystems and by understanding the various connections between species, ecologists can understand the impact of species loss on other organisms within the ecosystem[46]. Mushrooms and insects are two groups of diverse organisms that form a range of interactions. Given their importance in ecosystems worldwide, it is important to understand and document how they interact.

2. Methods

2.1. Mushroom collection

Mushrooms were collected over three seasons: fall 2018 (September to October 2018), summer 2019 (June to August 2019) and fall 2019 (September to November 2019). Sampling locations included forests in Hamilton (Cootes Paradise and the Dundas Valley Conservation Area) and the Tillsonburg, Ontario region (the Lowrie, Sandor and Wirth properties, three private forest properties). Additional mushroom samples were contributed by labmates and friends, collected from the McMaster University campus (primarily behind the David Braley Athletic Centre, DBAC), various subdivisions near McMaster University and Barrie, Ontario.

2.1.1. Hamilton

Cootes Paradise

Cootes Paradise, a nature sanctuary of the Hamilton Royal Botanical Gardens, is an old-growth forest that surrounds the Cootes Paradise Marsh. This region is a local biodiversity hotspot with a range of vegetation communities that are classified into 24 different ecological land classifications[45]. The surveyed areas were from land that is classified as deciduous forest and mixed forest. Surveys were conducted along the entirety of the Chegwin Trail (~1 km in length) and the Ravine Road Trail (~1.2 km in length), both of which begin at the McMaster University campus. Sampling involved walking along the trails and collecting mushrooms that grew within two metres of the trail on either side.

Occasionally, sampling involved walking up to four metres from the trail to collect a mushroom, if the mushroom was spotted from the trail.

Dundas Valley Conservation Area

The Dundas Valley Conservation Area is a 1,200-hectare plot of Carolinian forest to the west of Hamilton. Surveys were conducted along approximately the first 1 km of the Monarch Trail, and several of the side trails, starting at Lynden Ave., Dundas, Ontario. Sampling involved walking along the trail and collecting any mushrooms growing within a few metres on either side of the path. In areas where vegetation was sparse, sampling involved wandering up to 10 metres off the trail to search for and collect mushrooms growing on the forest floor, including on the slopes of ravines, or under fallen logs.

2.1.2. Tillsonburg Area

Lowrie Property

This property is located about 10 km south of Tillsonburg, Ontario and spans eight hectares of undisturbed forest with a large ravine. This forest is dominated by broadleaf deciduous trees. Mushrooms were collected from the southern section of the property in an area spanning about three hectares. The undergrowth in the forest was sparse, so surveys were conducted by walking back and forth across the forest area and along the side of the ravine, collecting any mushrooms found under fallen logs, at the base of trees or on the forest floor. No mushrooms were collected during the summer of 2019 because an infestation of horse flies prevented access to the property.

Sandor Property

This property spans about eight hectares in Norfolk County, Ontario, and is located about 26 km south of Tillsonburg. The property is dominated by forest, part of which is considered provincially significant wetland. The most abundant tree species are maple, cherry and ash, though most of the ash is dead because of the emerald ash borer. There is one region on the south side that is dominated by pine trees. Sampling involved walking along trails that run throughout the forest, as well as randomly sampling regions off the trails where there was sparse undergrowth and vegetation. No mushrooms were found in the wetland portion of the forest. The forest was logged in September of 2019, which greatly disturbed the area and reduced access to many of the regions that were sampled during the fall 2018 and summer 2019 surveys.

Wirth Property

Mushrooms were collected from this two-hectare plot of land located about five kilometers south of Tillsonburg, Ontario. The woodlot is composed almost entirely of pine trees that were planted about 50 years ago and is surrounded by crop field on three sides. Surveys involved walking up and down the rows of pine trees and collecting mushrooms growing between the rows and underneath trees. Some mushrooms were collected from the edge of the woodlot near the fields.

2.1.3. Additional notes

Mushroom fruiting is highly dependent on the weather and environmental conditions. Thus, it was not possible to predict when mushrooms would fruit, nor was it possible to collect mushrooms at regular intervals (e.g. each week). The two forests in

Hamilton (Cootes Paradise and the Dundas Valley Conservation Area) were surveyed every one to two weeks throughout the sampling period, depending on the weather. However, some surveys yielded no mushrooms. The properties in the Tillsonburg Area were each surveyed between one and five times each season. Overall, mushrooms were collected on 64 different days from September 2018 to November 2019.

During each survey, the objective was to collect as many different mushrooms species as possible. Therefore, almost every observed mushroom was collected. However, if multiple fruiting bodies with a similar morphology were found growing in close proximity to one another (within 10 centimeters), a random sample of fruiting bodies was usually chosen and the rest were left untouched. In these cases, all fruiting bodies likely represented the same genetic individual and were labelled with the same sample number.

Mushrooms were carefully picked by digging up the volva from the ground to ensure that the complete mushroom was obtained. Each mushroom, or group of mushrooms, was wrapped gently in aluminum foil to ensure that any organisms resting on or in the mushrooms remained with the associated sample. Mushrooms were dissected within 36 hours of collection. If it was not possible to examine the mushrooms on the same day they were collected, the mushrooms were stored at 4˚C until they could be processed.

2.2. Mushroom processing following collection

Mushrooms were labelled and photographed in the lab, then examined under a dissecting microscope. Sterile tweezers were used to pull apart the mushroom tissue to search for any arthropods on the outside or interior of the fruiting body. It was often difficult to tell the difference between insects and non-insect arthropods. Therefore, any animals

found in association with the mushrooms were collected and placed into individually labelled 1.5 ml Eppendorf tubes. Slugs or snails were released. Arthropods with different morphologies (e.g. larva vs beetle) were placed into separate tubes. In addition, if larvae from the same sample appeared to have different morphologies, such as different colours, black head/no black head or a different size, larvae of each type were placed into separately labelled tubes. Generally, all observable organisms were removed from each mushroom. Occasionally, a fruiting body would be infested with larvae, in which case only a sample of larvae were collected. If larvae were very tiny and difficult to pick up, a small piece of mushroom tissue containing the larvae was collected.

The tubes were placed in -20˚C to both kill the insects and store the samples until the DNA could be extracted. Dissected mushroom specimens were dried overnight in a fruit and vegetable dehydrator at 65˚C.

2.3. DNA extractions

DNA was extracted from the mushrooms using the standard protocol for isolating DNA from higher fungi[47]. A small piece of mushroom tissue, weighing approximately 0.1-0.2g, was used for the extraction. Briefly, each sample was placed in a 1.5 mL Eppendorf tube, which was submerged in liquid nitrogen for ten seconds. A sterile plastic pestle or a pipette tip was used to crush each sample into a fine powder before 600 µl of CTAB extraction buffer was added to each tube. The tubes were vortexed individually for 30 seconds. After incubating at room temperature for 30 minutes in the fume hood, 600 µl of chloroform-isoamyl alcohol (24:1) was added to each sample and they were vortexed again for 30 seconds. Samples were centrifuged for five minutes at 13,000 rpm.

Following centrifugation, 500 µl of the supernatant was removed and transferred to a fresh 1.5 mL Eppendorf tube. To precipitate out the DNA, 550 µl of ice-cold isopropanol was added to each tube, which was then gently inverted and centrifuged for one minute at 13,000 rpm. The supernatant was removed and 50 µl of 70% ethanol was added and the tubes were left to sit for two minutes. After pouring off the ethanol, each tube was inverted on paper towel overnight. The next day, 60 µl of TE buffer (pH 8) was added to each tube.

For the arthropods, DNA was extracted from a single organism each time. For smaller organisms that were collected with mushroom tissue, DNA was extracted from the entire contents of the tube. A modified CTAB extraction protocol was used, following the same method as described, but with reduced volumes. Individual specimens were placed in a 1.5 mL Eppendorf tube and 150 µl of CTAB extraction buffer was added. A sterile pestle was then used to crush the specimen in the buffer. After incubating a room temperature in the fume hood for 30 minutes, 150 µl of chloroform-isoamyl alcohol (24:1) was added and the tubes were vortexed for 30 seconds. Samples were centrifuged at 13,000 rpm for five minutes, after which the supernatant was removed (about 110 µl) and gently mixed with 120 µl of ice-cold isopropanol. After centrifuging for one minute at 13,000 rpm, the supernatant was poured off and 50ul of 70% ethanol was added to each tube. The ethanol was removed after two minutes and the tubes were left inverted overnight to dry. The next day, 20-25 µl of TE buffer was added to each sample.

Following the extractions, all samples were run on a 1% agarose gel to determine the success of the extraction and quantity the DNA. The stock DNA was stored at -20˚C before being diluted for PCR.

2.4. PCR amplification and sequencing of the DNA barcoding loci

The universal fungal barcoding primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3')[48] were used to amplify the ITS region, which spans the ITS1 and ITS2 intergenic regions and the 5.8s rRNA gene. Either 12 µl or 25 µl volume reactions were run with the following protocol: a pre-denaturation step at 95˚C for 10 minutes, 45 cycles with a denaturation step at 95˚C for one minute, a 30 second annealing step at 60˚C and a one-minute elongation at 72˚C, followed by a final elongation step at 72˚C for 10 minutes, then a 4 ˚C hold. Samples were stored at 4˚C.

The DNA primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') were used to amplify about 680 base pairs of the CO1 gene from the arthropods. These universal primers have a high success rate amplifying this fragment of the CO1 locus from metazoan invertebrates[49]. The PCR protocol consisted of an initial pre-denaturation step at 94˚C for one minute, followed by five cycles with a 94˚C denaturation step for one minute, one and a half minute annealing step at 45˚C and an elongation step for one and a half minutes at 72˚C. Following this, there were thirty-five cycles with a 94˚C denaturation step for one minute, one and a half minutes with an annealing temperature of 45˚C and an elongation step at 72˚C for one minute. After a final elongation step at 72˚C for five minutes, the samples were stored at 4˚C. The protocol was modified from Hebert (2003)[36].

For any mushroom samples from the fall 2018 collection for which no corresponding insect sequence was obtained, the CO1 primers were used with the fungal DNA as a template. This approach was successful in amplifying several CO1 sequences, likely from the DNA of eggs or insect tissue that was embedded in the mushroom and extracted with the mushroom DNA. Due to time constraints, this could not be completed for any samples collected in 2019. Following PCR, all mushroom ITS and insect CO1 products were run on a 1% agarose gel.

All successfully amplified samples were sent for Sanger sequencing. Samples from the fall 2018 collection and some from the 2019 collection were purified using microClean and sent to the Mobix Laboratory at McMaster University. The majority of samples from the 2019 collection were sent as crude products to Eurofins Genomics, Kentucky. Samples were sequenced in the forward direction (ITS1 and LCO1490 primers). Several samples from the 2018 collection were re-sequenced in the reverse direction (ITS4 primer) if sequencing in the forward direction failed to give a clean sequence read.

2.5. Challenging sequences

One challenge with using the ITS sequence as a barcoding locus for fungi is the prevalence of intrastrain heterogeneity. To obtain the ITS sequence for two mushrooms from the 2018 collection, the ITS region was amplified and cloned into a pMiniT 2.0 plasmid and transformed into competent *E. coli* using the NEB PCR Cloning Kit. Colony PCR was performed on the resulting *E. coli* cells, grown on an ampicillin selection plate, using the provided primers. One resulting sequence from each of the two cloned mushroom samples was sent for Sanger sequencing.

A sequence was not obtained for every collected sample due to a combination of failed DNA extractions, unsuccessful PCRs or unreadable sequencing results. Due to time constraints, it was not possible to troubleshoot every sample, particularly those from the 2019 collection. As a result, multiple identifications of both mushrooms and insects are missing from the dataset.

2.6. DNA barcode sequence analysis and species identification

2.6.1. Mushrooms and the UNITE database

All ITS and CO1 sequence chromatogram files were viewed and edited in FinchTV. Bases were trimmed from the start and ends of the read and all sequences were manually scanned for double peaks, indicating heterogeneity. These bases were re-labelled with the appropriate IUPAC code for ambiguous bases.

Multiple ITS sequences were obtained from heterogeneous mushrooms with different copies of the ITS region. The first 80 to 600 bases of the chromatograms were typically clean and readable, followed by overlapping and messy peaks for the remainder of the read. These overlapping peaks usually had the same or similar intensity, making it impossible to correctly identify the nucleotide at each position. When possible, the readable portions of these sequences were edited and saved for further analyses. Sequences that were unreadable throughout the entire read were discarded.

Mushroom specimens were identified through a BLAST search of the ITS sequences against the UNITE (User-Friendly Nordic ITS Ectomycorrhiza) database, a curated, open source database containing over two million full-length fungal ITS sequences (https://unite.ut.ee/index.php). The sequences within the database are clustered into species

hypotheses, which are groups of sequences with high similarity that lead to the approximation of fungal OTUs at the species level[50]. The current full "UNITE+INSDC" dataset[51] was downloaded from the UNITE website. The dataset was last updated on February 4th, 2020 and contains 714, 329 ITS sequences, which includes all ITS sequences from both the UNITE and INSDC (International Nucleotide Sequence Database Collaboration) databases that are represented in UNITE species hypotheses. This dataset was chosen because it contains more ITS sequences than the General FASTA Release dataset, which contains only reference and representative sequences for all of the species hypotheses. In addition, the default BLAST search tool on the UNITE analysis page includes the INSDC dataset.

The dataset was converted into a BLAST database using the BLAST+ software downloaded from NCBI (https://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/). FASTA files of the edited mushroom ITS sequences were searched against this database using the blastn application with default settings. Only the top 20 alignments and matches were examined. For mushroom samples that were sequenced in both the forward and reverse directions, generally the longest fragment out of the two was chosen for analysis.

2.6.2. Mushroom identification criteria

There are few definitive guidelines for assigning fungal species-level identifications based on the ITS region. Therefore, criteria that has previously been used for assigning identifications to fungi, including a diverse range of mushrooms, based on the ITS region was adopted[52,53]. A few modifications were made to the criteria to incorporate samples with heterozygous bases or partial sequences. The final criteria are as follows:

Species-level identifications:

A sample was identified to the species level if:

1. The sequence matched with 100% similarity to a species, with no matches to other species with 99% similarity or greater.
2. The sequence matched with 99% similarity to a species (with no matches to other species with 99% similarity or greater) and the only difference between the query and subject sequences were heterozygous bases or a single mismatch.

A sample was identified to the species level with the cf. suffix (meaning it is possibly this species) if:

1. The sequence matched with 99% similarity to the top species due to more than one mismatch or gap, with no matches to other species with 99% similarity.
2. The sequence matched to only one species in the top 20 hits, but with 98% similarity because of multiple mismatches, all due to heterozygous bases in either the subject or query sequences.

A sample was identified to the species level with the aff. suffix (meaning a related species, but not this exact species) if:

1. The sequence matched with 96% to 98% similarity to a species with no matches to other species with the same similarity score.

A few additional species-level identification criteria:

1. If two different species from the same genus met the above criteria for a given query sequence, it was not possible to confidently distinguish between the two species without more information. Both species were searched in the Index Fungorum

database to check if they are actually the same species but with a synonymous name. If not, the sample was identified as both species (e.g. *Leucoagaricus leucothites/subcretaceus*).

2. If a sequence matched with equal similarity to both a sample only identified to the genus level and a sample with a species-level identification, but of a different genus, the species-level identification was chosen.
3. If the BLAST output contained an 'outlier' species, meaning a single match to a species from a family or order that was different from the most common species match (but with an equivalent similarity score), photos of both species were compared to the photo of the collected specimen. In most cases, it was possible to confidently rule out the match to the 'outlier' species due to obvious morphological differences between this species and the specimen collected.
4. Samples that were identified based on a partial sequence with less than 300 clean bases were only identified to the species level with no suffixes if the species matched with 100% similarity (or 99% due to one mismatch or heterozygous base) and all species-level identifications within the top 20 hits were to this species.

Genus-level identifications:

A sample was identified to the genus level if:

1. The sequence matched with 96% to 100% similarity to a specimen only identified to the genus level, with no matches to a species with 96% similarity or higher. There are multiple sequences included in the UNITE database that are only identified to the genus, family or order level.

2. The sequence matched to three or more species from the same genus, all with the same similarity score, ranging from 96% to 100%. Even if the matches varied slightly in their bit scores due to a variable number of mismatches, if the similarity scores were identical, it was not possible to confidently assign a species-level identification.
3. The sequence matched to one or more species from the same genus all with less than 96% similarity.

Family-level identifications:

A sample was identified to the family level if the sequence matched with equal similarity to species of different genera within the same family.

Additional identification notes

Any specimens that matched to the top hit with less than 90% similarity were searched manually against the online UNITE + INSDC database using the BLAST analysis feature of the UNITE website. The online search includes locked sequences, which are hidden from the downloadable dataset.

All species were searched in the Index Fungorum database (http://www.indexfungorum.org/names/names.asp) to obtain the current family and order classifications. If there was any ambiguity in the identification of a specimen, the sample was identified to a higher taxonomic level to avoid incorrect species-level identifications. All ITS sequences obtained in this study will be submitted to GenBank.

Due to the university shut down, clean ITS sequences could not be obtained for all of the mushroom samples collected during 2019. As a result, many mushroom specimens

that had associated insect identifications were missing ITS sequences. Photos of these specimens were sent to Dr. Yang-Yang Cui at the Kunming Institute of Botany, Chinese Academy of Sciences, China, for identification. Dr. Cui has extensive experience identifying mushrooms based on morphology. However, identifying a mushroom based on a photograph is difficult and mushroom identification involves examining many features of the mushroom, such as the smell, texture, size and spore morphology, which are all features that a photograph cannot capture. Thus, identifications based on the photographs are estimates or 'best-guesses' of the identities of these mushrooms to the family or genus level. Each specimen was identified twice: once by Dr. Cui, then again by Dr. Cui with consultation from one of her colleagues. When the identifications were consistent between both attempts, the identification was adopted. However, if the two identifications were inconsistent, a higher taxonomic identification was used, most commonly the order level.

2.6.3. Insects and the BOLD database

Insect specimens were identified through queries of the partial CO1 sequences against the BOLD database (The Barcode of Life Data System)[54], which contains over seven million animal CO1 barcodes of more than 200,000 species. BOLD uses a clustering algorithm to group all sequences deposited in the database based on sequence similarity, initially a 2.2% similarity cut-off. Each group of sequences is assigned its own BIN (Barcode Index Number)[55]. These BINs generally align with species boundaries and provide a way to group sequences within the database. Initially, all sequences from insects collected in 2018 were searched manually using the sequence analysis search tool on the BOLD website. Sequences were first searched against the 'Species Level Barcodes Records'

database, then against the 'All Barcode Records on BOLD' database if the first search revealed no species-level match. For insects collected in 2019, searches were conducted in R (version 3.6.3) using the package 'bold'. Since most of the insects from the 2018 collection could not be identified to the species level, all 2019 samples were queried against the 'COX1' database, which is equivalent to the 'All Barcode Records' database on the BOLD website. The top 10 hits were examined. Any samples with no species-level match were re-queried against the 'COX1_SPECIES' database, which contains only CO1 sequences of samples identified to the species level, to confirm that no possible species-level identifications were missed. To ensure consistency, all 2018 samples were also queried against the two databases using the 'bold' package in R and compared with the results obtained from the manual blast searches.

The majority of the insect identifications (554/568) were unambiguous. Searches would typically result in all ten matches to a species, genus or family-level identification with 100% similarity. Any insect sequences that matched to a species with 98% similarity or above were classified to the species level. For ambiguous identifications, where a sequence matched with low similarity to multiple species, genera or families, the sample was identified to a higher taxonomic level.

Many samples (172/568) matched with 99% or 100% similarity to sequences that are only identified to the genus or family level, with no species-level identification, within the 'All Barcodes Records on BOLD' database. To further examine the within-genera and within-family diversity of these samples, all sequences were searched on the BOLD website and the BIN of the top match with the highest similarity score was recorded. Samples within

each genus or family were then sorted based on the BIN of the top match. The samples matching to each BIN group were assigned a "variation number" (e.g. *Exechia* sp. Var. 1, *Exechia* sp. Var 2, etc.) to provide a more specific identification for these insect samples.

For samples that matched with less than 95% similarity to any sequence in BOLD, all sequences from the BIN of the highest similarity match were downloaded. The samples were aligned with these BIN sequences using the Muscle algorithm in Mega7. Kimura-2-parameter pairwise genetic distances were computed to confirm that the pairwise genetic distances between each sample to those in the BIN exceeded the maximum within-BIN distance recorded on BOLD. Each of these sequences were also assigned a variation number. All partial CO1 sequences obtained in this study will be submitted to GenBank.

2.7. Spatial and temporal species abundance patterns

Spatial and temporal patterns in the mushroom and insect taxa collected from each of the five main sampling locations (Cootes Paradise, Dundas Valley, and the three properties in the Tillsonburg area) and during each season were examined. Since multiple mushroom specimens could not be identified to the species level, only those with a species-level identification were included in the analysis. Venn Diagrams were constructed in R to summarize the mushroom and insect taxa that were collected from multiple locations and seasons versus those unique to one location or season. Pairwise Bray-Curtis dissimilarity indices were calculated for the mushroom and insect communities from each location in R using the package 'picante' The Bray-Curtis dissimilarity index quantifies differences in species composition between different sites and ranges from 0 (all shared species between sites) to 1 (no similarities in species composition between sites). The index takes into

account the abundance of species as well as the number of species shared between sites. One assumption for this index is that samples are collected from sites that are the same physical size. While the total region surveyed in Cootes Paradise is likely slightly larger than the other four sites, mushrooms were collected over approximately the same area from the other regions. Cluster dendrograms were created to visualize the Bray-Curtis dissimilarity values.

2.8. Interaction analyses

To examine the interactions between mushrooms and their associated insects, a reduced dataset was created to include only samples with both a mushroom and insect identification. Any samples missing either identification were excluded. If multiple larvae/insects from the same mushroom were identified as the same species, one representative insect specimen was retained and any duplicates or triplicates from the sample were removed. Multiple mushroom samples (221/760) could not be identified to the species level due to ambiguity in the identification or because the mushrooms were identified based on a photograph. To maximize the number of mushroom species that could be included in any species-level analyses, ambiguous samples were assigned a sample number based on the BLAST result, for example, *Russula* sp. 1 (for samples identified as *Russula aff.pelargonia/clariana/innocua*) and *Russula* sp. 2 (for samples identified as *Russula aff. graveolens/aeruginea/amoenipes*). This could not be done for any samples identified based on a photograph because there was no way to know if these samples are unique species or the same species as mushrooms that were identified based on the ITS region.

A simple linear regression was conducted in R to examine the relationship between the number of specimens collected of a given species and the number of specimens that contained an identified insect. Interaction matrices were created to count the number of interactions between different taxa for all combinations of mushroom and insect taxa (i.e. mushroom species/insect species, mushroom genera/insect species, mushroom families/insect orders, etc.).

2.9. Network analyses

Network analyses were conducted in R using the package 'bipartite'[43], which allows the user to visualize ecological networks and calculate various network-level and species-level indices.

2.9.1. Network and species-level specialization

The network involving mushroom and insect species was the primary focus of analysis. First, the network-wide specialization index (H'_2) was calculated. This index quantifies the degree of a network specialization on a scale of 0 (completely generalized interactions) to 1 (completely specialized interactions). It is calculated based on how the distribution of interactions within the network differs from the expected distribution if all species interact proportionally to their frequency[41,56]. This index takes into account the strength of each interaction (i.e. the number of documented interactions), which makes it more informative than other indices that use binary data[56]. This index is also not largely affected by the size of the network, meaning it can be used to estimate the level of specialization for smaller, incomplete matrices that are representative of a larger network[56]. Species-level specialization, measured as the standardized Kullback-Leiber distance (d'),

was also calculated for each insect and mushroom species of the network. This index quantifies the degree of specialization of each species by taking into account the distribution of its interactions and the availability of its partners[56]. The index also ranges from 0 (a species has completely generalized interactions) to 1 (completely specialized interactions). To determine if the observed H'_2 and d′ values were significant, 1,000 null networks were generated using the r2table function in R. This method keeps the total number of interactions for each species the same as the original network, but randomizes the interactions and has previously been used to generate null models for network analyses of fungal-beetle interactions[41]. H'_2 and d′ values were calculated for each of the null networks. The observed values were compared to the null models by calculating a Z statistic. Values were considered significant if the Z score was greater than the critical value of 1.96 ($Z_{0.025} = 1.96$).

Network and species-level specialization values were calculated for a few additional networks. First, H'_2 was calculated for a species-species network with all single species interactions removed (i.e. interactions involving a single mushroom and a single insect species that both are represented by a single specimen and only interact with each other). H'_2 was also calculated for different networks with mushroom taxa grouped by genus, family and order to examine if the degree of network-level specialization changes when mushrooms are grouped by higher taxonomic levels. These calculations were repeated with a reduced dataset that included only mushroom samples with a complete identification at each taxonomic level and d′ values of each insect species were calculated.

A smaller network involving only flies (order Diptera) and their associated mushrooms was also considered and H'_2 was calculated. Finally, H'_2 was calculated for networks involving each the most commonly collected mushroom families and their associated insects.

2.9.2. Modularity

Modularity is a measure of how well a network can be structured into smaller subgroups, where species within each group or module interact with each other more frequently than with species in a different module[43]. Modularity (Q) was computed using the LPAwb+ algorithm, the default algorithm in bipartite, which is recommended for exploratory analyses[57]. The index also ranges from 0 (non-modular) to 1 (completely modular) and is calculated by combining groups of interacting species until a local maximum in modularity is reached, then combining modules in different combinations to maximize the global modularity of the network. The process is stochastic and the number of modules and species within each module varies each time modularity is calculated. Thus, modularity was calculated ten times using the metaComputeModules function, which runs the algorithm multiple times with each run, and the average value was reported. Modularity was computed for 50 null networks and a Z statistic was calculated. The modules and overall structure of the network was visualized.

2.9.3. Genetic distance analyses

To further investigate the taxonomic relationships between mushrooms and insects assigned to each of the modules, pairwise genetic distances of taxa within each module were calculated and compared. One representative ITS and CO1 sequence was chosen for

each of the mushroom and insect species. Only complete, unambiguous sequences were chosen for the analysis. Multiple mushroom species were identified based on heterozygous sequences. For these samples, the top sequence match from the UNITE database was downloaded instead. If the identification of the sample was ambiguous between two or more species, the sequence of the first match in UNITE was chosen. In addition, some partial sequences matched with low similarity to any sequence in UNITE (e.g. a partial ITS sequence matched to *Cuphophyllus colemannianus* with 92% similarity as the top match). For these samples, the top UNITE sequence was downloaded anyway. Using a complete ITS sequence from the actual collected specimen would have been preferable. However, when it was not possible to obtain a complete sequence, a sequence representing a close match to each of these samples was sufficient for these preliminary analyses. For two insect species, a truncated partial CO1 sequence was obtained, so representative sequences were downloaded from BOLD.

Mushroom ITS and insect CO1 sequences were aligned separately using MAFFT with default settings (https://www.ebi.ac.uk/Tools/msa/mafft/). Alignments were downloaded and the pairwise Kimura-2-parameter distances were calculated in Mega7 for the mushrooms and insects separately. The ITS region is highly divergent, so the resulting alignment contained multiple gaps. If all sites with gaps or missing data had been deleted, only 192 bases total would have been used to calculate the genetic distances. Thus, genetic distances were calculated using a pairwise deletion setting for gaps and missing data. Unrooted Neighbour-Joining trees were then constructed in Mega7 for the ITS and CO1 alignments separately, with 1,000 bootstrap replications, also using the pairwise deletion

setting for gaps and missing data. The goal was to confirm that species from the same genera and families clustered together based on similarity at the barcoding locus.

Mushroom and insect species were then assigned to communities, based on the module groupings that were estimated previously, as described above. For example, all mushroom species placed into the first module were assigned to community one, all species in the second module were assigned to community two, etc. Each species was only assigned to one community and the communities consisted of only mushrooms or insects, not both. The abundance of each species was not considered. The R package 'picante' was used to calculate the mean pairwise genetic distance between all species within each community. The pairwise distances were compared to the expected distances within each module for 1,000 null models, generated by keeping the species within each module the same, but randomizing the labels of the taxa in the distance matrices.

3. Results

3.1. Summary of mushroom specimens collected

In total, 1,017 mushroom specimens were collected. **Table 1** summarizes the number of mushroom samples collected during each season from every location. The number of samples collected on each of the 64 collection days ranged from one to over 60 samples each time, depending on how many mushrooms were growing. An approximately equal total number of samples were collected from both Hamilton and the Tillsonburg Area (504 from Hamilton and 499 from the Tillsonburg Area). However, the number of samples from each specific location within these regions differed. For example, within Hamilton, more than twice as many samples were collected from Cootes Paradise (297 specimens total) than the Dundas Valley Conservation Area (136 total), with more samples collected during the summer of 2019 than the other seasons (**Table 1**). In fact, the greatest number of samples overall were collected from Cootes Paradise. This primarily reflects sampling effort. Due to the close proximity of Cootes Paradise to McMaster, the forest was surveyed more frequently with 18 collection days compared to only nine collection days for Dundas Valley.

There were also seasonal and temporal differences in the number of mushroom specimens collected. It was noticeably harder to find mushrooms during fall 2019 than fall 2018 and fewer mushrooms were growing along the same collection routes. This is illustrated by the fact that slightly fewer mushrooms were collected in fall 2019 (353)

compared to fall 2018 (380), yet there were almost twice as many collection days (**Table 1**). Within the Tillsonburg Area, fewer mushrooms were found on the Sandor property in fall 2019 compared to the other seasons largely because the forest was logged. No samples were collected from the Lowrie property in summer 2019.

Approximately 50% of mushroom specimens collected contained at least one arthropod, with mushrooms collected during fall 2019 having the greatest estimated proportion of associated organisms (**Table 2**, estimates are based on rough notes taken as the mushrooms were dissected). More than 90% of organisms within the mushroom fruiting bodies were larvae, with at least 57 mature beetles and a few mature flies captured from the surface of the mushroom. Springtails (Collembola) were also common. While some mushroom specimens contained only a single arthropod within the fruiting body, others were full of larvae. The larvae were usually buried within the tissue of the mushroom under the gills or pores, at the junction of the stipe and cap or within the tissue of the stipe or volva. In contrast, the majority of the mature insects and other non-insect arthropods were generally found on the surface of the mushroom or in between the gills. Often smaller mushrooms, even those only a few centimeters tall, contained a single larva in the stipe or gills, while multiple large fruiting bodies did not contain any visible larvae. In general, it was difficult to predict, based on size or morphology, which mushrooms contained larvae prior to dissecting the mushrooms.

Table 1: Overall summary of the number of mushroom specimens collected and identified from each location and season.

Location	Fall 2018 (15 collection days)		Summer 2019 (21 collection days)		Fall 2019 (28 collection days)		Total	
	Mushrooms collected	Mushrooms identified	Mushrooms collected	Mushrooms identified	Mushrooms collected	Mushrooms identified	Mushrooms collected	Mushrooms identified
Hamilton								
Cootes Paradise	96	90	137	87	64	48	297	225
Dundas Valley	43	38	46	33	47	30	136	101
McMaster, DBAC	10	10	3	2	32	16	45	28
McMaster, Other	0	0	2	2	3	3	5	5
Dundas/Rail Trail	0	0	13	0	3	0	16	0
Hamilton, Various	0	0	5	4	0	0	5	4
Total	149	138	206	128	149	97	**504**	**363**
Tillsonburg Area								
Lowrie Property	49	37	0	0	93	61	142	98
Sandor Property	111	96	21	14	43	35	175	145
Wirth Property	57	45	58	52	66	50	181	147
Norfolk County, Other	0	0	0	0	1	1	1	1
Total	217	178	79	66	203	147	**499**	**391**
Other								
Barrie	13	11	0	0	0	0	13	11
Welland	1	1	0	0	0	0	1	1
Total	14	12	0	0	0	0	**14**	**12**
Overall Total	**380**	**328**	**285**	**194**	**352**	**244**	**1017**	**766**

Table 2: Summary of the number of mushroom specimens found in association with arthropods and the number of insects and non-insect arthropods/organisms identified during each of the three collection seasons.

Season	Minimum number of mushrooms that contained arthropods[1]	Total number of mushrooms collected	Proportion of mushrooms collected to contain an arthropod	Number of arthropods examined[2]	Number of insect specimens identified	Number of non-insect arthropods/nematodes identified
Fall 2018	181	380	48%	321	161[3]	54[3]
Summer 2019	174	285	61%	389	209	39
Fall 2019	183	352	52%	464	198	39
Totals	**538**	**1017**	**53%**	**1,174[4]**	**568**	**132**

[1]These numbers are estimates based on rough notes taken as the mushrooms were dissected. For a few collection dates, no notes were taken.
[2]These numbers are based on the number of arthropod samples for which the DNA was extracted and the CO1 region amplified.
[3]These numbers include the 12 insects and seven non-insects (three springtails and four nematodes) that were identified by amplifying the CO1 region from total fungal DNA.
[4]More than 1, 174 organisms in total were removed from the mushrooms, but when multiple larvae were removed from a single fruiting body, only one individual was analyzed. If the larvae had different morphologies, one representative specimen of each type was examined.

3.2. The ITS region as a barcoding marker

3.2.1. ITS sequencing

Of the 1,017 mushroom specimens collected, 680 were successfully identified using the sequence at the ITS locus. **Table 3** provides a summary of the sequencing and identification success rate for the mushroom samples using the ITS region. A complete ITS sequence was obtained for 444 samples, which is less than half of all mushrooms collected. Some of these complete ITS sequences had background signal throughout the chromatograph, possibly due to the amplification of other fungal contaminants in the mushroom or the failure to remove all primers. However, their sequences were still readable (**Figure 1A**).

One challenge with using the ITS region as a barcoding marker is the prevalence of within strain heterogeneity, where an individual mushroom fruiting body contains two different versions of the multi-copy gene region. If the copies only differ by a few bases due to nucleotide substitutions, the overall sequence is readable with a few heterogeneous bases throughout, which were denoted with the IUPAC ambiguity codes (**Figure 1B**). However, if either of the copies contains an indel, the two copies will be out of frame starting at the location of the indel and the rest of the chromatogram will be unreadable with overlapping and double peaks (**Figure 1C**). A total of 210 specimens had extensive heterogeneity within the ITS region and thus, only a partial ITS sequence of varying length was obtained for these specimens (**Table 3**).

For most of the heterogeneous samples, the chromatograms followed the same pattern: clean and readable bases at the start of the read, anywhere from 100-600 bases in length, followed by overlapping and double peaks for the rest of the read. However, thirteen samples had a slightly different pattern (**Figure 1D**). These chromatograms were generally clean overall, with small sections of overlapping peaks, each spanning a few dozen bases, before reverting back to readable bases. For these samples, only the readable bases following the regions with double peaks could be used to identify the specimens. Over half of these samples were from the family Mycenaceae.

A total of 337 mushroom samples failed to be identified based on the ITS barcoding region (**Table 3**). Most of these samples were never sent for sequencing due to an unsuccessful DNA extraction or failure to amplify the ITS region for sequencing (i.e. no amplification product, insufficient quantity amplified or multiple products amplified). For example, particularly tough fruiting bodies, such as the polypore or bracket fungi, and soft mushrooms, such as the puffballs, were difficult to grind into small enough pieces during the first step of the extraction procedure. Other reasons for a missing identification included a failed sequencing reaction, the amplification of a contaminant rather than the mushroom ITS region (such as a mold or yeast) or an unreadable sequence, likely due to within strain heterogeneity.

Table 3: Summary of the identification and sequencing success rate using the ITS region as a DNA barcode for the collected mushrooms

Mushroom samples that were successfully identified		**Notes**
No heterogeneity observed	444	*7 samples were identified with the reverse sequence
Heterogeneous ITS sequence - sequence cloned	2	
Heterogeneous ITS sequence with 100 readable bases or less	31	
Heterogeneous ITS sequence with 100 – 300 readable bases	60	*4 samples were identified with the reverse sequence fragment
Heterogeneous ITS sequence with 300 – 650 readable bases	119	*35 samples were identified with either a fragment of the reverse sequence or fragments from both the forward and reverse sequences
Partially messy sequence read, with at least 250 readable bases	21	
Incomplete sequence read or truncated, with at least 150 readable bases	3	
Total number identified using the ITS barcoding locus	**680[1]**	

Mushroom samples not successfully identified		**Notes**
Completely heterogeneous throughout - sequence not readable	37	*9 of these samples were identified using a photograph
A contaminant (yeast, mold or another fungal species) amplified	6	
Failed sequencing reaction	70	*24 of these samples were identified using a photograph
Heterogeneous or truncated, with 100 readable bases or fewer and an identification was not possible	6	
Never sequenced (due to failed DNA extraction or PCR)	218	*53 of these samples were identified using a photograph
Total number of samples that failed to be identified using the ITS barcoding locus	**337**	

Total number of mushrooms collected	**1017**

[1]In total, 766 mushroom samples were identified: 680 based on the sequence of the ITS barcoding locus and 86 samples based on a photograph.

Figure 1A: ITS chromatogram of a read with background throughout. All bases were called with little to no ambiguity and the background was ignored. The background is possibly caused by remaining primers or the amplification of a secondary product or contaminant. Mushroom identification: *Amanita muscaria*.

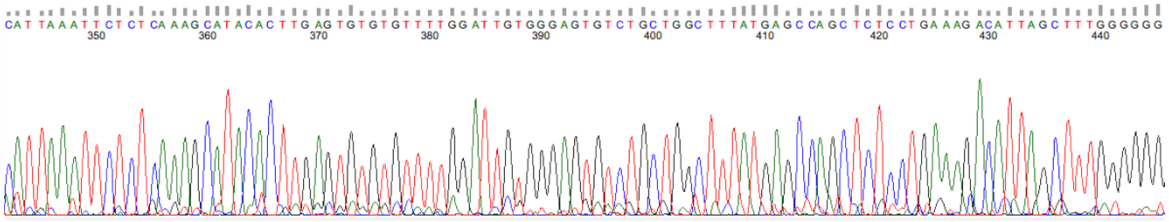


Figure 1B: ITS chromatogram for a sequence with double peaks, indicating heterogeneity of the ITS region. This sequence was perfectly clean and readable overall, with no background. This region has four double peaks, indicating two slightly different ITS sequences. The bases were labelled using the IUPAC ambiguity codes. Mushroom identification: *Boletus subluridellus/subvelutipes*.

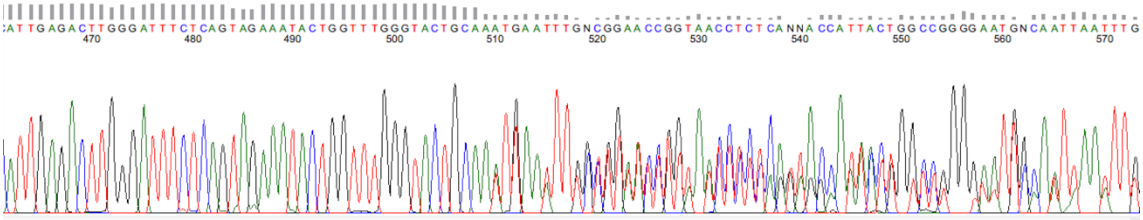


Figure 1C: ITS chromatogram of a sequence that displays partial heterogeneity. The first 510 bases of the read were of high quality with single, non-overlapping peaks. The two variations of the ITS region likely differ starting at the 510th base, resulting in an unreadable sequence with double peaks of equal intensity. This could be due to the presence of an indel in one of the copies. The readable portion of the sequence was used to identify the sample while the second portion of the sequence was discarded. Mushroom identification: *Gymnopus dryophilus*.

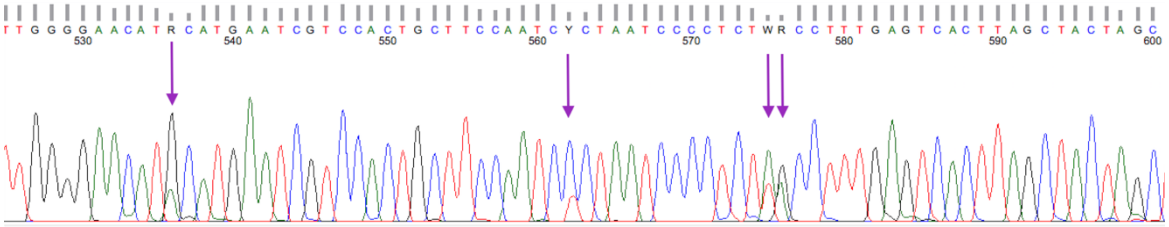
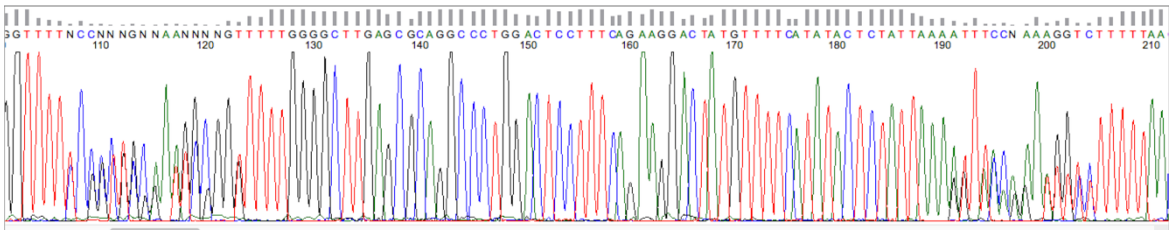


Figure 1D: ITS chromatogram of a sample with two regions of heterogeneity. This sequence does not follow the pattern of heterogeneity typically observed for most samples (such as the example in Figure 1C). The sequence contains double peaks in two short regions where the two versions of the ITS region differ, spanning bases 107 to 123, then again from bases 191 to 204. Outside of these regions, the sequence is readable with no ambiguous or double peaks. Mushroom identification: *Roridomyces roridus*



3.2.2. ITS sequence-based identification

Complete and partial ITS sequences were queried against the UNITE and INDSC fungal database. The majority of samples with at least 100 readable bases were identified to the species level, often with little to no ambiguity in the identification (i.e. the sequence matched with 99% or 100% similarity to the ITS region of only one species). For example, sequences that matched to the species *Amanita muscaria* and *Scleroderma citrinum* were consistently unambiguous, typically with most of the top 20 BLAST results matching to this species, even when short 100-200 base fragments were used as a query sequence. A total of 355 specimens were identified to the species level (either with or without a cf./aff. suffix) with no ambiguity in the identification.

However, it was not possible to confidently identify every specimen to a single species. It was common for a query sequence to match to two different species, often from the same genus, with identical or nearly identical similarity and bit scores. As a result, these samples were identified as both specimens. The two most common ambiguous results were sequences that matched to *Leucoagaricus leucothites* and *L. subcretaceus* (same genus, equivalent similarity and bit scores, labelled as *Leucoagaricus leucothites/subcretaceus*) and *Calocybe carnea* and *Rugosomyces persicolor* (different genera, equivalent similarity and bit scores, labelled as *Calocybe carnea/Rugosomyces persicolor*). Of the 680 specimens that were identified based on an ITS sequence, 191 were ambiguous between two different species.

It was also common for the identification to be ambiguous between three or more species. A total of 72 specimens were only identified to the genus level because the sequences matched to three or more different species from the same genus with a similar or equivalent similarity and bit score. An additional six specimens were identified to the family level because the sequence matched with high similarity to three or more species from different genera. For these samples, it was not possible to confidently assign a species-level identification because the BLAST results were too similar. The most common ambiguous identifications were for mushrooms within the genera *Agaricus* (*Agaricus nivescens/arvensis/excellens/sylvicola/crocodilinus* - all collected from beside DBAC at McMaster), *Armillaria* (*Armillaria gallica/calvescens/cepistipes/sinapina*), *Inocybe* (*Inocybe phaeoleuca/ochroalba/appendiculata/splendentoides* – all collected from the Wirth property), *Lactarius* (*Lactarius deliciosus/torminosus/pallescens* – all collected from the Wirth property) and *Pluteus* (*Pluteus pellitus/petasatus/cervinus*).

3.2.3. Summary of the mushroom identifications based on photographs

A total of 101 specimens that failed to be identified based on the ITS region were instead identified using a photograph. Of the 101 specimens sent to Dr. Cui, 15 specimens could not be identified. Twenty were ultimately identified to the order level (order Agaricales) because the identification to the genus level changed between the two identification attempts. In all, eighty-six samples were identified based on a photograph.

3.2.4. Overall totals

Overall, 680 of the 1,017 of mushrooms collected (67%) were identified using the sequence of the ITS locus. Including those identified based on a photograph, 766 mushroom

specimens (75%) were identified in total (**Table 1**). The overall mushroom identification rate was higher for the 2018 collection than both the summer 2019 and fall 2019 collections. Of the 380 mushrooms collected during the fall 2018 season, 328 were identified (86% of samples). In contrast, only 68% and 69% of mushrooms collected during the summer 2019 and fall 2019 collections were successfully identified (**Table 1**). This is primarily because it was not possible to troubleshoot any of the samples collected in 2019 for which an ITS sequence could not be obtained due to time constraints.

3.3. Summary of the mushroom identifications

The 766 identified mushrooms are from eight orders, approximately 53 different families and more than 200 species. **Supplementary Table 2** provides a complete list of all samples identified. The exact number of families could not be determined for two reasons. First, 20 specimens could not be identified beyond the order level (order Agaricales). The majority of these specimens were identified based on a photograph and without using more accurate identification techniques, it is not possible to know if these mushrooms are from families already collected or from different families. Second, the family level classification for nine of the genera is uncertain, denoted as *Incertae sedis* on Index Fungorum, so family-level classifications are unclear.

Similarly, it was not possible to determine the exact number of species collected. Of the 766 identified mushrooms, 220 specimens could not be identified to the species level for one of three reasons: 1) the specimens were identified based on a photograph, 2) the identification from a query against the UNITE database was ambiguous due to high similarity matches to multiple species or 3) the sample had no species-level record in

UNITE. Of the 546 specimens with a species-level identification, there were approximately 204 unique identifications, if cf. suffixes are removed (e.g. *Lepiota cf. cristata* is counted as the same identification as *Lepiota cristata*). Thus, 204 provides a rough estimate of the number of different species collected. However, the total number of species likely exceeds 204. Interestingly, just over half of these 204 unique species-level identifications are represented by a single specimen.

The majority of the mushrooms are from the order Agaricales, with 544 specimens, roughly 70% of all specimens collected. The order Russulales is the next highest represented order with 113 specimens, followed by Boletales (58 specimens) and Polyporales (37 specimens). The other four orders each have fewer than eight specimens: Hymenochaetales (8), Theleophorales (4), Cantharelles (1) and Pezizales (1). While mushrooms from the order Russulales accounted for only about one fifth of samples from the order Agaricales, the family Russulaceae was the most abundant family with 103 specimens. Only three families within the order Agaricales are represented by 50 or more specimens: Agaricaceae (70), Amanitaceae (50) and Mycenaceae (57). In contrast, eleven families are only represented by a single specimen. The most commonly collected mushroom species were *Leucoagaricus leucothites/subcretaceus* (29 specimens), *Calocybe carnea/Rugosomyces persicolor* (27), *Amanita muscaria* (21) and *Gymnopus dryophyllus* (17).

3.3.1. Potentially uncharacterized and new mushroom species

Several specimens could only be identified to the genus or family level, possibly because they represent either uncharacterized species or newly documented ITS sequences.

For twenty-three of these specimens, queries against the UNITE database yielded no species-level matches with greater than 95% similarity. Instead, these sequences shared 98% to 100% similarity with sequences in the UNITE database that were only identified to the genus or family level.

In contrast, thirty-seven samples from the orders Agaricales and Russulales did not match to any sequence in the UNITE database with greater than 95% similarity (**Table 4**). Most of the samples were collected from Cootes Paradise and the Sandor and Lowrie properties. Twenty-seven had complete ITS sequences with clean and readable chromatograms, meaning the reason for a lack of high similarity match is not because the sequences were incomplete or messy. An additional ten samples were identified with a partial ITS sequence, ranging from 106 to 539 readable bases. Without a high similarity match, these samples were identified to the genus, family or order level based on the BLAST results. For example, one sequence matched with less than 95% similarity to multiple *Hygrocybe* species, so the specimen was identified as *Hygrocybe* sp. Two samples matched with low similarity to multiple sequences from different families and were therefore identified as Agaricales sp. to the order level. Thirteen samples from the genus *Entoloma* matched with less than 93% similarity to any *Entoloma* sequence in UNITE. These *Entoloma* samples were all collected on the same day from the Dundas Valley Conservation Area.

Table 4: List of thirty-seven specimens with no high similarity sequence matches in the UNITE database. Ten samples were identified based on a partial ITS sequence due to the sample being heterogeneous or incomplete. Numbers in brackets indicate the number of specimens, if more than one specimen from the same location had an identical result.

Mushroom Order	Mushroom Family	Mushroom Genus	Location and Season Collected	Notes about top BLAST matches in UNITE
Identifications based on complete ITS sequences				
Agaricales	(Agaricales sp.)	(Agaricales sp.)	Fall 2019, Dundas Valley	*Pholiota abieticola*, *Galerina* sp., *Galerina badipes*, *Pholiota alnicola* and various other *Galerina* species with 88% and 87% similarity
		(Agaricales sp.)	Fall 2018, Sandor Property	*Tephrocybe confusa*, *Tephrocybe* sp., *Leucopaxillus pulcherrimus*, *Clitocybe vibecina* and *Tricholoma atrosquamosum* with 88% similarity and a low query cover (450/676 bases)
	Biannulariaceae	(Biannulariaceae sp.)	Fall 2019, Cootes Paradise	*Callistosporium pinicola*, *Callistosporum* sp., *Callistosporum luteo-olivaceum* and *Pleurocollybia* sp. with 91% similarity or less
	Crepidotaceae	*Crepidotus* sp.	Fall 2018, Sandor Property	*Crepidotus subverrucisporus*, *Crepidotus* sp., *Crepidotus luteolus* and various *Crepidotus* species with 93-94% similarity or less
	Entolomataceae	*Entoloma* sp. (8)	Summer 2019, Dundas Valley	*Entoloma pallidocarpum* with 93% similarity, then at least eight other *Entoloma* species with 88% to 91% similarity
		Entoloma sp. (2)	Summer 2019, Dundas Valley	*Entoloma subsinuatum* with 92% similarity, then at least six other *Entoloma* species with 88% to 92% similarity
		Entoloma sp. (2)	Summer 2019, Dundas Valley	*Entoloma rhodopolium* with 93% similarity, then at least five other *Entoloma* species with 88% to 93% similarity
	Hygrophoraceae	*Hygrocybe* sp.	Summer 2019, Cootes Paradise	*Hygrocybe* sp. with 96% similarity, the *Hygrocybe concia* (92%) and Hygrophoraceae sp. (90%)
		Hygrocybe sp.	Fall 2019, Sandor Property	*Hygrocybe conica* with 95% similarity, then *Hygrocybe* sp., Hygrophoraceae sp., and *Hygrocybe singeri* with 94% to 95% similarity.
		(Hygrophoraceae sp.) (3)	Fall 2018, Sandor Property	Hygrophoraceae sp. with 96% similarity. The rest of the matches were to *Clitocybe nebularis* with 82% similarity, then various other species from different families with low similarity and query covers.

		(Hygrophoraceae sp.[1]) (2)	Fall 2019, Lowrie Property	*Camarophyllus* sp., *Cuphophyllus fornicatus* with 87% similarity or less
	Inocybaceae	*Inocybe* sp.	Fall 2018, Sandor Property	*Inocybe* sp., *Inocybe glabrescens* and *Inocybe nitidiuscula* with 90% to 91% similarity.
		Inocybe sp.	Fall 2019, Cootes Paradise	*Inocybe* sp. with 85% to 86% similarity
	Mycenaceae	*Mycena* sp.	Fall 2019, Lowrie Property	*Mycena laevigata* with 95% similarity, then various *Mycena* species with 94% similarity or less
Russulales	Russulaceae	*Russula* sp.	Fall 2019, Lowrie Property	*Russula* sp. and Russulaceae sp. with 96% similarity, then *Russula crustosa* with 92% similarity
Identifications based on partial ITS sequences				
Agaricales	Agaricaceae	*Melanophyllum* sp.	Fall 2019, Sandor Property	408 readable bases - *Melanophyllum haematospermum* with 94% similarity
	Amanitaceae	*Amanita* sp.	Fall 2018, Sandor Property	106 readable bases – *Amanita oberwinklerana* with 91% similarity
	Entolomataceae	*Entoloma* sp.	Summer 2019, Dundas Valley	460 readable bases - *Entoloma rhodopolium*, *Entoloma sinuatum* and *Entoloma pallidocarpum* with 94% similarity
	Hygrophoraceae	*Cuphophyllus* sp.	Fall 2019, Lowrie Property	192 readable bases - *Cuphophyllus colemannianus* with 92% similarity or less
		Cuphophyllus sp.	Fall 2019, Lowrie Property	186 readable bases - *Cuphophyllus colemannianus* with 92% similarity or less
		Hygrocybe sp.	Fall 2019, Sandor Property	539 readable bases – *Hygrocybe* sp., *Hygrocybe conica* and Hygrophoraceae sp. with 95% similarity or less
		Hygrocybe sp.	Fall 2019, Lowrie Property	130 readable bases – *Hygrocybe* sp. with 88% similarity or less
		Hygrocybe sp.	Summer 2019, Cootes Paradise	179 readable bases – *Hygrocybe conica* and *Hygrocybe* sp. with 92% similarity or less
		(Hygrophoraceae sp.)	Fall 2018, Sandor Property	126 readable bases –Hygrophoraceae sp. with 97% similarity, then to various species, genera and family with 79% similarity or less
	Mycenaceae	*Mycena* sp.	Fall 2018, Cootes Paradise	403 readable bases - *Mycena amicta* with 95% similarity, then *Mycena* sp., *Mycena cyanorhiza* and Mycenaceae sp. with 93% similarity or less

[1]Samples have a small region of heterogeneity (with less than 40 unreadable bases) about 200 bases from the start of the sequence

3.4. Insect barcoding and identification

3.4.1. CO1 as a barcoding marker

Approximately 1,174 animals were analyzed from all 1,017 mushrooms collected. Of these, 681 were identified, with 556 insects, 121 non-insect arthropods and four nematodes (**Table 2**). An additional 19 specimens were identified from the fall 2018 collection using the CO1 primers to amplify and isolate insect sequences from extracted mushroom DNA, resulting in an additional 12 insect, four nematode and three springtail identifications. The overall DNA extraction and PCR amplification success rate was about 58% (681 individuals identified out of the approximately 1,174 processed). However, the sequencing success rate of amplified CO1 products was quite high. On average, only one or two samples per 100 CO1 samples sequenced produced an unreadable chromatogram, either due to low signal or a failed sequencing reaction. While some of the sequences contained a few double peaks scattered throughout the chromatogram, CO1 sequences were obtained for almost every sample.

3.5. Summary of the insect species identifications

In total, 568 insect specimens were identified based on the sequence of the partial CO1 gene. These insects represent five different orders within the class Insecta (Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera) and include at least 35 different insect families. Over half of the specimens were identified to the species level, with 65 different species-level identifications. However, one hundred fifty-nine samples could only be identified to the genus level, three to the subfamily level and 28 to the family level. In

addition, three specimens from the order Coleoptera could not be identified beyond the order level.

Many specimens matched with high similarity to sequences in BOLD that have not been classified to the species level. For example, multiple samples matched with greater than 99% sequence similarity to samples identified as *Megaselia sp., Exechia sp., Cordyla sp., Allodia sp., Mycetophila sp.* or *Cecidomyiidae sp.,* among others, with no corresponding species identification. Thus, samples within each genus or family were sorted according to the corresponding BIN of the top sequence match on BOLD. For example, samples identified as *Allodia sp.* fell into three different groups based on the BIN ID of the top matches. Samples identified as *Megaselia sp.* fell into 20 groups. BINs are generally reflective of species groupings, suggesting that these samples fall into three *Allodia* species, two *Brachypeza* species, eight Cecidomyiidae species, two *Cordyla* species, four *Dinotrema* species, seven *Exechia* species, three Keroplatidae species, twenty *Megaselia* species, two *Mycetophila* species, one *Pegomya* species, two *Platypezidae* species and two *Scaridae* species. In addition, there were multiple single-count specimens that could only be identified to the genus or family level, and each of these is likely a different species. This brings the approximate total number of insect species collected to 133.

Flies (order Diptera) were the most commonly collected insects, with 494 specimens. Within this order, most of the specimens were from the family Mycetophilidae (269 specimens), followed by Phoridae (165 specimens). *Mycetophila fungorum* (family Mycetophilidae, with 98 specimens representing over one sixth of all insects collected) and

the *Megaselia pulicaria* complex (family Phoridae, 61 specimens) were the most frequently collected insect species. The genus *Exechia* (Mycetophilidae) was the most represented genus with 127 specimens including *Exechia subfrigida* (35 specimens), *Exechia nigroscutellata* (27 specimens) and *Exechia dorsalis* (21 specimens) as the most common species. Beetles (order Coleoptera) were the next most commonly collected, though there were only 54 specimens in total. Except for *Tachnius fimbriatus* with 11 specimens, all other species within this order had five or fewer specimens. The other three orders, Hemiptera, Hymenoptera and Lepidoptera, each had fewer than 10 specimens. Overall, the total insect collection consists of a few highly abundant insect species that account for the majority of the specimens collected, combined with dozens of rare species, each represented by less than three specimens. **Supplementary Table 2** provides a complete list of the insects identified.

3.5.1. Potentially uncharacterized or new species

Fourteen CO1 sequences of samples from two orders (Diptera and Coleoptera) and at least five families, matched with less than 96% similarity to any sequence within BOLD (**Table 5**). For example, seven sequences matched with less than 90% similarity to the family Cecidomyiidae (four different BINs) with no genus or species-level identifications. The pairwise genetic distances of each sequence to all other sequences in the BIN group exceeded the maximum within-BIN distance documented on BOLD. Similarly, one specimen matched to the genus *Megaselia* with around 96% similarity and the pairwise distances between this sequence and others in the corresponding BIN group were greater than the maximum within-BIN distance. The other samples from the families

Mycetophilidae and Staphylinidae, as well as the order Coleoptera, matched to one or more species, genera or families with around 92% similarity or below. The lack of a high similarity match in BOLD suggests that these are these are all new sequences, potentially undocumented species, that are not yet part of this database.

Table 5: List of fourteen insect specimens with no high similarity species-level match in BOLD. Notes about the top matches from the BOLD search are included.

Insect Order	Insect Family	Insect Genus	Location and Season Collected	Notes about BOLD search
Diptera	Cecidomyiidae	Cecidomyiidae sp.	Fall 2018, Wirth Property	Matches with 86.54% similarity to Cecidomyiidae (BOLD:ACZ3621). Genetic distance of this sequence to sequences of this BIN exceed the maximum within BIN distance.
		Cecidomyiidae sp.	Summer 2019, Cootes Paradise	Matches with 88.31% similarity to Cecidomyiidae (BOLD:ACI5183). Genetic distance of this sequence to sequences of this BIN exceed the maximum within BIN distance.
		Cecidomyiidae sp.	Fall 2019, Dundas Valley	Matches with 90.16% similarity to Cecidomyiidae (BOLD:AAN5275). Genetic distance of this sequence to sequences of this BIN exceed the maximum within BIN distance.
		Cecidomyiidae sp.	Fall 2019, Dundas Valley	Matches with 89.66% similarity to Cecidomyiidae (BOLD:AAN5275). Genetic distance of this sequence to sequences of this BIN exceed the maximum within BIN distance.
		Cecidomyiidae sp.	Fall 2019, Dundas Valley	Matches with 89.66% similarity to Cecidomyiidae (BOLD:AAN5275). Genetic distance of this sequence to sequences of this BIN exceed the maximum within BIN distance.
		Cecidomyiidae sp.	Fall 2019, Dundas Valley	Matches with 88.3% similarity to Cecidomyiidae (BOLD: ADJ5086). There is only one sequence in this BIN.
		Cecidomyiidae sp.	Fall 2019, Cootes Paradise	Matches with 88.3% similarity to Cecidomyiidae (BOLD: ADJ5086). There is only one sequence in this BIN.
	Phoridae	*Megaselia*	Fall 2018, Sandor Property	Matches with 95.95% similarity to *Megaselia* (BOLD:AAN8683). Genetic distances of this sequence to sequences of the BIN exceed the maximum within BIN distance.
	Mycetophilidae	Mycetophilidae sp.	Summer 2019, Wirth Property	Top matches are to the species *Allodonsis rustica* with 92.11% similarity and below, then to the genus *Allodonsis* with less than 92% similarity. The top sequences are locked (private).
		Mycetophilidae sp.	Summer 2019, Wirth Property	Top matches are to the species *Allodonsis rustica* with 91.79% similarity and below, then to the genus *Allodonsis* with less than 92% similarity. The top sequences are locked (private).
Coleoptera	Staphylinidae	Staphylinidae sp.	Summer 2019, Cootes Paradise	Top match is to the species *Phanerota fasciata* with 90.65% similarity.

Coleoptera sp.	Coleoptera sp.	Summer 2019, Dundas Valley	Top match is to the family Staphylinidae with 87.88% similarity, then to the genus *Laccophilus* (family Dytiscidae) with 87.72% similarity. Rest of the matches are to various taxa within the family Staphylinidae and one from the family Dytiscidae, all with 87% similarity or less
Coleoptera sp.	Coleoptera sp.	Fall 2019, Lowrie Property	Top matches are to the species *Orthocis festivus* with 85.58% similarity and below. The next matches are to three different families within the order Coleoptera with less than 86% similarity.
Coleoptera sp.	Coleoptera sp.	Fall 2019, Lowrie Property	Top matches are to the species *Orthocis festivus* with 85.37% similarity and below. The next matches are to three different families within the order Coleoptera, then various species within the genus *Orthocis* with 84% to 85% similarity.

3.6. Summary of the arthropod species collected

Since the CO1 primers are not specific to insects, it was possible to amplify the barcoding locus from many non-insect arthropods and other animals found in association with the mushrooms. In total, 124 additional arthropods were identified from five classes: Arachnida (21 specimens), Chilopoda (two specimens), Collembola (79 specimens), Diplopoda (12 specimens) and Malacostracans (10 specimens). Eight nematode specimens that were mistaken for insect larvae were also identified (phylum Nematoda, class Chromadorea). Overall, the arthropod family Hypogastruridae (Collembola) was the most abundant with 49 specimens. The other organisms included mites and millipedes. Of the 132 total specimens, only 13 were identified to the species or genus level, while the rest had no species-level match in BOLD and were identified to family, order or class. Several specimens matched with less than 95% similarity to any sequences present in BOLD. For example, of the 49 Hypogastruridae specimens, 30 matched to this family with less than 95% similarity to the top hit.

3.7. The mushroom communities

3.7.1. Mushroom species by season and temporal patterns

Overall, the greatest number of mushroom species, genera and families were collected during fall 2018 (110 species, 64 genera and 42 families). Interestingly, over 75% of all mushroom species (159 of 204 species total) were collected during a single season, with little overlap in the taxa collected between collection periods (**Table 6**). However, most of these species were represented by a single specimen. Eight mushroom species were

collected during all three seasons: *Amanita muscaria, Gymnopus dryophilus, Pleurotus cornucopiae/pulmonarius* and *Pluteus hongoi* (order Agaricales)*, Loweomyces fractipes* (Polyporales)*, Russula aff. risigallina* (Russulales) and *Scleroderma citrinum* and *Tapinella atrotomentosa* (Boletales). Out of all possible combinations, the two fall seasons (fall 2018 and fall 2019) had the greatest overlap in the mushroom species collected, sharing 26 species in total.

Four of the main sampling locations were surveyed during all three collection seasons: Cootes Paradise, Dundas Valley and the Sandor and Wirth properties. A few temporal patterns in the mushroom community composition were observed at these locations. For example, during the fall 2018 survey of Cootes Paradise, a total of 27 mushrooms of the genus *Mycena* were collected, which accounted for 30% of the 90 mushrooms identified from Cootes Paradise during this season. In contrast, only a single *Mycena* specimen was identified during each of the summer 2019 and fall 2019 collections, representing less than 2% of all mushrooms identified during these two seasons (87 mushrooms total for the summer 2019 and 48 mushrooms for the fall 2019). In contrast, only two mushrooms from the genus *Russula* were identified from Cootes Paradise during the fall 2018 collection, compared to 13 during summer 2019 and 10 during fall 2019, representing 15% and 21% of mushrooms identified during the summer and fall 2019 seasons.

At the Sandor property, multiple species from the families Hymenogastraceae and Amanitaceae, in addition to the species *Lactarius vinaceorufescens* and *L. vinaceorufescens/chrysorrheus* (15 specimens in total, family Russulaceae) were collected

during the fall of 2018, but no species from these families were identified from either of the other two seasons. In contrast, the fall 2019 mushroom community was dominated by species from the family Inocybaceae. However, it is a bit challenging to compare seasonal patterns at this location because 111 samples were collected during the fall 2018 collection, while only 21 and 43 samples total were collected during the summer and fall seasons of 2019.

An approximately equal number of mushrooms were identified from the Wirth property from all three collection seasons. *Leucoagaricus leucothites/subcretaceus* (17 specimens) and mushrooms from the genus *Lactarius* (11 specimens) accounted for over 60% of mushrooms from the fall 2018 collection. However, none of these samples were identified from either the summer 2019 or the fall 2019 collections. Instead, the property was dominated by *Calocybe carnea/Rugosomyces persicolor* and mushrooms from the family Omphalotaceae during summer 2019 and *Inocybe* species in the fall of 2019.

Table 6: Summary of the number of mushroom species collected from each season and the number of species unique to each season. The numbers in brackets indicate the number of species represented by a single specimen. In total, approximately 204 mushroom species were collected over the course of all three seasons.

	Number of mushroom species collected	**Number of mushroom species unique to each season**
Fall 2018	110	73 *(46)*
Summer 2019	53	26 *(20)*
Fall 2019	94	60 *(41)*
Number of species collected from more than one season		**45**

3.7.2. Mushroom species by location and geographical patterns

Overall, the greatest number of mushroom taxa were collected from Cootes Paradise (77 species, 47 genera, 36 families), followed by the Sandor property. Close to 80% of mushroom species were only collected from one location, resulting in minimal overlap in species composition between sampling regions (**Table 7**). In fact, the fungal communities from each of the five main sampling locations (Cootes Paradise, Dundas Valley and the Sandor, Lowrie and Wirth properties) are highly dissimilar, according to the Bray-Curtis dissimilarity values of pairwise comparisons between regions. The lowest value, 0.8, was observed between the Lowrie and Sandor properties, meaning the mushroom communities in these two regions are only 20% similar. All other locations share less than 20% similarity with another region (**Figure 2**, **Table 8**). On average, the fungal community at the Wirth property showed the highest degree of dissimilarity to the other locations.

Less than a quarter of mushroom species were collected from two or more regions and no mushroom species was collected from all of the main sampling locations. *Leucoagaricus leucothites/subcretaceus,* with 31 specimens, was the only sample collected from four of the five main locations. In addition to having the greatest overall number of taxa, Cootes Paradise had the greatest number of unique species, genera and families.The high levels of dissimilarity in fungal communities between locations can likely be explained by the fact that over half of species collected from one location are represented by a single specimen. In general, the most commonly collected mushroom species were

obtained from multiple locations, while the rarer species were not common across multiple sampling regions. There are a few notable exceptions. For example, *Calocybe carnea/Rugosomyces persicolor*, the second-most commonly collected species with 27 specimens, was only found on the Wirth property, while *Lactarius vinaceorufescens* and *L. vinaceorufescens/chrysorrheus,* with 15 specimens combined, were only collected from the Sandor property. While no mushroom species were common to all of the main sampling locations, specimens from the genera *Lepiota* and *Mycena*, as well as the families Agaricaceae and Physalacriaceae, were collected from all regions within Hamilton and the Tillsonburg area.

Taken together, these results suggest that each geographical region has its own distinct macrofungal community at the species level. This is interesting considering that Cootes Paradise and the Dundas Valley Conservation Area are less than five kilometers away from each other, yet many species are unique to each area and the fungal communities are only about 14% similar (**Table 7**). In addition, the Wirth and Lowrie properties are less than 2% similar, with only a single mushroom species collected from both sites, even though these collection areas are about four kilometers away from each other.

Table 7: Summary of the number of mushroom species collected from each location and the number of species unique to each location. The numbers in brackets indicate the number of species represented by a single specimen. In total, approximately 204 mushroom species were collected over the course of the study.

	Number of mushroom species collected	**Number of mushroom species unique to each location**
Cootes Paradise	77	49 *(30)*
Dundas Valley	37	18 *(11)*
Lowrie Property	40	24 *(18)*
Sandor Property	51	32 *(22)*
Wirth Property	32	24 *(13)*
Other	23	15 *(12)*
Number of species collected from more than one location		**42**

Figure 2: Dendrogram showing the main sampling locations clustered based on the Bray-Curtis dissimilarity values for the mushroom communities.

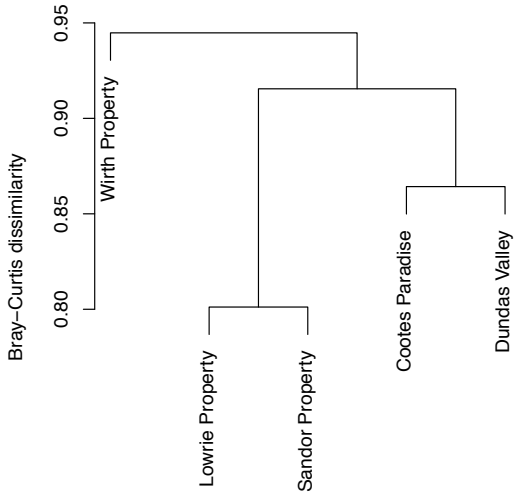


Table 8: Pairwise Bray-Curtis dissimilarity values based on the mushroom communities at all of the five main sampling locations. The Bray-Curtis dissimilarity index ranges from 0 (completely identical communities) to 1 (no shared species).

	Cootes Paradise	Dundas Valley	Lowrie Property	Sandor Property	Wirth Property
Cootes Paradise					
Dundas Valley	0.864				
Lowrie Property	0.884	0.933			
Sandor Property	0.905	0.940	0.801		
Wirth Property	0.971	0.898	0.989	0.921	

3.8. The insect communities

3.8.1. Insect species by season and temporal patterns

Overall, more insect species, genera and families were collected during both the summer 2019 and fall 2019 collection periods compared to fall 2018 (**Table 9**). Eight species from the order Diptera and one from the order Coleoptera were collected during all three seasons. Of these, *Mycetophila fungorum* and *Megaselia pulicaria* complex were the most frequently collected insects overall. Interestingly, the third and fourth most commonly collected insects, *Exechia subfrigida* (35 specimens) and *Exechia nigroscutellata* (27 specimens) were only collected during the two fall collection seasons, but not during the summer. Only 32 of the approximately 133 insect species were collected during more than one season. More than half of all species (73 out of 133) are represented by a single specimen.

There are a few temporal differences in insect abundance. For example, during the fall 2018 and fall 2019 surveys of Cootes Paradise, only five (fall 2018) and six (fall 2019) specimens from the genus *Megaselia* were identified. In contrast, fifty *Megaselia* specimens were collected during the summer 2019 survey. In addition, more than 30 insect species collected from Cootes Paradise during the summer 2019 collection were not identified during either of the fall surveys. A similar pattern was observed for Dundas Valley. For example, 19 specimens of *Exechia dorsalis* were collected during summer 2019, but none were collected during the other seasons. At the Wirth property, ten specimens each of *Megaselia* sp. n.2 SH-2015 and *Mycetophila* sp. Var. 1 were collected during fall

2018, representing a combined two thirds of all insects collected from the site during 2018. Neither insect was identified from the summer or fall 2019 collections.

Interestingly, *Mycetophila fungorum* was more abundant during the fall collections than the summer collection, with 47 and 35 specimens collected during fall 2018 and fall 2019, respectively, while only 16 specimens were identified from the summer 2019 collection.

Table 9: Summary of the number of insect species collected from each season and the number of species unique to each season. The numbers in brackets indicate the number of species represented by a single specimen. In total, 133 insect species were collected over the course of all three seasons.

	Number of insect species collected	**Number of insect species unique to each season**
Fall 2018	41	21 *(15)*
Summer 2019	69	43 *(31)*
Fall 2019	64	37 *(27)*
Number of species collected from more than one season		**32**

3.8.2. Insect species by location and geographical patterns

The greatest number of insect taxa were collected from Cootes Paradise (61 species, 25 genera and 19 families), followed by Dundas Valley and the Sandor property. Only around 25% of all insect species were collected from more than one location (**Table 10**). Interestingly, the insect communities between the main sampling locations were overall more similar than the mushroom communities. For example, while no mushroom species were collected from all of the main sampling regions, *Mycetophila fungorum* was collected from all locations. In addition, two species, *Megaselia pulicaria* complex and *Exechia subfrigida*, were each collected from four of the five sampling locations. The greater similarity in the insect communities is also reflected by the lower Bray-Curtis dissimilarity values than what was observed for the mushroom communities. Cootes Paradise and the Lowrie property had the most similar insect communities, with about 50% similarity (**Figure 3**, **Table 11**). In contrast, the mushroom communities at both locations were only about 12% similar. For every pairwise comparison between locations, the insect communities showed a greater similarity than the mushroom communities. Cootes Paradise and the Wirth property had the least similar insect communities (dissimilarity value of 0.91: 9% similarity).

Around three quarters of all insect species were collected from a single location and most of these species are represented by a single specimen. Specimens of three genera (*Exechia*, *Megaselia*, *Mycetophila*) and three families (Mycetophilidae, Phoridae, Staphylinidae) were widespread and collected from all of the five main sampling locations.

These genera and families were also the most frequently collected, accounting for 72% and 83% of all insects collected, respectively.

Table 10: Summary of the number of insect species collected from each location and the number of species unique to each location. The numbers in brackets indicate the number of species represented by a single specimen. In total, 133 insect species were collected over the course of the study.

	Number of insect species collected	**Number of insect species unique to each location**
Cootes Paradise	61	37 *(28)*
Dundas Valley	33	16 *(11)*
Lowrie Property	25	11 *(8)*
Sandor Property	31	14 *(13)*
Wirth Property	22	9 *(4)*
Other	24	10 *(9)*
Number of species collected from more than one location		**36**

Figure 3: Dendrogram showing the main sampling locations clustered based on the Bray-Curtis dissimilarity values for the insect communities.

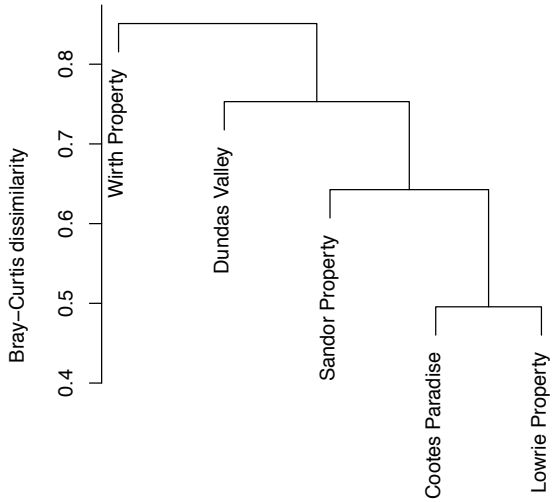


Table 11: Pairwise Bray-Curtis dissimilarity values based on the insect communities at all of the five main sampling locations. The Bray-Curtis dissimilarity index ranges from 0 (completely identical communities) to 1 (no shared species).

	Cootes Paradise	Dundas Valley	Lowrie Property	Sandor Property	Wirth Property
Cootes Paradise					
Dundas Valley	0.738				
Lowrie Property	0.496	0.691			
Sandor Property	0.685	0.829	0.600		
Wirth Property	0.91	0.803	0.865	0.826	

3.9. Mushroom - insect interactions

3.9.1. Overall summary

An interaction between a mushroom and an insect could only be mapped if both the mushroom and the associated insect were identified. Despite close to half of the collected mushrooms containing insects or non-insect arthropods, it was not possible to analyze every interaction, since multiple mushroom and insect samples were not successfully identified. Of the total 568 insects that were identified, 110 of these were duplicates, meaning larvae or insects that were sequenced from the same mushroom that were actually the same species. These duplicates were removed for the interaction analyses, as they do not represent unique interactions between the two species. An additional 49 individuals were associated with mushroom specimens (36 specimens in total) that failed to be identified. Thus, these samples could not be included in any interaction analyses. Most of these excluded insect samples were common species, meaning the same species were also found in association with successfully identified mushrooms and therefore included in the interaction analyses. However, nine specimens represented rare species that therefore could not be included in additional analyses.

The remaining 409 insect specimens were found in association with 321 identified mushrooms, representing less than half of the 766 mushroom specimens that were identified and about one third of all mushrooms collected. These 321 mushrooms are from all eight orders collected, 41 families and 57 genera. Samples from the order Agaricales were the most highly represented with 231 specimens. These mushrooms were found in

association with insects from all five orders, with the order Diptera being the most highly represented (347 specimens out of the 409 total). The insects are from 33 different families and 55 genera with 127 unique species identifications. Thus, despite the fact that not all of the identified mushrooms and insects were included in the interaction analyses, all orders and the majority of families and genera that were collected for both the mushrooms and insects are represented in the mapped interactions.

Overall, the majority of mushroom species were only found in association with one insect species (**Figure 4.1**). However, most of these species were represented by a single specimen. The mushroom species with the greatest number of documented interactions include: *Gymnopus dryophilus* (19 interactions total, 9 different insect species), *Leucoagaricus leucothites/subcretaceus* (17 interactions total, 8 different insect species) and *Amanita muscaria* (16 interactions total, 3 different insect species) When grouped by genus, the genera *Russula* and *Lactarius* have both the greatest number of total interactions and greatest number of interactions with different species (*Russula*: 38 interactions total, 14 different species; *Lactarius*: 33 interactions total, 13 different species). Similarly, the family Russulaceae has the most documented interactions with 76 interactions in total to 25 different insect species.

A similar pattern is observed for the insects, where the majority of insect species were only found in association with one mushroom species (**Figure 4.2**). Most of these are also represented by a single specimen. The insect species with the greatest number of interactions are *Mycetophila fungorum* (associated with 33 mushroom species, 19 genera, 18 families and three orders), *Megaselia pulicaria* complex (19 mushroom species, 15

genera, 12 families and three orders) and *Exechia subfrigida* (17 mushroom species, 11 genera, families and two orders) These were also the most commonly collected insect specimens, accounting for almost 50% of all 409 insects with associated identified mushrooms. Around 70 insect species were found in association with mushrooms that could not be identified to the species level because they were identified based on a photograph.

Figure 4.1: Bar plot showing the number of mushroom species that were found in association with one or more insect species. For example, 69 mushroom species were only found in association with one insect species whereas one mushroom species (*Gymnopus dryophilus*) was found in association with nine different insect species.

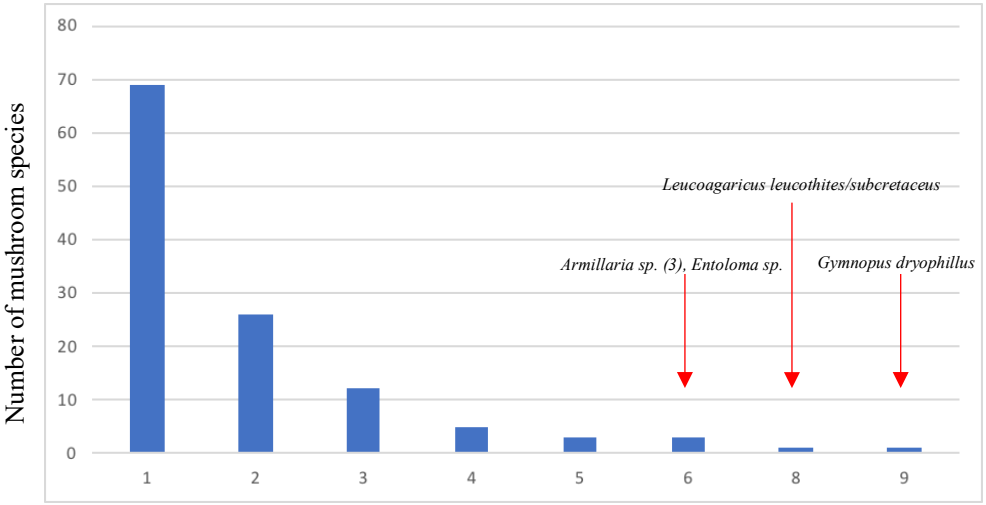
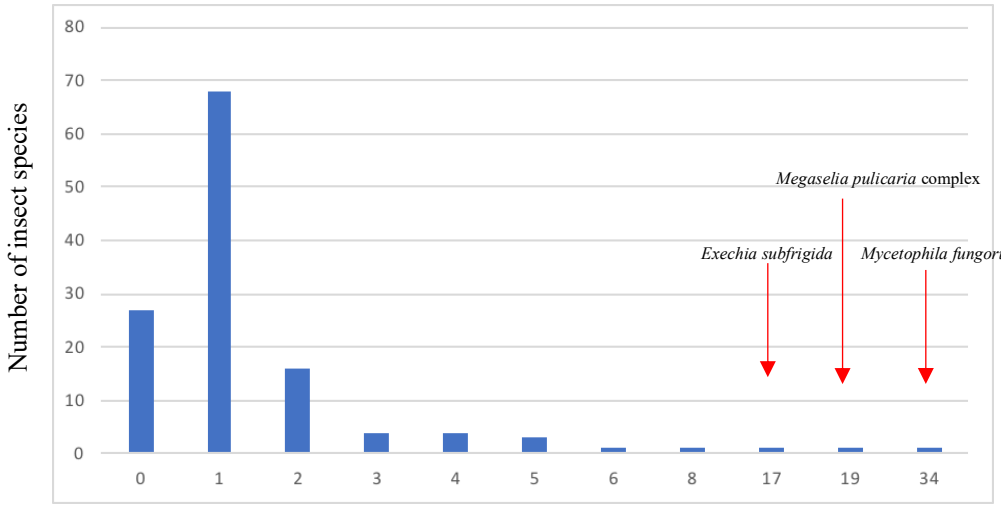


Figure 4.2: Bar plot showing the host range of insect species. The plot illustrates the number of insect species that were found in association with one or more mushroom species. For example, 68 insect species were only found in association with one mushroom species, whereas one insect species (*Mycetophila fungorum*) was found in association with 34 different insect species. Twenty-seven insect species were found in association with mushrooms that could not be identified to the species level.

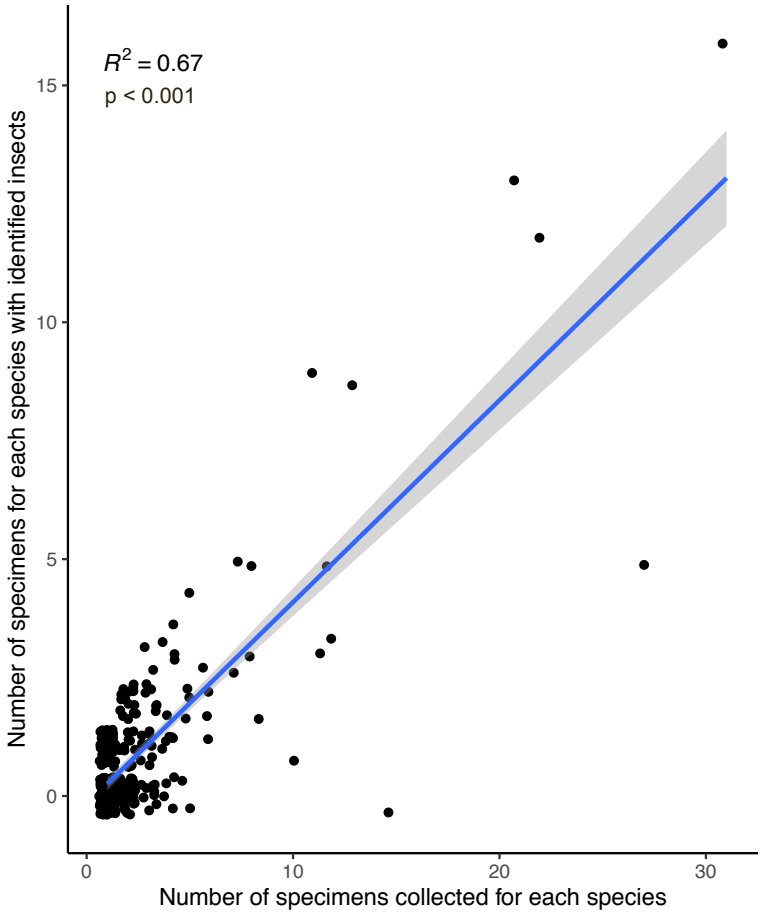


3.9.2. Correlation between number of samples and documented interactions

There is a positive correlation between the number of mushroom specimens collected for a given species and the number of specimens with an associated insect record ($R^2 = 0.67$, $p < 2.2e\text{-}16$, **Figure 5**). In general, the more frequently collected mushroom species contained more specimens for which an insect was identified. Thus, these species are more highly represented in the interaction analyses. For example, a total of 16 *Leucoagaricus leucothites/subcretaceus* (and *Leucoagaricus cf. leucothites/subcretaceus*) specimens have an associated insect record. This was also the most commonly collected mushroom species, with 31 specimens in total. However, there are a few exceptions. Twenty-seven *Calocybe carnea/Rugosomyces persicolor* specimens were collected, yet only five have an associated insect record. Notes that were taken as the mushrooms were dissected suggest that an additional three specimens contained at least one larva each, but these insects were not successfully identified. Other exceptions include *Lactarius vinaceorufescens* (12 specimens collected, three with identified insects), *Scleroderma citrinum* (10 specimens collected, one with an identified insect) and *Tapinella atrotomentosa* (15 specimens collected, none with an identified insect). None of the *L. vinaceorufescens* or *S. citriunum* specimens that lack an insect record were observed to contain any insects upon collection. Two *T. atrotomentosa* samples likely contained insects, based on recorded observations, but none of these were identified. Overall, the majority of mushroom species collected are represented by less than five specimens, with only a few of these specimens containing an identified insect.

Generally, only one insect species was identified from each individual mushroom fruiting body. However, 49 mushroom specimens contained two different insect species within the same fruiting body. Similarly, 13 mushroom specimens contained three insect species and seven specimens contained four insect species. One specimen, the only specimen identified as *Omphalotus cf. illudens*, was found in association with six different insect species from six families and two orders. Various Diptera species were commonly found together in the same fruiting body. For example, multiple mushrooms were inhabited two different *Megaselia* or *Exechia* species.

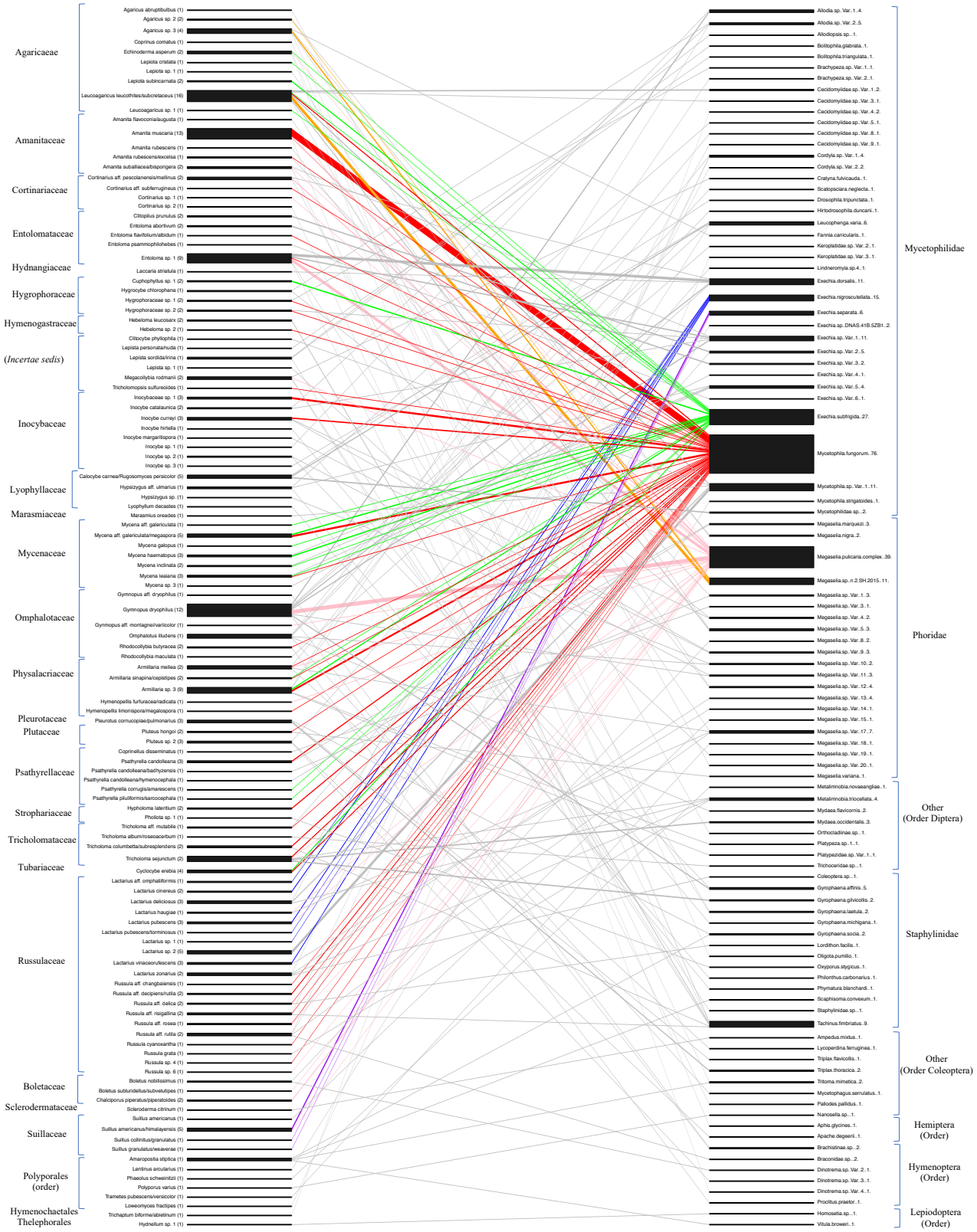
Figure 5: Scatterplot of the number of specimens collected for a given species with the number of specimens that have an associated insect record. In general, the more specimens that were collected for a given species, the more specimens there were with an associated insect record.



3.10. Visualizing the network

Figure 6 provides a visual representation of the documented interactions between the mushroom and insect species, incorporating 308 interactions in total. The species are organized by order, family and genus, with the mushroom species along the left and the insect species on the right. Each line represents an interaction, with the thickness proportional to the number of documented interactions between those two species. The wide host ranges of *Mycetophila fungorum* (red), *Megaselia pulicaria* complex (pink) and *Exechia subfrigida* (green) are illustrated by the multiple lines radiating from these species to several different mushroom species. **Supplementary Figure 1** provides a complete summary of the number of interactions documented between all species, represented as a matrix. The greatest number of documented interactions between any two species was observed between *Amanita muscaria* and *Mycetophila fungorum*, with 12 total interactions, followed by six between both *Gymnopus dryophyllus* and *Megaselia pulicaria* complex and *Leucoagaricus leucothites/subcretaceus* and *Megaselia* sp. n2 SH 2015.

Figure 6 (next page): Web of 308 interactions documented between mushroom and insect species. The mushroom species are on the left side, arranged by family and order. Numbers in brackets indicate the number of specimens. Insect species are on the right side, arranged by family (not every family is labelled). The last number at the end each species label indicates the number of specimens collected. The size of each box is proportional to the number of individual specimens with a documented interaction. Lines connect species that were found in association with one another, with the thickness proportional to the number of interactions. The coloured lines highlight interactions that will be discussed in the next sections.


Agaricaceae
Amanitaceae
Cortinariaceae
Entolomataceae
Hydnangiaceae
Hygrophoraceae
Hymenogastraceae
(Incertae sedis)
Inocybaceae
Lyophyllaceae
Marasmiaceae
Mycenaceae
Omphalotaceae
Physalacriaceae
Pleurotaceae
Plutaceae
Psathyrellaceae
Strophariaceae
Tricholomataceae
Tubariaceae
Russulaceae
Boletaceae
Sclerodermataceae
Suillaceae
Polyporales (order)
Hymenochaetales
Thelephorales
Agaricus abruptibulbus (1)
Agaricus sp. 2 (2)
Agaricus sp. 3 (4)
Coprinus comatus (1)
Echinoderma asperum (2)
Lepiota cristata (1)
Lepiota sp. 1 (1)
Lepiota subincarnata (2)
Leucoagaricus leucothites/subcretaceus (16)
Leucoagaricus sp. 1 (1)
Amanita flavoconia/augusta (1)
Amanita muscaria (13)
Amanita rubescens (1)
Amanita rubescens/excelsa (1)
Amanita suballiacea/bisporigera (2)
Cortinarius aff. pescolanensis/mellinus (2)
Cortinarius aff. subferrugineus (1)
Cortinarius sp. 1 (1)
Cortinarius sp. 2 (1)
Clitopilus prunulus (2)
Entoloma abortivum (2)
Entoloma flavifolium/albidum (1)
Entoloma psammophilohebes (1)
Entoloma sp. 1 (9)
Laccaria striatula (1)
Cuphophyllus sp. 1 (2)
Hygrocybe chlorophana (1)
Hygrophoraceae sp. 1 (2)
Hygrophoraceae sp. 2 (2)
Hebeloma leucosarx (2)
Hebeloma sp. 2 (1)
Clitocybe phyllophila (1)
Lepista personata/nuda (1)
Lepista sordida/irina (1)
Lepista sp. 1 (1)
Megacollybia rodmanii (2)
Tricholomopsis sulfureoides (1)
Inocybaceae sp. 1 (3)
Inocybe catalaunica (2)
Inocybe curreyi (3)
Inocybe hirtella (1)
Inocybe margaritispora (1)
Inocybe sp. 1 (1)
Inocybe sp. 2 (1)
Inocybe sp. 3 (1)
Calocybe carnea/Rugosomyces persicolor (5)
Hypsizygus aff. ulmarius (1)
Hypsizygus sp. (1)
Lyophyllum decastes (1)
Marasmius oreades (1)
Mycena aff. galericulata (1)
Mycena aff. galericulata/megaspora (5)
Mycena galopus (1)
Mycena haematopus (3)
Mycena inclinata (2)
Mycena lealana (3)
Mycena sp. 3 (1)
Gymnopus aff. dryophilus (1)
Gymnopus dryophilus (12)
Gymnopus aff. montagnei/varicolor (1)
Omphalotus illudens (1)
Rhodocollybia butyracea (2)
Rhodocollybia maculata (1)
Armillaria mellea (2)
Armillaria sinapina/cepistipes (2)
Armillaria sp. 3 (9)
Hymenopellis furfuracea/radicata (1)
Hymenopellis limonispora/megalospora (1)
Pleurotus cornucopiae/pulmonarius (3)
Pluteus hongoi (2)
Pluteus sp. 2 (3)
Coprinellus disseminatus (1)
Psathyrella candolleana (3)
Psathyrella candolleana/badhyzensis (1)
Psathyrella candolleana/hymenocephala (1)
Psathyrella corrugis/amarescens (1)
Psathyrella piluliformis/sarcocephala (1)
Hypholoma lateritium (2)
Pholiota sp. 1 (1)
Tricholoma aff. mutabile (1)
Tricholoma album/roseoacerbum (1)
Tricholoma columbetta/subresplendens (2)
Tricholoma sejunctum (2)
Cyclocybe erebia (4)
Lactarius aff. omphaliiformis (1)
Lactarius cinereus (2)
Lactarius deliciosus (3)
Lactarius haugiae (1)
Lactarius pubescens (3)
Lactarius pubescens/torminosus (1)
Lactarius sp. 1 (1)
Lactarius sp. 2 (5)
Lactarius vinaceorufescens (3)
Lactarius zonarius (2)
Russula aff. changbaiensis (1)
Russula aff. decipiens/rutila (2)
Russula aff. delica (2)
Russula aff. risigallina (2)
Russula aff. rosea (1)
Russula aff. rutila (2)
Russula cyanoxantha (1)
Russula grata (1)
Russula sp. 4 (1)
Russula sp. 6 (1)
Boletus nobilissimus (1)
Boletus subluridellus/subvelutipes (1)
Chalciporus piperatus/piperatoides (2)
Scleroderma citrinum (1)
Suillus americanus (1)
Suillus americanus/himalayensis (5)
Suillus collinitus/granulatus (1)
Suillus granulatus/weaverae (1)
Amaropostia stiptica (1)
Lentinus arcularius (1)
Phaeotus schweinitzii (1)
Polyporus varius (1)
Trametes pubescens/versicolor (1)
Loweomyces fractipes (1)
Trichaptum biforme/abietinum (1)
Hydnellum sp. 1 (1)
Mycetophilidae
Phoridae
Other (Order Diptera)
Staphylinidae
Other (Order Coleoptera)
Hemiptera (Order)
Hymenoptera (Order)
Lepiodoptera (Order)
Allodia.sp..Var..1..4.
Allodia.sp..Var..2..5.
Allodiopsis.sp..1.
Bolitophila.glabrata..1.
Bolitophila.triangulata..1.
Brachypeza.sp..Var..1..1.
Brachypeza.sp..Var..2..1.
Cecidomyiidae.sp..Var..1..2.
Cecidomyiidae.sp..Var..3..1.
Cecidomyiidae.sp..Var..4..2.
Cecidomyiidae.sp..Var..5..1.
Cecidomyiidae.sp..Var..8..1.
Cecidomyiidae.sp..Var..9..1.
Cordyla.sp..Var..1..4.
Cordyla.sp..Var..2..2.
Cratyna.fulvicauda..1.
Scatopsciara.neglecta..1.
Drosophila.tripunctata..1.
Hirtodrosophila.duncani..1.
Leucophenga.varia..6.
Fannia.canicularis..1.
Keroplatidae.sp..Var..2..1.
Keroplatidae.sp..Var..3..1.
Lindneromyia.sp.4..1.
Exechia.dorsalis..11.
Exechia.nigroscutellata..15.
Exechia.separata..6.
Exechia.sp..DNAS.41B.SZB1..2.
Exechia.sp..Var..1..11.
Exechia.sp..Var..2..5.
Exechia.sp..Var..3..2.
Exechia.sp..Var..4..1.
Exechia.sp..Var..5..4.
Exechia.sp..Var..6..1.
Exechia.subtrigida..27.
Mycetophila.fungorum..76.
Mycetophila.sp..Var..1..11.
Mycetophila.strigatoides..1.
Mycetophilidae.sp..2.
Megaselia.marquezi..3.
Megaselia.nigra..2.
Megaselia.pulicaria.complex..39.
Megaselia.sp..n.2.SH.2015..11.
Megaselia.sp..Var..1..3.
Megaselia.sp..Var..3..1.
Megaselia.sp..Var..4..2.
Megaselia.sp..Var..5..3.
Megaselia.sp..Var..8..2.
Megaselia.sp..Var..9..3.
Megaselia.sp..Var..10..2.
Megaselia.sp..Var..11..3.
Megaselia.sp..Var..12..4.
Megaselia.sp..Var..13..4.
Megaselia.sp..Var..14..1.
Megaselia.sp..Var..15..1.
Megaselia.sp..Var..17..7.
Megaselia.sp..Var..18..1.
Megaselia.sp..Var..19..1.
Megaselia.sp..Var..20..1.
Megaselia.variana..1.
Metalimnobia.novaeangliae..1.
Metalimnobia.tricellata..4.
Mydaea.flavicornis..2.
Mydaea.occidentalis..3.
Orthocladiinae.sp..1.
Platypeza.sp..1..1.
Platypezidae.sp..Var..1..1.
Trichoceridae.sp..1.
Coleoptera.sp..1.
Gyrophaena.affinis..5.
Gyrophaena.gilvicollis..2.
Gyrophaena.laetula..2.
Gyrophaena.michigana..1.
Gyrophaena.socia..2.
Lordithon.facilis..1.
Oligota.pumilio..1.
Oxyporus.stygicus..1.
Philonthus.carbonarius..1.
Phymatura.blanchardi..1.
Scaphisoma.convexum..1.
Staphylinidae.sp..1.
Tachinus.fimbriatus..9.
Ampedus.mixtus..1.
Lycoperdina.ferruginea..1.
Triplax.flavicollis..1.
Triplax.thoracica..2.
Tritoma.mimetica..2.
Mycetophagus.serrulatus..1.
Pallodes.pallidus..1.
Nanosella.sp..1.
Aphis.glycines..1.
Apache.degeerii..1.
Brachistinae.sp..2.
Braconidae.sp..2.
Dinotrema.sp..Var..2..1.
Dinotrema.sp..Var..3..1.
Dinotrema.sp..Var..4..1.
Procilitus.praetor..1.
Homosetia.sp..1.
Vitula.broweri..1.

3.11. Interesting interaction patterns

There are a few potentially interesting patterns that emerged in the interactions. First, the insect species *Exechia nigroscutellata* has nine documented interactions, all with various *Lactarius* species, illustrated in blue in **Figure 6**. When the mushrooms are grouped by genus, 11 interactions were documented between *E. nigroscutellata* and the genus *Lactarius*, with three additional interactions between this insect and the genus *Russula*. This insect was not found in association with any mushroom species outside of the family Russulaceae. Similarly, *Exechia separata* was only found in association with *Suillus* species, with six interactions in total. These interactions are highlighted in purple in **Figure 6.** *Megaselia* sp. n.2 SH-2015 has a total of nine documented interactions, but only with three mushroom species from the family Agaricaceae (*Agaricus* sp. 2, *Agaricus* sp. 3 and *Leucoagaricus leucothites/subcretaceus*, illustrated in orange in **Figure 6**). When considering all mushrooms identified to the family level, the number of interactions between this insect and the family Agaricaceae increases to 11 in total, with no documented interactions to any other family.

While *Mycetophila fungorum* has a large range and interacts with 33 different mushroom species, the greatest number of all documented interactions are between *M. fungorum* and *Amanita muscaria* (12 interactions), suggesting this species may show some preference towards this species. This is illustrated by the thick red line in **Figure 6** between these two species. When the mushrooms are grouped by genus, *M. fungorum* has 16 documented interactions with the genus *Russula* and 15 interactions with the genus *Amanita*. Interestingly, *Amanita muscaria* is a toxin-producing mushroom that is poisonous

and hallucinogenic to humans. Additional toxin-producing mushrooms that are lethal to humans and also contained insects include *Amanita bisporigera/suballiacea, Lepiota subincarnata* and possibly *Lepiota cristata. Galerina marginata* is also lethal, but no interactions were documented for this species.

3.12. Network Analyses

A total of 409 interactions were documented between identified mushrooms and insects. However, as described above, multiple mushroom specimens were only identified to the genus, family or order level and are therefore missing identifications at lower taxonomic levels. Thus, it was not possible to analyze all 409 interactions when considering taxa identified to the species-level. The overall total number of interactions that could be examined differed depending on how the mushroom and insect taxa were grouped. Any combination involving mushrooms grouped at the order-level with insects identified to the species, family or order-level allowed for all 409 interactions to be included (**Table 12**). However, given that there are eight mushroom orders represented in the interactions and the majority of samples are from the order Agaricales, grouping the mushrooms by order level would not be that meaningful for most analyses. Thus, the species-species network was the focus for the majority of the network analyses.

3.12.1. Network and species-level specialization

Species-species network

When mushroom and insect are considered at the species level, the network is moderately generalized (H'_2 = 0.341). However, the network is significantly more specialized than the expected networks based on comparisons to the null model (mean H'_2

for 1,000 null networks is 0.095 ± 0.018, Z >1.96). When considering degree of specialization (d′) of the mushroom and insect species individually, only 13 of the 120 mushroom species had significantly specialized interactions. The majority of these 13 species had a d′ value greater than 0.8, indicating a high level of specialization, and most are represented by fewer than five specimens. The d′ value was also significant for two of the most commonly collected species that have the greatest number of documented interactions: *Gymnopus dryophilus* (d′ = 0.52) and *Leucoagaricus leucothites/subcretaceus* (d′ = 0.56). Fewer insect species showed significantly specialized interactions with seven species in total: *Exechia nigroscutellata* (d′ = 0.89), *Exechia separata* (d′ = 0.94), *Exechia* sp. Var. 2 (d′ = 0.92), *Exechia* sp. Var. 3 (d′ = 1), *Megaselia* sp. Var. 12 (d′ = 1), *Mycetophila fungorum* (d′ = 0.56) and *Mycetophila* sp. Var. 1 (d′ = 0.76).

In total, 21 insect species and 22 mushroom species had a specialization index of 1 (i.e. the insect species only interacts with a subset of mushroom species, which in return only interact with that insect species). Of these, 19 involved mushroom and insect species each represented by a single specimen. These 19 mushroom and insect species were therefore removed from the interaction matrix. The H'_2 value was calculated again to observe the effect on the network-level specialization if single species involved in highly specialized interactions were removed. The H'_2 value decreased slightly to 0.338 (mean H'_2 for 1,000 null networks is 0.107± 0.018, Z > 1.96), which was still significantly more specialized than the null models.

Networks with additional species groupings

Network-level specialization was also calculated for networks where mushrooms were grouped by genus, family and order to allow for the inclusion of more interactions than the species-species network. For all networks, insects were analyzed at the species level in order to retain as much specificity as possible with the interactions. All networks were significantly specialized with the highest H'_2 value achieved when the mushrooms were grouped by order (H'_2 is 0.624, **Table 13.1**). In general, network-level specialization increased as the mushrooms were grouped by higher taxonomic levels.

For a stronger comparison of how H'_2 values change as mushrooms are grouped to different taxonomic levels, a reduced dataset was created to only include mushroom specimens with a complete identification (i.e. order, family, genus and species-level identifications). This reduced dataset included 240 mushroom specimens (out of the total 388 identified specimens with insects) involved in a total of 304 interactions. A similar pattern was observed where the H'_2 values increased overall as the mushrooms were grouped by higher taxonomic levels (**Table 13.2**). In contrast, the degree of specialization (d') for most insect species decreased as the mushrooms were grouped by higher taxonomic levels. Average d' values for the 99 insect species were 0.628, 0.445, 0.375 and 0.136 as the mushrooms were grouped by species, genus, family and order, respectively. Thus, while the network overall becomes more specialized, the interactions of each insect species decrease in specialization.

Dipteran network

Flies were the most common organisms collected, accounting for over 85% of all insect specimens. As discussed in the Introduction, flies and beetles can use mushrooms for slightly different purposes. For example, flies use mushrooms as a place for larval development, while beetles primarily use mushrooms as a source of food. The interactions between mushrooms and these two groups of organisms could be classified as two different networks. Thus, H'_2 was calculated for a reduced network that included only flies (order Diptera) and their associated mushrooms. This network was also significantly specialized with an H'_2 value of 0.399 (mean H'_2 for 1,000 null networks is 0.118± 0.021, $Z > 1.96$). This network is slightly more specialized than the network that includes all insect species.

Networks for different mushroom families

Finally, network level specialization indices were calculated for all species within the most commonly collected mushroom families. This is potentially not very informative, since fewer than 45 interactions could be included in each of these networks, which is less than 10% of all 409 interactions. **Table 13.3** summarizes the H'_2 values for the five most abundant mushroom families: Agaricaceae, Amanitaceae, Entolomataceae, Omphalotaceae and Russulaceae. The networks were all significantly more specialized than 1,000 null networks ($Z > 1.96$), except for the family Agaricaceae. Interestingly, networks for these six mushroom families were more specialized than the species-species network incorporating all mushroom species collected. The family Entolomataceae had the most specialized network ($H'_2 = 0.88$), though only 20 interactions were included.

Table 12: Total number of interactions incorporated into each network based on how the mushroom and insect taxa are grouped. The maximum number of interactions is 409, which are all incorporated when the mushrooms are grouped by order and the insects are grouped by species, family or order.

	Insect Species	**Insect Genera**	**Insect Families**	**Insect Orders**
Mushroom Species	308	296	308	308
Mushroom Genera	370	355	370	370
Mushroom Families	390	370	390	390
Mushroom Orders	409	388	409	409

Table 13.1: H'_2 values for networks involving mushrooms grouped by species, genus, family and order and their corresponding insect species. * means significant based on a comparison to 1,000 null networks.

Network	H'_2	Statistics
Mushroom species/insect species	0.341*	Null mean: 0.095 (± 0.018) **Z = 13.68**
Mushroom genera/insect species	0.412*	Null mean: 0.116 (±0.015) **Z = 19.43**
Mushroom family/insect species	0.398*	Null mean: 0.128 (±0.016) **Z = 17.18**
Mushroom order/insect species	0.624*	Null mean: 0.250 (±0.029) **Z = 12.79**

Table 13.2: H'_2 values for networks involving mushrooms grouped by species, genus, family and order and their corresponding insect species for a reduced dataset. * means significant based on comparisons to 1,000 null networks.

Network	H'_2	Statistics
Mushroom species/insect species	0.344*	Null mean: 0.095 (±0.017) **Z = 14.40**
Mushroom genera/insect species	0.436*	Null mean: 0.140 (±0.016) **Z = 18.10**
Mushroom family/insect species	0.410*	Null mean: 0.148 (±0.017) **Z = 15.44**
Mushroom order/insect species	0.627*	Null mean: 0.252 (±0.041) **Z = 9.081**

Table 13.3: H'_2 values for networks involving the five most commonly collected mushroom families. * means significant based on comparisons to 1,000 null networks.

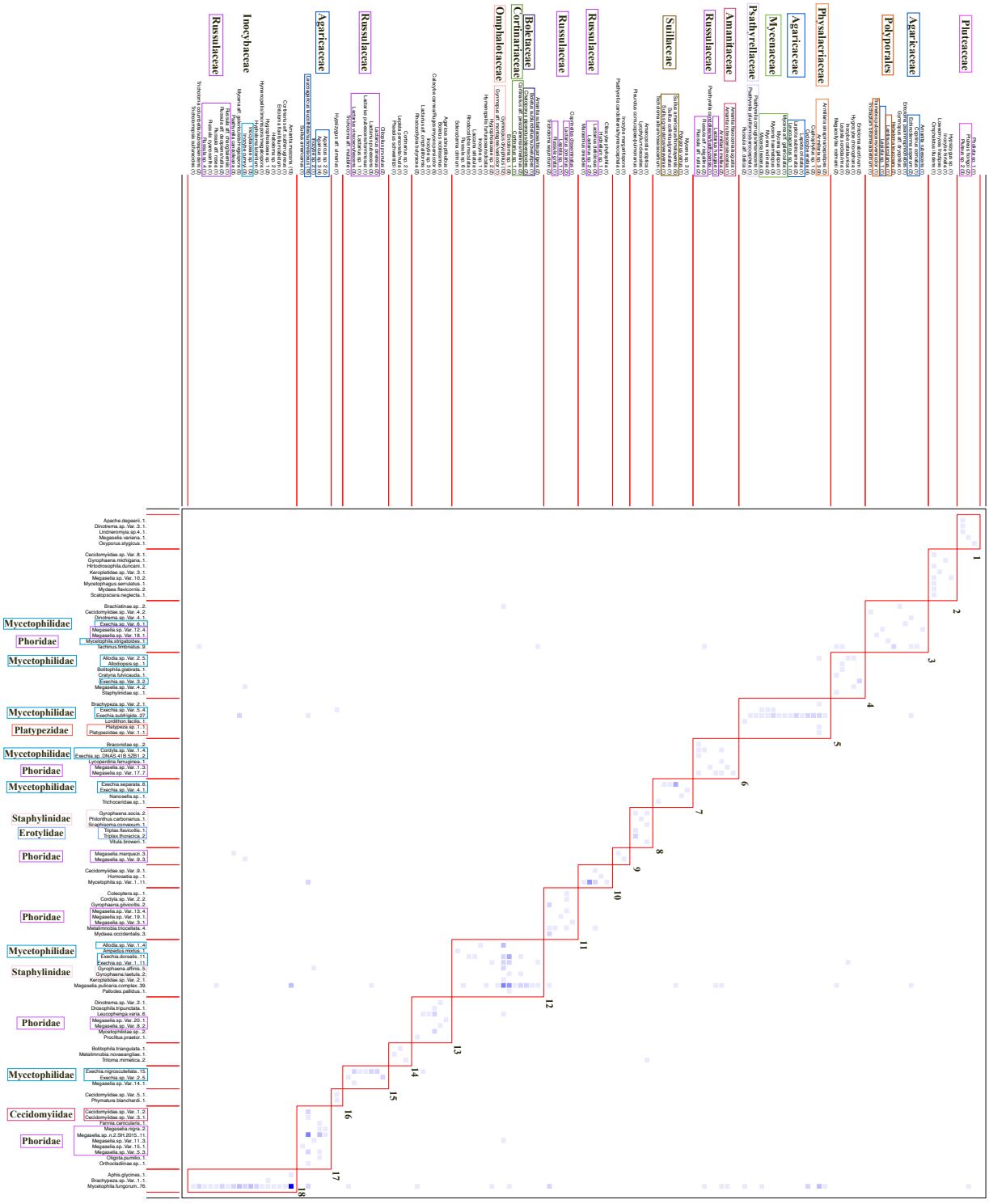
Mushroom family	Number of specimens with species-level identifications	Number of interactions in network	H'_2	Statistics
Agaricaceae	31	37	0.379 (n.s.)	Mean: 0.244 (±0.077) **Z = 1.760**
Amanitaceae	18	23	0.772*	Mean: 0.229 (±0.160) **Z = 3.395**
Entolomataceae	15	20	0.881*	Mean: 0.258 (±0.147) **Z = 4.240**
Omphalotaceae	18	30	0.672*	Mean: 0.379 (±0.127) **Z = 2.303**
Russulaceae	28	45	0.387*	Mean: 0.1443 (±0.059) **Z = 4.142**

3.12.2. Modularity

Modularity quantifies the structure of a network. The average modularity value for 10 calculations based on the species-species network was 0.715 (±0.002). These networks are significantly more modular than the expected modularity for 50 null networks (mean 0.56± 0.013, $Z > 1.96$). Between the 10 computations of modularity, the number of modules ranged from 18 to 41, with an average of about 27 modules per run. The exact species assigned to each module also changed. **Figure 7** shows the 18 modules for the trial that had the highest modularity value ($Q = 0.72$, slightly higher than the other nine trials). Module #18 contains the greatest number of mushroom species with 18 species in total. All mushrooms within this module associate with *Mycetophila fungorum*.

Overall, there is no clear association between taxonomy and module placement. For example, five modules (modules 3, 7, 10, 12 and 13) each contain mushrooms from three different taxonomic orders. In addition, most mushrooms from the same genus and family are not found in the same module. There are a few exceptions. Three of the four *Suillus* species fall into module #7, while five of the seven total *Mycena* species are grouped together in module #5. While mushrooms from the family Russulaceae are found in seven different modules, half of all *Lactarius* species fall into module 15 and half of the *Russula* species are in module 18. For the insects, species from the families Mycetophilidae and Phoridae are widely distributed across the modules and each family is represented in 11 out of the 18 total modules. Module 17 contains the greatest number of *Megaselia* species, accounting for half of all species from this genus.

Figure 7: Module plot for the trial that resulted in the highest modularity value. Mushroom and insect species within each red box interact more frequently with each other than species in a different module. Every blue box is an interaction with the darker colour meaning more interactions. Mushrooms are on the top (left-hand side when the page is rotated sideways.



3.12.3. Quantifying taxonomic similarity between species within modules

To quantify the similarity between species within each module, mean pairwise genetic distances of species in each module were calculated. Unrooted Neighbour-Joining trees were constructed based on the resulting ITS and CO1 alignments to check for inconsistencies in the placement of taxa. **Figure 8.1** shows the unrooted Neighbour-Joining tree for the mushroom samples based on the ITS sequence. With a few exceptions, species from each genus, and for the most part each family, cluster together. This suggests that the ITS region is sufficient for a broad sorting or clustering of samples based on taxonomy (i.e. more similar species from the same family are generally separated by smaller distances). However, there are some exceptions, highlighted in red. For example, *Amanita muscaria* falls into a different clade than the other four *Amanita* species, clustering with *Cyclocybe erebia* instead. Similarly, two Inocybaceae samples (Inocybaceae sp.1 and *Inocybe curreyi*) are in a separate clade than the other three Inocybe species. The species *Trichaptum biforme/abientinum* (order Hymenochaetales), *Amaropostia stiptica* (order Polyporales), *Calocybe carnea/Rugosomyces persicolor* (family Lyophyllaceae) and *Hygrocybe chlorophana* (family Hygrophoraceae) either all fall within clades dominated by species from a different family or fail to cluster with other species of the same family.

Figure 8.2 shows the unrooted Neighbour-Joining tree for the insect samples aligned based on the CO1 sequence. Most insect taxa within the same genus and family cluster together, with a few exceptions. First, the species *Lindneromyia* sp. 4 does not fall into the same clade as the two other taxa from the family Platypezidae while the species

Fannia canicularis also falls within the same clade that is primarily composed of *Megaselia* species. None of the three species from the order Lepidotera cluster together.

Despite a few inconsistencies in both the ITS and CO1 trees, overall, most species that are closely related taxonomically show lower pairwise genetic distances and therefore cluster together, as expected. Pairwise genetic distances for species within each module were computed to examine the genetic similarity of taxa assigned to each group.

Only four out of the 18 modules (modules 1, 3, 5 and 15) shown in **Figure 7** were composed of mushroom species that had a significantly lower mean pairwise genetic distance than what would be expected based on the null models. Module 15 is composed of seven species, five of which are from the genus *Lactarius*. Module 3 is composed of ten species from three different orders, yet the mean pairwise genetic distance is significantly smaller than expected.

Since the number of modules and species assigned to each module varied between the ten modularity calculations, the mean pairwise distances within each module were calculated for the other nine trials. The maximum number of modules with a significantly lower genetic distance than the null models ranged from three (trials 9 and 10) to six (trial 3) (**Table 14**). Overall, less than one quarter of all assigned modules in each of the 10 trials contained mushroom species with a significantly lower average genetic distance than expected based on comparisons to the null model. Interestingly, the five *Lactarius* species, *Lactarius cinereus, L. pubescens*, *L. pubescens/torminosus*, *Lactarius* sp. 1, *L. vinaceorufescens,* were always assigned to the same module across all ten trials and the modules containing these species consistently had a significantly lower genetic distance

than the null expectations. There were additional groups of mushroom species that were consistently observed together in modules with significantly lower genetic distances that expected. These groups include: *Armillaria sinapina/cepistipes, Armillaria* sp. 3, *Cuphophyllus* sp.1, *Cyclocybe erebia, Lepiota cristata, L. subincarnata, Leucoagaricus* sp.1, *Mycena* aff. *galericulata*, *M. galopus*, *M. haematopus*, *Psathyrella corrugis/amarescens* and *P. piluiformis/sarcocephala* (all 10 trials), *Coprinus comatus, Echinoderma asperum, Hebeloma leucosarx* (six of the ten trials), *Suillus americanus/himalayensis, S. collinitus/granulatus. S. granulatus/weaverae* (five of the ten trials) and *Agaricus* sp. 2, *Agaricus* sp. 3, *Leucoagaricus leucothites/subcretaceus* (five of the ten trials).

The insect species within each module display a much lower degree of taxonomic similarity. For seven out of the ten trials, either zero or only one module had a significantly lower average genetic distance than expected (**Table 14**). For the trial illustrated in **Figure 7**, modules 9 and 17 were the only significant modules. Both are dominated by *Megaselia* species. Across all trials, it was not common to observe the same insect species grouped together in the modules that had significantly lower genetic distances. Thus, overall, there is a lack of taxonomic similarity between insect species within each module.

Figure 8 (next pages)**:** Neighbour-Joining trees constructed based on the ITS (**Figure 8.1**) and CO1 (**Figure 8.2**) loci. The trees were constructed to assess the ability of the ITS and CO1 regions to separate taxa based on family and to assess if genetic distance estimates based on these loci could be used to estimate the similarity between species in each module. Branch support is based on 1,000 bootstrap replications. The ITS tree was constructed using the pairwise deletion method for gaps and missing data since the ITS region is highly divergent between taxa.

Figure 8.1

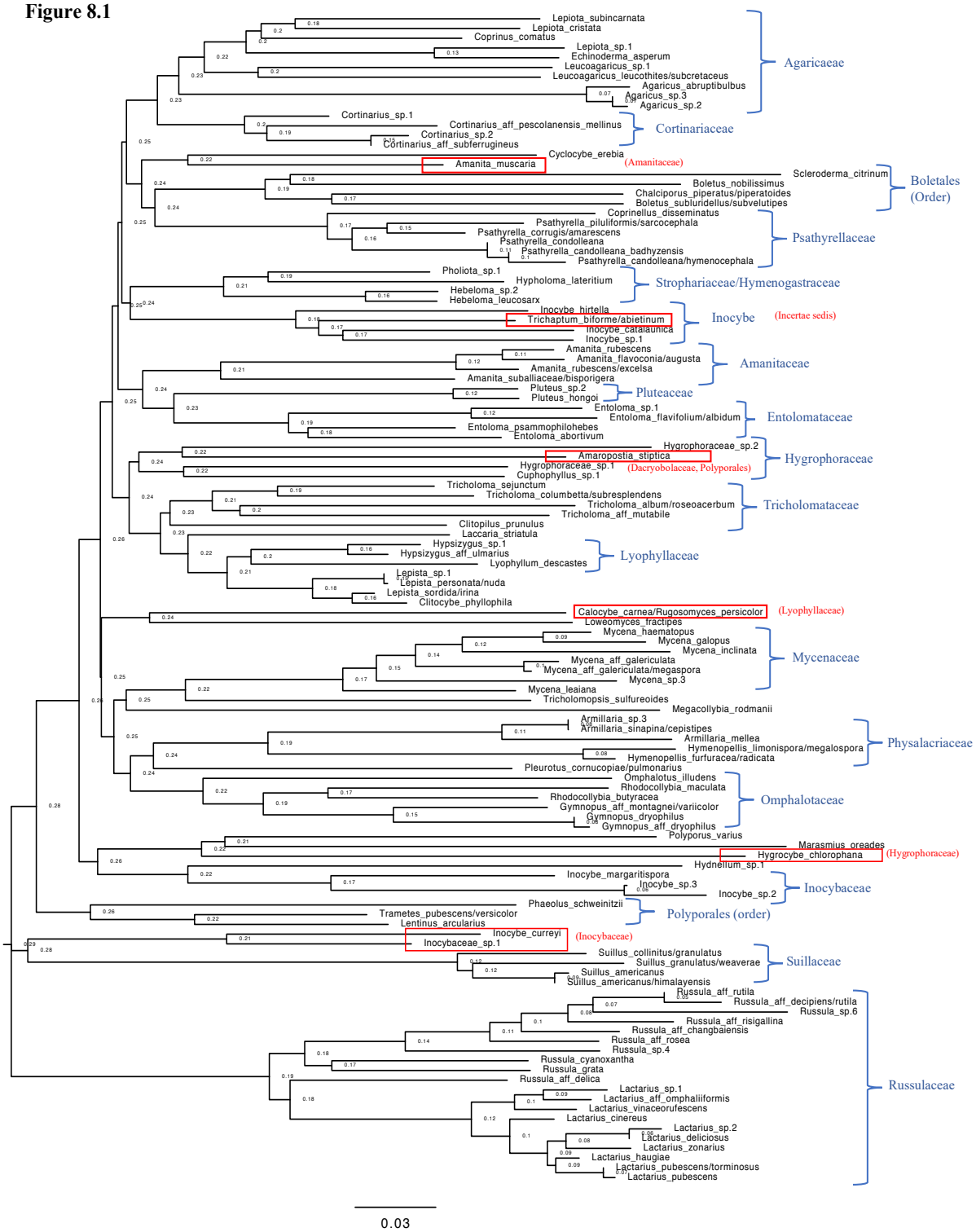

Lepiota_subincarnata
Lepiota_cristata
Coprinus_comatus
Lepiota_sp.1
Echinoderma_asperum
Leucoagaricus_sp.1
Leucoagaricus_leucothites/subcretaceus
Agaricus_abruptibulbus
Agaricus_sp.3
Agaricus_sp.2
Agaricaceae
Cortinarius_sp.1
Cortinarius_aff_pescolanensis_mellinus
Cortinarius_sp.2
Cortinarius_aff_subferrugineus
Cortinariaceae
Cyclocybe_erebia
Amanita_muscaria
(Amanitaceae)
Scleroderma_citrinum
Boletus_nobilissimus
Chalciporus_piperatus/piperatoides
Boletus_subluridellus/subvelutipes
Boletales (Order)
Coprinellus_disseminatus
Psathyrella_piluliformis/sarcocephala
Psathyrella_corrugis/amarescens
Psathyrella_condolleana
Psathyrella_candolleana_badhyzensis
Psathyrella_candolleana/hymenocephala
Psathyrellaceae
Pholiota_sp.1
Hypholoma_lateritium
Hebeloma_sp.2
Hebeloma_leucosarx
Strophariaceae/Hymenogastraceae
Inocybe_hirtella
Trichaptum_biforme/abietinum
Inocybe_catalaunica
Inocybe_sp.1
Inocybe
(Incertae sedis)
Amanita_rubescens
Amanita_flavoconia/augusta
Amanita_rubescens/excelsa
Amanita_suballiaceae/bisporigera
Amanitaceae
Pluteus_sp.2
Pluteus_hongoi
Pluteaceae
Entoloma_sp.1
Entoloma_flavifolium/albidum
Entoloma_psammophilohebes
Entoloma_abortivum
Entolomataceae
Hygrophoraceae_sp.2
Amaropostia_stiptica
Hygrophoraceae_sp.1
Cuphophyllus_sp.1
(Dacryobolaceae, Polyporales)
Hygrophoraceae
Tricholoma_sejunctum
Tricholoma_columbetta/subresplendens
Tricholoma_album/roseoacerbum
Tricholoma_aff_mutabile
Tricholomataceae
Clitopilus_prunulus
Laccaria_striatula
Hypsizygus_sp.1
Hypsizygus_aff_ulmarius
Lyophyllum_descastes
Lyophyllaceae
Lepista_sp.1
Lepista_personata/nuda
Lepista_sordida/irina
Clitocybe_phyllophila
Calocybe_carnea/Rugosomyces_persicolor
(Lyophyllaceae)
Loweomyces_fractipes
Mycena_haematopus
Mycena_galopus
Mycena_inclinata
Mycena_aff_galericulata
Mycena_aff_galericulata/megaspora
Mycena_sp.3
Mycena_leaiana
Mycenaceae
Tricholomopsis_sulfureoides
Megacollybia_rodmanii
Armillaria_sp.3
Armillaria_sinapina/cepistipes
Armillaria_mellea
Hymenopellis_limonispora/megalospora
Hymenopellis_furfuracea/radicata
Physalacriaceae
Pleurotus_cornucopiae/pulmonarius
Omphalotus_illudens
Rhodocollybia_maculata
Rhodocollybia_butyracea
Gymnopus_aff_montagnei/variicolor
Gymnopus_dryophilus
Gymnopus_aff_dryophilus
Omphalotaceae
Polyporus_varius
Marasmius_oreades
Hygrocybe_chlorophana
(Hygrophoraceae)
Hydnellum_sp.1
Inocybe_margaritispora
Inocybe_sp.3
Inocybe_sp.2
Inocybaceae
Phaeolus_schweinitzii
Trametes_pubescens/versicolor
Lentinus_arcularius
Polyporales (order)
Inocybe_curreyi
Inocybaceae_sp.1
(Inocybaceae)
Suillus_collinitus/granulatus
Suillus_granulatus/weaverae
Suillus_americanus
Suillus_americanus/himalayensis
Suillaceae
Russula_aff_rutila
Russula_aff_decipiens/rutila
Russula_sp.6
Russula_aff_risigallina
Russula_aff_changbaiensis
Russula_aff_rosea
Russula_sp.4
Russula_cyanoxantha
Russula_grata
Russula_aff_delica
Lactarius_sp.1
Lactarius_aff_omphaliiformis
Lactarius_vinaceorufescens
Lactarius_cinereus
Lactarius_sp.2
Lactarius_deliciosus
Lactarius_zonarius
Lactarius_haugiae
Lactarius_pubescens/torminosus
Lactarius_pubescens
Russulaceae
0.03

Figure 8.2


Megaselia_sp_var_5
Megaselia_sp_n2_sh2015
Megaselia_sp_var_8
Megaselia_sp_var_20
Megaselia_nigra
Megaselia_sp_var_15
Megaselia_sp_var_14
Megaselia_sp_var_13
Megaselia_sp_var_4
Megaselia_variana
Megaselia_sp_var_3
Megaselia_sp_var_1
Megaselia_sp_var_12
Megaselia_sp_var_9
Megaselia_sp_var_11
Megaselia_sp_var_10
Megaselia_pulicaria_complex
Megaselia_sp_var_17
Megaselia_sp_var_19
Megaselia_marquezi
Phoridae
Fannia_canicularis
(Fanniidae)
Lindneromyia_sp4
(Platypezidae)
Megaselia_sp_var_18
Mydaea_occidentalis
Mydaea_flavicornis
Muscidae
Leucophenga_varia
Drosophila_tripunctata
Hirtodrosophila_duncani
Drosophilidae
Metalimnobia_triocellata
Bolitophila_triangulata
Bolitophila_glabrata
Bolitophilidae
Metalimnobia_novaeangliae
Platypezidae_sp_var_1
Platypeza_sp_1
Platypezidae
Trichoceridae_sp
Orthocladiinae_sp
Mycetophila_sp_var_1
Mycetophila_fungorum
Cordyla_sp_var_2
Mycetophila_strigatoides
Mycetophilidae_sp
Allodiopsis_sp
Exechia_sp_dnas
Cordyla_sp_var_1
Brachypeza_sp_var_2
Brachypeza_sp_var_1
Allodia_sp_var_2
Allodia_sp_var_1
Exechia_subfrigida
Exechia_separata
Exechia_dorsalis
Exechia_sp_var_6
Exechia_nigroscutellata
Exechia_sp_var_1
Exechia_sp_var_2
Exechia_sp_var_3
Exechia_sp_var_5
Exechia_sp_var_4
Mycetophilidae
Scatopsciara_neglecta
Cratyna_fulvicauda
Sciaridae
Keroplatidae_sp_var_3
Keroplatidae_sp_var_2
Keroplatidae
Vitula_broweri
(Lepidoptera, Order)
Mycetophagus_serrulatus
Nanosella_sp
Oxyporus_stygicus
Scaphisoma_convexum
Gyrophaena_michigana
Gyrophaena_gilvicollis
Gyrophaena_laetula
Gyrophaena_affinis
Staphylinidae_sp.
Coleoptera_sp.
Gyrophaena_socia
Phymatura_blanchardi
Tachinus_fimbriatus
Lordithon_facilis
Philonthus_carbonarius
Ampedus_mixtus
Pallodes_pallidus
Staphylinidae
Oligota_pumilio
(Staphylinidae)
Lycoperdina_ferruginea
Triplax_thoracica
Tritoma_mimetica
Triplax_flavicollis
Erotylindae
Cecidomyiidae_sp_var_5
Cecidomyiidae_sp_var_9
Cecidomyiidae_sp_var_8
Cecidomyiidae_sp_var_4
Cecidomyiidae_sp_var_3
Cecidomyiidae_sp_var_1
Cecidomyiidae
Homosetia_sp
(Lepidoptera, Order)
Hemiptera (Order)
Apache_degeerii
Aphis_glycines
Proclitus_praetor
Brachistinae_sp
Dinotrema_sp_var_3
Braconidae_sp
Dinotrema_sp_var_4
Dinotrema_sp_var_2
Hymenoptera (Order)
0.03

Table 14: Summary of the 10 metacompute modularity trials. This table summarizes the total number of modules estimated for each trial and the modularity likelihood (Q) value for those modules. The last column summarizes the number of modules for which the mean pairwise genetic distances of mushrooms and insects within each module were significantly lower than expected based on the null models. The trial highlighted in red is the trial illustrated in **Figure 7**.

Metacompute modularity trial	Total number of modules	Modularity Likelihood (Q)	Number of modules with a significant MPD value	
1	34	0.714	Mushrooms	5
			Insects	1
2	35	0.716	Mushrooms	4
			Insects	1
3	34	0.716	Mushrooms	6
			Insects	1
4	18	0.721	Mushrooms	4
			Insects	2
5	23	0.715	Mushrooms	4
			Insects	2
6	20	0.713	Mushrooms	4
			Insects	2
7	27	0.715	Mushrooms	5
			Insects	0
8	41	0.717	Mushrooms	5
			Insects	0
9	18	0.713	Mushrooms	3
			Insects	1
10	23	0.713	Mushrooms	3
			Insects	0

4. Discussion

4.1. Overall summary

The goal of this project was to conduct a broad survey of mushroom species and their associated insects from forests in Hamilton and the Tillsonburg areas. Over the course of three collection seasons, from fall 2018 to the end of fall 2019, a diverse group of at least 200 mushroom species from approximately 53 families and eight orders were collected. These mushrooms harboured a variety of organisms including insects (approximately 133 species from five orders), mites, millipedes, springtails and nematodes. The collected mushroom and insect species varied between sampling periods and each of the five main sampling locations had their own distinct mushroom and insect communities. While there are a few highly specialized interactions, the overall network is moderately generalized. There is limited evidence to suggest that taxonomic similarity between species with similar interactions influences the structure of the network.

4.2. Mushroom identifications and patterns

4.2.1. The ITS region as a barcoding marker

Of the 1,017 mushroom samples collected, only 680 specimens were identified through DNA barcoding of the nuclear internal transcribed spacer (ITS) region. While DNA barcoding theoretically presents a rapid and efficient method for identifying species, there are many challenges associated with using the ITS region as a barcoding marker. For example, within-strain heterogeneity makes it difficult to obtain a complete ITS sequence.

In this study, 210 samples were identified based on a partial ITS sequence and another 43 samples could not be identified because the chromatograms were not readable. The ITS region is a multi-copy nuclear gene. The entire region from the large subunit ribosomal RNA to the small subunit ribosomal RNA is repeated in tandem multiple times[58]. It is common for fungal species, including mushrooms, to have different copies of the ITS region within the same fruiting body that differ from one another as a result of indels or substitutions in some of the copies[34,58-60]. It is also possible for individuals that are diploid to have inherited a different sequence variation of the ITS region from each parent. The widespread heterogeneity at the ITS region across multiple groups of fungi reduces the efficiency and ease with which it can be used to identify different species. Additional steps, such as cloning, must be used in order to obtain complete ITS sequences for these samples. Due to time constraints, it was not possible to clone every sequence that failed to give a complete, readable ITS sequence.

Despite the prevalence of heterogeneity at the ITS locus among samples collected in this study, many were still identified with a partial ITS fragment, usually the first part of the sequence which encompasses ITS1. Recent studies have shown that within the Basidiomycota, the lineage that contains most mushroom species, identifications based on either the ITS1 or ITS2 fragments individually can yield an accurate species identification[35]. On average, ITS1 is more variable than ITS2[61] and both can have a sufficient barcoding gap. However, the fragment with a more distinct barcoding gap depends on the mushroom genus[35]. Thus, while a complete ITS sequence would have ideally been obtained for each mushroom sample in this study, identifications based on partial ITS fragments are still valid.

Another challenge with using the ITS region to identify species is the overall lack of consensus about the sequence similarity cut-off that can be used to assign species identifications. One universal cut-off cannot be applied to all species. The average intraspecific genetic distance at the ITS region for Basidiomycota species is estimated to be about 3.33% with a standard deviation of 5.62[61]. However, some species can have up to a 25% intraspecific sequence divergence[61], making it difficult to define one cut-off value. In contrast, a 97% sequence similarity cut-off is too low to accurately assign species identifications for other fungal species (summarized in [53]). Without clear or universal guidelines, criteria described by Hofstetter et al. (2019) were adopted in this study, which have previously been used to identify a range of mushroom species[53]. Using this criteria, 355 specimens were identified unambiguously to the species-level. For example, sequences of *Amanita muscaria* and *Scleroderma citrinum* mushrooms consistently yielded 100% similarity matches to the top hits in UNITE with no ambiguity in the identification. For these species, the ITS region is likely a strong barcoding marker.

However, the identifications of many specimens were ambiguous between two or more species, all of which had equally high similarity matches. This suggests that the ITS region is too similar between taxa of these genera to allow for a species-level identification, even with a 99% similarity cut-off. Several of these samples were from the genera *Agaricus, Hymenopellis, Lepista, Psathyrella, Russula* and *Suillus.* This is slightly inconsistent with previous research, which has shown that the ITS region is a good barcoding marker for species within these genera, based on the high probability of correct identification and clear barcoding gap[35]. In contrast, there were multiple specimens with an ambiguous

identification from the genera *Armillaria, Hebeloma, Inocybe, Mycena, Pluteus* and *Lactarius*. Species from all of these genera are generally observed to have an intermediate or poor barcoding gap, meaning the ITS region cannot clearly separate species within these genera[35].

To overcome these challenges associated with using the ITS region, many mycologists have proposed that multiple fragments could be used to barcode mushrooms. For example, combining sequences of *RPB1* with the ITS region can be highly effective at identifying many fungal species[34]. Other markers, including *TEF1*, have also been considered, since single copy protein-coding genes are less likely to have intrastrain heterogeneity (reviewed in [62]). Thus, further analyses would be required to confirm the identifications of the ambiguous specimens collected in this study.

DNA barcoding is time consuming and to thoroughly identify each species, additional tools such as phylogenies, careful reviews of taxonomic literature and a strong understanding of fungal systematics are required[53]. In this study, analyzing the BLAST results and evaluating which species hit was the best match accounted for a large proportion of the total time spend on data analysis. Given the ambiguity in many of the species identifications, it was not possible to define one similarity cut off for all samples, which would have sped up the analysis. In addition, for 86 samples that failed to be identified through DNA barcoding, traditional morphological techniques were used instead to identify the mushrooms based on photographs. However, despite these challenges, DNA barcoding is a valuable method for species identification. It would have been practically impossible to identify these 680 mushroom specimens using morphological techniques,

given our lack of extensive knowledge and experience in morphological-based mushroom identification and taxonomy.

4.2.2. Mushrooms of Southern Ontario

Over 200 mushroom species from 53 families and eight orders were collected from forests in Hamilton and the Tillsonburg regions, with mushrooms from the order Agaricales being the most common, followed by Russulales. The diversity of macrofungi within Southern Ontario is fairly well studied. At least two field guides have been published about the mushrooms of Ontario[63,64], both primarily focusing on edible mushrooms, and additional books and government documents have been written about mushrooms from across Canada and North America[9,65]. The Mycological Society of Toronto is one of many groups that frequently lead mushroom forays throughout Southern Ontario covering forests in St. Catherines, Cambridge, Guelph, the GTA, Barrie, Orangeville, Shelburne and areas in between. The mushrooms collected from each foray are documented (https://www.myctor.org/forays/past-forays). Over 600 species have been collected so far, with mushrooms from the orders Agaricales and Russulales accounting for over two thirds of the species collected. This is consistent to what was observed during this study. During spring 2019, the most recent report available, 114 different species were collected over the course of 15 forays from the Toronto area. Thus, it is not surprising that in this study, over 200 mushroom species were collected from just five main sampling locations. Many of the mushroom species and genera collected during this study have been previously documented during the forays over the past decade and a half. Interestingly though, several of the most commonly collected species during this study are not included on the foray list, such as

Leucoagaricus leucothites/subcretaceus, Calocybe carnea/Rugosomyces persicolor and *Tapinella atrotomentosa.* Foray records such as these could potentially be a valuable resource for examining large-scale distribution patterns of mushroom diversity across the province.

4.2.3. Potentially new and uncharacterized mushroom species

Multiple mushrooms could not be identified to the species level based on the ITS sequence. This was unexpected, given that the mushrooms within the Great Lakes Region, including Ontario, are well-documented. For example, twenty-three specimens matched with high similarity (98% to 100%) to sequences within UNITE that were identified to the genus or family level. Thus, these sequences, or highly similar sequences, are already documented in UNITE, but without a species-level identification or a match to one of the species hypotheses. An additional thirty-seven sequences generated in this study have no high similarity match in UNITE. All 37 sequences matched with 95% similarity or less to any sequence currently in the database, suggesting that these are new ITS sequences that have not yet been deposited into UNITE. As such, none of these samples could be identified to the species level, given the criteria outlined in the Methods section.

There are three possible explanations for the lack of a species-level identification or high similarity match in UNITE. First, it is possible that some of these sequences represent mushroom species that have previously been identified morphologically, but no representative ITS sequence has yet been obtained. For example, while there are an estimated 2.2 to 3.8 million fungal species, there are only about 459,000 species hypotheses in UNITE[50]. It is therefore plausible that samples collected in this study have no match in

UNITE because the UNITE database is incomplete. A second possibility is that these are species that lack a clear barcoding gap. For the majority of these sequences, there were low similarity matches to different species. For example, one specimen from the Sandor property matched with 94% similarity to *Crepidotus subverrucisporus* as the top match (**Table 4**). The ITS region often cannot be used to differentiate *Crepidotus* species due to high levels of intraspecific variation within species of this genus[35]. Similarly, among species within the *Mycena* and *Inocybe* genera, high levels of intraspecific variation have been reported, meaning the ITS region shows an intermediate barcode gap[35]. In this study, two *Inocybe* specimens and two *Mycena* specimens matched with 95% similarity or less to the top matches within UNITE. Therefore, identifying these species would require a closer examination of the intraspecific variation at the ITS locus for species within these genera.

The third possibility is that some of these sequences represent new, undocumented species. For example, two specimens matched with less than 88% similarity to multiple species from different families and were therefore identified to the order Agaricales. There were also multiple specimens from the genera *Hygrocybe*, *Entoloma* and *Russula* that lacked a high similarity species-level match in UNITE (**Table 4**). The ITS region shows a clear barcoding gap overall for species within these genera, meaning it should be a strong enough marker to distinguish between species[35]. Therefore, the lack of a species-level identification in UNITE could suggest that the specimens represent new species. However, additional morphological analyses and comparisons with closely related species are needed to confirm their taxonomic uniqueness. Mycologists typically evaluate a range of mushroom properties to identify mushrooms including fruiting body size, colour, texture,

odour, shape of the cap and pilus, veil remnants and the shape and position of the annulus (the ring around the stipe of the mushroom), in addition to multiple microscopic characterizations of the hyphae and spores[9]. The samples collected in this study have been dissected and dried. While some micromorphological features may be obtained from these dried specimens, fresh samples are needed to characterize other morphological characteristics of these mushrooms.

Regardless, it was an interesting observation that several of these ITS sequences have not yet been documented in UNITE. As a next step, full sequences of these samples will be submitted to GenBank. In all, these findings suggest that the diversity of mushrooms in Southern Ontario could be greater than what has previously been documented.

4.3. Insect identifications and patterns

4.3.1. CO1 as a barcoding locus

Just over 1,100 animals were collected from within and on the surface of 1,017 mushrooms obtained during this study. Of these, 568 insects and 124 non-insect arthropods and nematodes were successfully identified using sequences of the partial CO1 gene. While the CO1 region is known to have a high amplification and sequencing success rate across a range of insect orders[38], only about 58% of animals collected (692 out of 1,174) were identified. This can likely be attributed to a low DNA extraction rate for many of the organisms collected, particularly the small larvae for which there was not enough sample to extract sufficient DNA for analysis using the CTAB extraction method.

The CO1 marker has a number of advantages over the ITS region in its utility for barcoding. First, in contrast to the ITS region, intrastrain heterogeneity within CO1

sequences is rare. The mitochondrial genome is generally inherited uniparentally and CO1 exists as a single-copy gene on the mitochondrial genome. While there were a few CO1 chromatograms with double, unreadable peaks, this was most likely because DNA from more than one individual was extracted and amplified. Thus, the vast majority of CO1 sequences were clean, readable and unambiguous throughout the whole read. Second, identifying the insects and other animals was much faster than identifying the mushrooms, largely because there was little ambiguity in the identifications. The identification process is also greatly facilitated by the Barcode of Life Data Systems (BOLD) which, unlike UNITE, provides probabilities that each identification is correct when sequences are queried online. The majority of sequences matched with 99-100% similarity to sequences within BOLD and generally, the identities of all high-similarity matches were the same.

4.3.2. Insects of Canada and Southern Ontario

The 568 insects successfully identified using DNA barcoding were from five orders, 35 families and at least 133 species. Flies (order Diptera) accounted for the majority of insects identified. Projects associated with the Barcode of Life initiative have been instrumental in increasing our understanding of insect diversity both globally and within Canada. However, the insect diversity in Canada is estimated to be far greater than what has been documented so far. For example, in 2016, one million insects from across Canada were collected and identified through DNA barcoding. Of these, an estimated 30, 000 species represented undescribed species[38]. Thus, it is not surprising that many of the insect samples collected in this study could not be identified to the species level. For the majority of the 193 samples without a species-level identification, the top matches on BOLD were

to specimens only identified to the genus, subfamily, family or order level. Further analyses of these samples suggest that they represent at least 56 different species, none of which have likely been described.

Fourteen samples matched with less than 96% similarity to any sequence in the database, suggesting that these are new sequences without an associated BOLD record. Some insect species have large intraspecific variability at the CO1 locus, meaning a 2% sequence similarity cut-off is too small to separate species[40]. Thus, some of these samples with low similarity matches may actually be the same species as the top matches in BOLD, if these species have high levels of intraspecific divergence. However, it is also possible that these samples represent previously undocumented species. Unfortunately, most of these insects were collected as larvae and all killed upon collection, meaning they cannot be reared to allow for morphological analyses to add more information about the possible identification of these species.

Finally, an additional 124 non-insect arthropods and nematodes were also identified, spanning six classes and 13 orders. The majority of these specimens could not be identified to the species level and several matched with low similarity to any sequences in BOLD. This suggests that these are relatively under-explored groups of organisms. Since insects were the focus of this study, the associations between mushrooms and the non-insect arthropods and nematodes were not examined further. However, previous surveys have documented the presence of springtails (Collembola)[42] and nematodes[66] in association with mushrooms.

Overall, these observations demonstrate that mushrooms are colonized and utilized by a wide range to animal taxa, many of which have yet to be described.

4.4. The mushroom communities

4.4.1. Seasonal and temporal patterns

Environmental conditions play a large role in determining where and when mushroom fruiting bodies will form. Mushroom field guides will generally provide information about the optimal conditions under which different species produce fruiting bodies (e.g. *Lepiota* species fruit in moist and relatively warm conditions, *Agaricus* species prefer moist and mild conditions, *Amanita muscaria* grows during different seasons throughout North America)[9]. While temporal patterns in mushroom abundance were not the focus of this study, some interesting patterns emerged. First, the mushrooms collected during each season differed greatly and the greatest number of species, genera and families were collected during the fall, a seasonal pattern which has previously been observed[67]. Temperature and rainfall can both influence mushroom abundance and growth[67-69] and higher temperatures in the summer can delay the fruiting of mushrooms later in the year[70]. Thus, variations in precipitation and temperature in Southern Ontario between 2018 and 2019 could potentially explain why the mushroom species that were collected differ between seasons and years. Environmental variables were not measured during this study, so no further analyses can be conducted to examine the specific influence of different factors on the observed seasonal patterns in mushroom diversity.

Second, there were changes in the dominant species, genera or families that were collected between sampling periods from locations such as in Cootes Paradise and the

Wirth and Sandor properties. This suggests that these forests experience some degree of species turnover, which is something that has been observed before in mushroom communities. For example, more than 70,000 mushrooms from 408 species were observed during weekly surveys of a single forest plot in Switzerland of the course of 21 years[70]. Only eight species were observed during all 21 years and most mushroom species were observed during fewer than half of the years. Thus, mushroom communities have the capacity to vary temporally.

It is important to note that the temporal and seasonal patterns observed in the current study are unlikely the result of over-harvesting certain mushrooms in one collection period. When a mushroom is picked, it has likely already released thousands of spores and if the mycelia beneath the soil is left intact, future growth of the mushroom will not be greatly affected[71]. A second long-term study in Switzerland demonstrated that the weekly collection of mushrooms from study plots over the course of more than 20 years did not result in a decrease in mushroom species richness or mushroom abundance compared to control plots[71].

Any temporal patterns of the mushroom communities at the Sandor property are likely because the forest was logged in September 2019. As a result, fewer specimens were collected from this forest in fall 2019 compared to fall 2018 and the majority of species collected in 2018 were missing from the 2019 collection. Previous studies have recorded a decrease in mushroom diversity in forests that have been thinned. This is because microclimate conditions are altered in areas where the trees are removed and ectomycorrhizal mushrooms lose their host tree species (reviewed in [72]).

From an insect's perspective, the unpredictability of mushroom fruiting due to seasonal variation and the minimal overlap in the mushroom species between seasons and years likely presents a challenge.

4.4.2. Geographical patterns

The mushroom communities also varied between sampling regions and these spatial patterns are likely the result of different environmental conditions and forest types. While no specific data was collected on tree and plant diversity in these regions, there were some obvious differences. For example, the Wirth property is composed almost entirely of pine trees. In contrast, both Cootes and the Lowrie property are dominated by deciduous trees. Several mushroom species, such as *Clitocybe* species and *Lactarius deliciosus*, grow best in pine forests[9]. *Lactarius* species, for example, are ectomycorrhizal fungi, so they require associations with pine tree hosts in order to grow[9]. Four *Clitocybe* species (10 specimens) and 11 *L. deliciosus* specimens (including samples that were ambiguous between *L. deliciosus* and two other *Lactarius* species) were all collected from the Wirth property and no other location. In addition, out of all pairwise comparisons, the mushroom community at the Wirth property was the least similar to both Cootes Paradise and the Lowrie property, according to the Bray-Curtis dissimilarity values. Thus, the presence or absence of the appropriate host tree for ectomycorrhizal mushrooms can likely influence where these mushrooms are found.

A recent global study of fungal communities demonstrated that soil texture, the age of vegetation and dominant host tree species can all significantly impact the composition of ectomycorrhizal mushroom communities[68]. Thus, differences in environmental

characteristics between the sampling regions would be a plausible explanation for the lack of overlap in species abundance between regions, even regions such as the Wirth and Lowrie properties that are only separated by a few kilometers.

Some mushroom species have a broad ecological distribution. For example, *Mycena* and *Russula* species are often widely distributed across a range of forests[9]. In this study, *Mycena leaiana* was the only species found in four out of the five locations and *Mycena* species in general were found in all of the five sampling locations. *Mycena* mushrooms are saprophytes, so they can grow on a range of substrates, such as logs, soil and leaves[9], and thus their distributions are not limited by the abundance of host tree species, as is the case for some ectomycorrhizal mushrooms. In contrast, *Russula*_species are ectomycorrhizal mushrooms. However, these species have a broad host range and can associate with many tree species[9], which could explain why they were also collected from almost every location. Outside of forests, species such as *Agaricus bitorquis* and *Lyophyllum decastes* are typically found in disturbed areas, such as roadsides and building sites[9]. Both of these species are also saprophytic and can survive on a range of substrates. Interestingly, two *A. bitorquis* samples were collected, one in a subdivision near McMaster and the other near the parking lot outside of LSB. Both of the *L.decastes* samples were collected from beside the track outside of DBAC on the McMaster campus. All of these regions are disturbed areas.

4.5. The insect communities

4.5.1. Seasonal and temporal patterns

Seasonal patterns, such as temperature, rainfall and the length of the growing season, can all influence insect abundance and lifecycles (reviewed in [74]). Thus, insect species in a given area will likely change between seasons and years. In this study, the composition of the insect communities also differed between collection seasons and many insects were only collected during one period. For example, two of the most highly abundant insect species, *Exechia subfrigida* and *Exechia nigroscutellata,* were only collected during the fall seasons. But, since most of the insect species that were collected during only one season are represented by a single specimen, it is difficult to draw any conclusions about seasonal variation in their abundance.

Temporal variation in the abundance in fungivorous insects has previously been documented. For example, the Coleoptera communities feeding on *Pleurotus ostreatus* mushrooms within experimental plots differed significantly between two years when observations were recorded[26]. Several studies have also reported patterns of insect abundance coinciding with seasonal patterns in host mushroom abundance. *Allodia* species in boreal forests were observed to have the highest levels of yearly flight activity in the spring, around the same time when various Pezizales species, a common host of *Allodia* flies, produce fruiting bodies[13]. More broadly, fungus gnats (family Mycetophilidae) in Britain are highly abundant during the fall at the same time when peak mushroom fruiting is generally observed[21]. Interestingly, while *Mycetophila fungorum* was collected during each sampling period, this fungus gnat was more commonly collected during the fall

collections (both 2018 and 2019) than the summer collection. However, the greatest number of insect taxa were collected during the summer 2019 season, even though the fewest number of mushroom taxa were collected during this same time. This is contrary to what would be expected based on these observations that mushroom abundance can influence insect community composition. Thus, potentially other environmental factors influenced the insect communities.

4.5.2. Geographical patterns

Similar to the pattern observed for the mushrooms, each of the main sampling locations had a distinct set of insect species. However, overall, insect communities were more similar between locations than the mushroom communities. For example, three fly species collected during this study, *Mycetophila fungorum, Megaselia pulicaria complex* and *Exechia subfrigida,* were widespread and frequently found in every or almost every location. One possible explanation for this observation is that these insects could have broad ecological niches and are therefore less affected by differences in regional environments than mushrooms. Fungus gnats can be found almost everywhere, particularly places with a moist environment, forest or fallen trees[21]. Thus, these species seem to have fewer ecological constraints on their habitat. Interestingly though, both the mushroom and insect communities at the Wirth property and Cootes Paradise are highly dissimilar (97% and 91% dissimilar for mushrooms and insects, respectively), suggesting that there is still some degree of habitat structuring in these communities. Regional differences in the insect communities isolated from mushrooms growing in different locations has previously been documented[66].

Spatial and temporal variability in mushroom abundance make them an unpredictable resource. If insect community composition were tightly linked to mushroom abundance at the species level (i.e. if insects displayed completely host-specific interactions), it would be expected that large differences in mushroom communities between locations would lead to large differences in the insect communities that use these mushrooms as a resource. Given the greater degree of similarity across regions for the insect communities than the mushroom communities, this suggests that insect abundance and occurrence patterns are influenced by factors other than the abundances of their hosts. Several researchers have suggested that given the unpredictability of mushroom fruiting, both temporally and spatially, insects likely evolved polyphagous feeding strategies, allowing them to utilize multiple mushroom species as a resource[15,75].

4.6. Mushroom-insect interactions

4.6.1. Overall trends

Mushrooms host a wide range of insect species from multiple taxonomic orders. In this study, flies (order Diptera) and beetles (order Coleoptera) were the most commonly observed taxa, followed by insects from the orders Hemiptera, Hymenoptera and Lepidoptera. It has been widely documented that many insect species from these orders readily use fungi as a resource. For example, species from the majority of Dipteran families that were collected during this study are known fungivores[14,76] and while larvae from the families Muscidae and Sciaridae are not generally fungivorous, some species are predators of other larvae[14]. Interestingly, all five Muscidae species and both Sciaridae species collected in this study were found in mushrooms that also contained at least one Diptera

species, generally a *Megaselia* or *Mycetophila* species, which would have been collected as larvae. Many Coleoptera species, particularly Staphylinidae species, feed on mushrooms and eat the mushroom tissue, hyphae and spores[31,77,78]. Ancient specimens preserved in amber suggest that these beetles have possibly been consuming fungi for over 125 million years[79]. Lepidoptera, Hymenoptera and Hemiptera, while less frequently observed, have also been documented to form associations with various mushroom species[42]. For example, several Lepidoptera species, mostly moths, are known fungal pests. Species that infest Shiitake mushrooms are particularly well-studied[80,81]. Hymenoptera species from the family Braconidae are known parasitoids of fungus gnats[23] and some species regulate fungus gnat populations within mushrooms, such as a common gnat pest of Shiitake mushrooms[82]. At least four of the nine Hymenoptera species (all family Braconidae) collected in this study were obtained from mushrooms that were also inhabited by Diptera larvae[23]. Overall, mushrooms present a versatile resource that is readily used by a range of insect taxa.

Multiple rearing experiments and field observations over the past decades have led to a large body of literature documenting mushroom-insect associations. Since the 1950's, numerous surveys have been published about Diptera and their associated mushrooms from across Europe[14,23]. Several studies have also examined associations between fungi and Coleoptera species. **Supplementary Table 1** presents a summary of previously documented mushroom-insect interactions, from twelve different studies, that involve mushroom species also collected in this study. The papers summarized in this table include two large-scales studies of Diptera and mushrooms from Finland (Hackman and Meinander,

1979) and the Czech Republic and Slovakia (Ševčík, 2010), in addition to a comprehensive review by Jakovlev (2012) covering most studies of mycetophilids that had previously been published in Europe up to 2012. The majority of the studies are from Europe and the primary focus is on Diptera species.

This table is not an exhaustive list and in fact, hundreds of Diptera species and many Coleoptera species have been documented to association with mushrooms. For many of the mushroom species, genera or families included in this table, dozens of other insect species have been reared from these mushrooms. In addition, multiple mushrooms species that were collected during this study are not included in the table because previously documented associations include insect species that were not observed in the current study. The overall conclusion from a brief comparison to these twelve studies is that overall, many of the main patterns of species interactions that were observed in forests of Southern Ontario are consistent with what has previously been documented in other countries, primarily in Europe. This is an interesting observation, considering these two regions are on different continents and separated by the Atlantic Ocean.

4.6.2. Specific patterns

Consistent with what was observed in this study, mushrooms from the orders Agaricales and Russulales are common hosts for many insect species. *Mycetophila fungorum* was the most commonly collected insect species and the species with the largest host range (**Figure 6**, highlighted in red). The extreme polyphagy of *M. fungorum* has been documented numerous times[13,14,23,24] and this insect has previously been collected from at least 100 different mushroom species overall[13]. In this study, the greatest number of

interactions between any two species was observed between *Amanita muscaria* and *M. fungorum*. Interestingly, *A. muscaria* is a poisonous, hallucinogenic mushroom that produces ibotenic acid and muscimol. Despite its toxicity to humans, associations between this mushroom and many different insect species have previously been documented[14,23,66]. Other poisonous *Amanita* mushrooms are known to be consumed and inhabited by different insect species. For example, lethal mushrooms, such as *Amanita phalloides* and *A. virosa,* produce the deadly toxin alpha-amanitin and several insect species have been reared from these mushrooms[14,25]. *A. bisporigera* and *A. suballiacea* also produce alpha-amanitin. These species were collected during this study and found in association with several Diptera insects. Alpha-amanitin targets RNA polymerase II. Some mycophagous *Drosophila* species have an altered RNA polymerase II molecular structure, which confers insensitivity to alpha-amanitin[83]. Perhaps the other insect inhabitants of the poisonous mushrooms possess a similar mechanism.

In addition to *M. fungorum*, two other insect species were observed to be polyphagous, as illustrated in **Figure 6**: *Exechia subfrigida* (in green) and *Megaselia pulicaria* complex (in pink). These two Diptera species were not mentioned in any of the twelve papers summarized in **Supplementary Table 1**. According to records on BOLD, the species range of both *E. subfrigida* and *M. pulicaria* complex includes North America and Europe, so these are not species that are specific to Canada. Without a more thorough search through the literature, it is unclear if these interactions have been documented before.

Interestingly, *Exechia fusca* is a polyphagous species that has been reared from many mushroom species[14,22,23]. However, this insect was not collected from any

mushrooms in this study. All *E. fusca* specimens on BOLD were obtained from Europe, except for two samples from the Middle East, suggesting that this species is possibly not found in Southern Ontario. Further studies could involve examining the geographical distributions of all insects collected in this study to determine if these species are specific to Canada. This could provide information about mushroom-insect associations that may be unique to this part of the world.

Drosophila species have also been frequently observed to inhabit mushrooms[14-17]. However, in this study, only one *Drosophila* species (one specimen) and eight additional specimens from the family Drosophilidae, were collected from mushrooms. It is unclear why so few *Drosophila* were observed. In addition, mushrooms from the order Polyporales are frequently inhabited and consumed by insects[13]. In the current study, only 19 insects were identified from 13 Polyporales specimens, so these interactions were not commonly observed. Since polypore mushrooms generally have tough fruiting bodies, it was often difficult to break them apart and look for insects, which perhaps explains why fewer interactions were documented for these mushrooms.

There are a few other interactions listed in **Supplementary Table 1** that were not observed in this study. In addition, there are several mushrooms species, such as *Tapinella atrotomentosa*[23], that have no documented associations in the current study, yet insects are known to inhabit them. It is important to note that the lack of a documented interaction does not mean the interaction does not exist. Given the rather low identification success rate of insects/arthropods that were collected from mushrooms (only 692 identified out of 1,174 analyzed) and the relatively small sample size (just over 1,000 mushroom specimens

compared to 3700 specimens in the Hackman 1979 survey), it is possible that some of these interactions were missed. In addition, the positive correlation between the number of specimens collected and the number with an insect record suggests that if more mushrooms were collected of some of the poorly sampled species, more interactions could have been documented.

4.6.3. Limited evidence of host-specificity

There is limited evidence of host-specificity from both the data collected in this study and in the literature. **Figure 6** shows that many of the insect species were found in association with more than one mushroom species. Of those that were not, most were represented by a single specimen, making it difficult to draw any conclusions about the degree of specificity in their interactions. In general consensus from the literature is that most fungivorous insect species are polyphagous[13,14,22,75] and those that do display some degree of host specificity are often found in association with wood-inhabiting polypores, which have more stable fruiting bodies[13,22]. The overall lack of host specificity is likely due to the unpredictable and short-lived nature of mushrooms. As discussed in earlier sections, the mushroom communities can vary greatly temporally and spatially. If an insect only eat a certain species of mushroom, the chances that this mushroom species fruits at the same time that the insect is ready to oviposit and in the same location would be low[75]. Thus, many insects have likely adapted to polyphagous strategies. For example, multiple species from the family Drosophilidae (*Drosophila busckii*, *D. putrida, D. testacea, Leucophenga varia* and *Mycodrosophila dimidiata*) feed on and lay their eggs in mushrooms. All of these species are polyphagous. In contrast, the *Drosophila* species *D. quinaria* is monophagous

on a specific species of skunk cabbage, the growth of which is highly predictable and appears in the same places at approximately the same time every year[25].

However, some fungivorous species appear to have a more limited host range. For example, *Exechia nigroscutellata* is considered a specialist species and interacts primarily with *Lactarius* species[13,14,24] as well as Russulaceae species in general[21]. In this study, all *E. nigroscutellata* specimens were found in association with only Russulaceae species, primarily *Lactarius* (**Figure 6**, highlighted in blue). The milk-like sap that *Lactarius* species produce could limit the ability of other insects to utilize the mushrooms[14]. *Exechia separata* is another known specialist that primarily interacts with mushrooms from the family Boletaceae and the genus *Suillus* (family Suillaceae)[13,14,21,24]. Here, all six specimens were found in association with *Suillus* species (**Figure 6**, highlighted in purple).

Megaselia nigra generally prefers to associate with *Agaricus* species[23] and both specimens in this study were found inhabiting *Agaricus* mushrooms. Interestingly, **Figure 6** shows that another *Megaselia* species, *Megaselia* sp. n.2 SH-2015, was only found in association with *Agaricus* and *Leucoagaricus* mushrooms from the family Agaricaceae, with the interactions highlighted in orange. Thus, this *Megaselia* species may display some degree of host preference for mushrooms within this family. There are only two sequences assigned to the BIN on BOLD that corresponds to *Megaselia* sp. n.2 SH-2015. Both samples were obtained in Sweden, but these flies were obtained from Malaise traps, so it is unknown if they ever inhabited mushrooms[84]. Interestingly, Phoridae species are among the most common inhabitants of *Agaricus* mushrooms, which are rarely observed to be

infested with fungus gnats[14]. In this study, no Mycetophilidae specimens were obtained from any *Agaricus* mushrooms.

Other possible examples of host specificity that were both previously documented and observed in this study include *Megaselia marquezi* and *Psathyrella candolleana*[20], *Mycetophila strigatoides* and *Polyporus* species[23] and *Allodia* and *Pezizales* species[13] (**Supplementary Table 1**). In general, however, many insect species have a wide host range and can interact with mushrooms across multiple taxonomic orders. Poldmaa (2016) suggested that host specialization among insect species feeding on mushrooms is likely a continuum, with a few specialist species on one end and a few generalists with a large host range on the other end and a range of feeding strategies in between[24]. This would be consistent with what was observed here.

4.7. Network analyses

Most of the studies summarized in **Supplementary Table 1** were primarily observational. One way to analyze species interactions is through bipartite network analyses, which have previously been used to study interactions between beetles and fungi[26,41]. Therefore, network analyses were conducted to provide more information about the structure of the mushroom-insect associations observed from these two regions in Southern Ontario. Overall, the mushroom-insect interactions are moderately generalized, with some species displaying significantly specialized interactions, and modular, which means the mushrooms and insects can be compartmentalized into groups with similar interactions.

4.7.1. Network level of specialization

A total of 308 interactions were mapped between mushroom and insect specimens at the species-level. While this network is significantly more specialized than what would be expected based on random interactions (H'_2 = 0.341, significant based on 1,000 null models), the network is moderately generalized. This aligns with expectations, given that multiple insect species collected in this study interacted with multiple mushroom taxa (**Figure 6**). However, the H'_2 also index takes into account the number of interactions between each species[56]. Thus, some degree of specialization is supported because certain species interacted more frequently than others. For example, *Amanita muscaria* has documented interactions with only three mushroom species and 12 of the 16 interactions are with *Mycetophila fungorum* (**Figure 6**). There are also numerous 'exclusive' interactions where two species, each represented by a single specimen, were only found in association with each other. Removing these nineteen mushroom and insect species resulted in a slight decrease in network-wide specialization (H'_2 decreased to 0.338 and was significantly more specialized than the null models).

The calculation of H'_2 is not affected by the size of the network and generally, incomplete networks that contain a subset of interactions of a larger network, can still yield an H'_2 value that is close to value of the larger network[56]. In addition, there is no correlation between the size of the network and the H'_2 value[85]. Thus, despite the fact that only 308 interactions could be included for this species-species network, the H'_2 value is still a strong estimate for the level of specialization of this network.

H'_2 specialization values are often used to characterize plant-pollinator networks and other networks involving interacting species. For example, Bluthgen et al. (2007) examined H'_2 values for 51 networks that included pollinator, seed dispersal, ant-myrmecophyte and ant-nectar plant networks. The H'_2 values ranged from 0.13 to 1.00 across all examined networks, and all networks, except for one, were significantly specialized[85]. Interestingly, the plant-pollinator networks, with H'_2 values ranging from 0.24 to 0.85 (average 0.55), were significantly more specialized than the networks involving seed-dispersal associations, which had H'_2 values ranging from 0.18 to 0.47 (average 0.29)[85]. One possible explanation for this observation is that plants may benefit from having more generalized interactions with multiple species so that the seeds are dispersed to different locations[85]. The mushroom-insect H'_2 value of 0.341 is close to the average specialization value for organisms engaged in seed-dispersal associations. Therefore, the mushroom-insect network could possibly be analogous to seed-dispersal networks that have previously been documented between plants and birds or mammals.

4.7.2. Specialists and generalists

In the broad sense, a generalist can be defined as a species that feeds or preys upon multiple species regularly, while a specialist's diet is limited to a single or a few species[86]. As discussed in previous sections, only a few insect species collected during this study display evidence of host specificity and therefore most species appear to have generalist properties. The species-level specialization index (d′) calculates specialization by taking into account both the strength of the interactions and number of interactions each partner species is engaged in[56], rather than simply counting the number of interactions. Seven insect

species had significantly more specialized interactions compared to the null models. Of these species, *Exechia nigroscutellata* and *Exechia separata* are known specialists of Russulaceae and Boletales mushrooms (**Supplementary Table 1**). The d′ values were 0.89 and 0.94 for *E. nigroscutellata* and *E. separata*, respectively, meaning they associate primarily with a set of host species, which, in turn, primarily associate with these insects. Thus, this provides quantitative evidence for the numerous observations that these species are engaged in specialized interactions. None of the other species discussed earlier that potentially display evidence of host specificity, such as *Megaselia sp. n.2 SH-2015*, had significantly specialized interactions. This is likely because the mushrooms species they associate with were typically found in association with multiple other insect species.

Surprisingly, *Mycetophila fungorum*, a known generalist, had a d′ value of 0.56, which was significantly more specialized than the null models. However, this can be explained by the fact that out of the 33 mushroom species that *M. fungorum* interactions with, ten were only found in association with this insect. Thus, these interactions would be considered specialized from the perspective of the mushroom. In addition, the greatest number of interactions between any two species was observed between *M. fungorum* and *Amanita muscaria*, suggesting there may be some degree of specificity or host-preference between these two species. Therefore, *M. fungorum* is a generalist, but overall has interactions that are significantly more specialized than expected.

Specialization values were also calculated for the mushroom species and 13 had significantly specialized associations. Of these, eight had a specialization value greater than 0.8, but all of these species were represented by a single specimen, making it difficult to

conclude that these mushroom species have highly specialized interactions. For example, *Omphalotus illudens* has a d′ value of 0.96. However, only one specimen was collected, which contained six different insect species, five of which are represented by a single specimen. Interestingly, none of the *Suillus* or *Lactarius* species found in association with *Exechia separata* or *Exechia nigroscutellata* had significantly specialized interactions. This is probably because each *Suillus* or *Lactarius* species individually only had one or two interactions with these insect species, but these insects were found in association with multiple *Suillus* and *Lactarius* species, indicating a low level of specialization. If examined at the genus level, interactions involving the *Suillus* and *Lactarius* genera would likely be significantly specialized.

4.7.3. Limitations and assumptions with the specialization indices

There are a few limitations and assumptions associated with the H'_2 and d′ indices. First, the calculations for these indices do not account for the taxonomic similarity of the taxa when quantifying specialization. For example, species A might be considered to have more generalized interactions than species B, despite the fact that species A interacts with multiple species from the same genus whereas species B interacts with a few species from different orders[56]. Bluthgen et al. (2006) suggest running the network analyses with organisms grouped to higher taxonomic levels to account for this. Overall, H'_2 values increased as mushrooms were grouped by the genus, family and order level, meaning the networks became more specialized. This is likely because species such as *Exechia separata*, *Exechia nigroscutellata* or *Megaselia sp. n.2 2015* were each found in association with multiple mushroom species but only one genus or family. The network with mushroom

taxa grouped to the order level had the highest H'_2 value of 0.624, but this is probably not that informative because grouping the mushrooms by order is too broad. Insect d′ values were also calculated when mushrooms were grouped by genus, family and order. Overall, the level of specialization decreased for most species as the mushrooms were grouped to higher taxonomic levels. This is not intuitive, given that the network-wide specialization generally increased. However, if mushrooms are grouped by order, for example, each insect species may associate with only one group. But each order, as a whole, forms associations with multiple insect species, meaning the interactions are not 'exclusive' or specialized. Networks for the most abundant mushroom families were also examined separately. All networks had larger H'_2 values than the complete mushroom-insect network. However, these networks were quite small, with few mushroom taxa and fewer than 45 interactions total.

The second limitation with calculating the specialization indices relates to the assumptions with using a null model. For the null models in this study, the total number of interactions for each species were kept constant while the interactions were randomized between species. Thus, these null models make the assumption each species can physically use every other species as a resource[56]. This might not quite be true. For example, while beetles primarily feed on the mushroom tissue, flies generally lay their eggs in mushrooms and consume the mushrooms they inhabit. Certain mushrooms that beetles feed on may not have properties that make them unsuitable for larval development, for example, if they are too tough. As a result, some null models may produce networks that are unrealistically generalized[87]. For this reason, the network properties were examined for a network that

only included flies and their associated mushrooms. This network was significantly specialized (H'_2 = 0.399) and had a higher H'_2 value than the larger mushroom-insect network. Further analyses could involve using different null models and comparing the resulting H'_2 averages to the observed network values to see if the level of significance is affected.

Third, when levels of specialization are examined for the whole network, there is the assumption that all species could theoretically interact with any other species and therefore the more common interactions indicate host preference. However, this may not be true if samples are collected over different geographical areas or time points[56], as was the case in this study. If interactions that are documented from different places and times are grouped together, the number of interactions will likely be an overestimate of possible interactions and differences between the two locations cannot be taken into account (reviewed in [88]). For example, a fly living on mushrooms at the Wirth property certainly will not have access to the same mushrooms growing in Cootes Paradise over 90 kilometers away. In addition, many factors can influence the properties of a network and H'_2 for a network can vary between years[88]. Given that the mushroom communities in each of the sampling locations differed from one another and between seasons, treating all 308 interactions as one network cannot take into account possible differences in network structure between locations or seasons. Ideally, the network analyses would have been conducted for each location during each season (e.g. Cootes Fall 2018, Cootes Summer 2019, etc). However, only around 20-30 interactions were documented for each

combination of location and season, so these networks would not have been that informative.

Lastly, a bipartite network is an over-simplification because it cannot account for complexities in interactions between species within the same group (i.e. insect-insect interactions). For example, *Drosophila* larvae can exhibit some degree of competition within mushroom fruiting bodies that they inhabit[15]. In addition, as discussed earlier, Muscidae and Sciaridae larvae eat the larvae of other Diptera species within mushrooms, while some Hymenoptera species are parasitoids of fungus gnats. Thus, there are possibly interactions occurring between insects that may affect which interactions are documented, but none of these potential interactions are taken into account if the network is simplified as a bipartite network.

Despite these limitations, examining the interactions between mushrooms and insects as one large network can still allow for overall observations about broad patterns, while allowing any instances of host-preference to be quantified.

4.7.4. Modularity

Modularity (Q) quantifies the degree to which a network can be organized into compartments or groups based on shared interactions. Examining modularity and visualizing the predicted modules is important because it can lead to the development of hypotheses about the factors that shape a network[89]. Generally, modularity and H'_2 are positively correlated[89], which is intuitive considering that if there are many specialized interactions within a network, the species will generally fall into more defined groups of commonly interacting organisms.

It is therefore a bit surprising that the modularity value for the mushroom-insect network was so high (0.72), given that the network-wide level of specialization is only 0.341. However, network size and the number of interactions can both influence modularity. An estimated 15-24 interactions per consumer (in this case, insects) would be required in order to obtain a modularity value that is close to the 'true' value of a more thoroughly sampled network[87]. For this mushroom-insect network, only three insect species had between 15 and 24 documented interactions, while the rest had fewer than 15. Thus, the high level of modularity may be an over-estimate because too few interactions for each insect species were documented. Regardless, patterns in the species that fall into each module can still be examined.

Modules reflect groups of species that interact more frequently with each other than with other species outside of the module. Many factors can influence which species group together in modules. One factor is evolutionary history. Species that are more closely related will generally share similar physical characteristics. There may be evidence of a phylogenetic signal in the structure of a network if these shared characteristics result in shared interactions with a similar subset of species[89,90]. Therefore, modularity of the mushroom-insect network was examined in relation to the evolutionary history of the taxa involved. Based on **Figure 7**, the mushroom and insect species within each module do not appear to closely follow taxonomic groupings (i.e. mushrooms from the same family are not consistently found within the same module). This is contrary to what would be expected if there were evidence of phylogenetic signal in the network.

4.7.5. Quantifying taxonomic similarity of species within modules

To quantify the structure of the network further, pairwise genetic distances were calculated between all of the mushroom and insect species within each module. Sequences of the ITS and CO1 loci broadly separate the taxa based on taxonomy (i.e. generally, taxa have lower pairwise genetic distances to taxa within the same family than to taxa from other families), as illustrated by the Neighbour-Joining trees in **Figure 8**. However, there are a few misplaced taxa that do not cluster as expected, suggesting that the sequences of these barcoding loci are highly variable between species within some mushroom and insect families. Ideally, additional genes would have been sequenced from both the mushrooms and the insects to allow for a multi-locus approach to estimate pairwise genetic distances between taxa.

Despite these inconsistencies, genetic distances were used to estimate the similarity of taxa assigned to each module across all ten estimates of modularity. For the majority of modules, the genetic distances between species within each module were not significantly smaller than expected, based on comparisons to null models. Thus, mushroom and insect species that share more similar interactions are generally not more taxonomically similar than expected, suggesting that other factors are influencing the structure of the network.

The species within a few of the significant modules are worth discussing. For example, the species *Lactarius cinereus, L. pubescens*, *L. pubescens/torminosus*, *Lactarius* sp. 1, *L. vinaceorufescens* and sometimes *L. aff. omphaliiformis* were consistently grouped into the same module across all ten trials. Regardless of what additional species were included in these modules, the taxa always had a significantly lower average pairwise

genetic distance than expected. All of these species were found in association with *Exechia nigroscutellata*. Thus, taxonomic similarity may play a role in influencing this aspect of the network.

Two other groups of mushrooms, *Suillus americanus/himalayensis, S. collinitus/granulatus* and *S. granulatus/weaverae,* as well as *Agaricus* sp. 2, *Agaricus* sp. 3, *Leucoagaricus leucothites/subcretaceus* (all from the family Agaricaceae) were frequently grouped together in the same modules. As discussed earlier, *Exechia separata* was observed to only interact with *Suillus* mushrooms and similarly, *Megaselia sp.* n.2 SH-2015 was only found in association with three species from the family Agaricaceae. However, modules that contained these *Suillus* and Agaricaceae species only had significantly lower average pairwise genetic distances for five out of the ten trials. This is likely because these species were often grouped with other species, which would increase the average genetic distances of taxa within the module. For example, in module 7 of **Figure 7**, the *Suillus* species are grouped with three additional species from two different orders.

Overall, there does not appear to be much taxonomic similarity of the insect species within each module, as very few had significantly lower pairwise genetic distances that expected. This suggests that closely related insect species (or species within the same genus or family, at least) do not interact with a similar subset of mushroom species. Take for example *Exechia separata, Exechia nigroscutellata* and *Exechia subfrigida*. These insects are all from the same genus, yet *E. separata* is specialized on Boletales mushrooms, *E. nigroscutellata* on *Russulales* mushrooms and *E. subfrigida* is a generalist that interacts

with multiple species from different orders. Previous studies have observed that the modular structure of a network is generally more strongly influenced by the taxonomic similarity of the 'host' species rather than their interaction partners[90].

It is important to note that all of these observations are preliminary and more sequence data would be required to accurately estimate genetic distances between taxa. In addition, if a strong, well-supported and rooted phylogeny were available, phylogenetic distances could be used instead of genetic distances. Analyzing the similarity between taxa this way would allow for complete evolutionary relationships to be taken into account when looking for evidence of phylogenetic signal in the structure of the network.

4.7.6. Other factors that influence modularity in networks

One factor that can influence the structure of a network is trait matching, where species interact because they have a specific set of complimentary traits[89]. Physical and/or behavioural traits have often been observed to influence the structure of plant-pollinator networks[91]. For example, hummingbird beak morphology has a significant influence on the species of flower that hummingbirds choose to eat. Longer-beaked hummingbirds preferentially feed on longer flowers, while the opposite is observed for shorter-beaked humming birds[92]. Trait matching is often associated with phylogenetic signal and coevolution, but it could result arise independently of these factors[89]. Thus, it would be interesting to examine the properties of mushrooms within each module to see if there are any shared traits that might influence their interactions with certain insects. Different levels of volatile production[29], colouration and fruiting body shape[33] between mushroom species have all been observed to influence the insects that interact with these fungi.

Module 5 of **Figure 7** would be particularly interesting to examine further. The mushroom species in this module had a significantly lower average pairwise genetic distance than the null models. Almost all of these mushrooms were found in association with *Exechia subfrigida.* Within this module, several of the *Armillaria* species, including *A. sinapina, A. cepistipes* and *A. gallica* (*Armillaria* sp. 3 is an ambiguous between *A. gallica* and the other *Armillaria* species) and many *Mycena* species, including *M. haematopus*, are members of a phylogenetic lineage of bioluminescent mushrooms[93,94]. Perhaps *Exechia subfrigida* is particularly attracted to the light emitted from these mushrooms, thus contributing to the high frequency of interactions between this insect and these mushroom species. Interactions between insects and bioluminescent mushrooms have been observed before. For example, the Brazilian mushroom *Neonothopanus gardneri* is bioluminescent and light trap experiments showed that the light from the mushroom attracts significantly more insects from various orders than control traps[95]. It is possible that these mushrooms use light as a way to attract insects, which in turn allows the mushroom to distribute its spores[95]. However, trait matching is difficult to quantify[89] and so further analysis would be required to determine if trait matching influences the structure of the observed modules in this study.

There are other factors that can influence the structure of a network, such as habitat separation. For example, modules could reflect which species are found in certain areas[89]. Each of the main sampling locations of this study had their own distinct mushroom and insect communities, with limited overlap in the species found in each location. There is overlap in the geographical distribution of mushroom and insect species for four modules

of **Figure 7** (modules 6, 8, 10 and 17). For example, the majority of specimens within module 6 were collected from Cootes Paradise (6 out of 7 samples) and of these, four were found in association with the species *Megaselia sp.* Var. 17. All *Megaselia sp.* Var. 17 specimens were collected from locations in Hamilton, thus suggesting that overlap in the geographical distributions of these two groups of organisms could influence their interactions. In other words, *Megaselia sp.* Var. 17 could interact frequently with these mushroom species because they happen to exist in the same location. In this scenario, the interactions occur irrespective of taxonomic similarity between the mushroom hosts.

Similarly, temporal dynamics can influence network structure[89]. If there is no overlap in the season or timeframe when two species exist, they cannot interact with one another. Seven of the eighteen modules display potential evidence of seasonal structuring: the majority of mushroom specimens within each of modules 5, 7, 10, 12, 15, 17 and 18 were primarily collected during only one season. Given the observed seasonal variation in mushroom fruiting, it is plausible that certain interactions between mushrooms and insects may be more commonly observed during some seasons than others, primarily due to overlap in seasonal abundance.

Overall, multiple ecological and evolutionary variables can influence the structure of a network and the interactions that are observed[89]. Teasing apart which factors have the strongest influence is difficult and would require more analyses.

4.7.7. Coevolution as a method to further analyze interactions

The original goal of this project was to examine evidence of coevolution between interacting mushroom and insect species. Phylogenetic patterns within modules are often

interpreted as evidence of coevolution[89] and thus far, coevolution analyses have not been conducted for interactions between mushrooms and insects. However, these analyses were not conducted in this study for a few reasons. First, not enough sequence data was obtained to accurately infer the phylogenies of the mushrooms and insects collected. A robust, well-supported phylogeny is a requirement for examining coevolution. For example, previous studies examining coevolution in other fungi-insect associations used multiple gene fragments to construct the insect and fungal phylogenetic trees and then used Bayesian methods to estimate the divergence times of the different branches[96]. The sequences of the ITS and CO1 loci alone are insufficient for constructing such phylogenies. The ITS sequences of mushrooms from different orders are highly divergent and hard to align, making it difficult to use this gene region to build a well-supported phylogeny.

Second, coevolution studies often focus on one genus or family of organisms. For example, analyses of coevolution between termites and mushrooms have focused on the one subfamily of termites that evolved to form symbiotic relationships with only one genus of mushroom[2]. These termites are a single group that are genetically isolated from their sister taxon that does not cultivate fungi. The mushroom dataset collected in this study is too broad and it spans too many fungal orders. In addition, since the mushrooms are only a small subset of all species from these genera, families and orders that have documented associations with insects, there would be no way to capture the intricacies of the interactions and evolutionary patterns with such a broad, yet poorly sampled dataset.

Third, given the low degree of network-wide specialization, limited evidence of host specificity and overall lack of taxonomic similarity for species within modules,

coevolution may not be that informative for understanding this mushroom-insect network. Hackman and Meinander (1979) note that there were no known instances of monophagy among mushrooms and insects that resulted from coevolution, since species that are thought to be monophagous are sometimes found in association with completely different mushroom hosts[14]. However, given that some species, such as *Exechia nigroscutellata* or *Exechia separata,* do appear to have a more limited host range, potentially future investigations could examine evidence of coevolution within specific groups of mushrooms, such as mushrooms within the Russulaceae or Suillaceae families.

4.8. Limitations with the current study

There are several limitations with the current study. First, the relatively low DNA extraction and sequencing success rate for many of the insect and mushroom samples, combined with the ambiguity in several mushroom identifications, severely reduced the dataset. Not every insect that was collected from a mushroom was identified and similarly, not every mushroom with an insect record was successfully identified. For example, the species-species network contained 308 interactions. In contrast, if the mushrooms of every identified insect were also identified to the species level, 458 interactions would have been documented (101 additional interactions mapped to mushroom species that were not identified to the species level, plus 49 interactions for insect species missing an associated mushroom identification). Second, by extracting larvae from mushrooms manually, not all interactions were likely captured. Any eggs or small larvae that were not visible under the dissecting microscope would not have been collected and since some of the collected larvae were too tiny, the DNA extraction often failed. In addition, multiple larvae were generally

only sequenced from the same fruiting body if they displayed different morphologies. Thus, it is possible that the true diversity of insects associated with these mushrooms was underestimated if some of the larvae that were not sequenced are actually different species.

Third, too few interactions were documented from each geographical region. Given the high levels of dissimilarity in both the insect and mushroom communities between locations, the network analysis should have probably considered each location individually. Dividing the larger network into smaller networks would give a better idea of local structuring of the network at a smaller spatial scale. An alternative research design could have involved collecting mushrooms and analysing the interactions from only one location, such as Cootes Paradise, but with more frequent surveys to collect as many mushrooms as possible. Lastly, no additional sequence data was obtained beyond the two barcoding loci, making it difficult to construct well-supported phylogenies of the mushroom and insect species to allow for thorough analyses of phylogenetic signal and coevolution in the structuring of the networks.

4.9. A recently described approach

In 2019, a paper entitled “Finding flies in the mushroom soup: Host specificity of fungus-associated communities revisited with a novel molecular method” by Koskinen et al. was published in “Molecular Ecology”[42]. The novel protocol outlined in this paper presents a solution to many of the limitations listed above and should be adopted in future studies of mushroom-insect interactions. Using 319 fruiting bodies from 12 mushroom species (from the genera Cortinarius, Russula, Boletus, Leccinum and Lactarius) collected from Finland, Koskinen et al. attempted to capture the diversity of arthropods, fungi and

bacteria found in association with these mushrooms. They developed a DNA extraction protocol that involves homogenizing entire mushroom fruiting bodies, then extracting total DNA from the mushroom. Three sets of primers targeting 157 bp of the CO1 gene, the ITS2 region and a small fragment of the 16S rRNA gene were used on the total fungal DNA to amplify any arthropod, fungal or bacterial DNA in the samples using metabarcoding.

The raw dataset contained over two million CO1 reads and more than three million reads for both the ITS and 16S regions. From just over 300 mushrooms, they were able to identify more than 200 insect species from 59 families and 12 orders, with commonly identified insect families including Phoridae, Muscidae, Chironomidae, Anthomyiidae, Mycetophilidae and Staphylinidae. Insect species from the orders Hymenoptera and Hemiptera were also identified, in addition to multiple Collembola species. More than 600 fungal OTUs (species other than the ones collected) and over 1,000 bacterial OTUs were also obtained. In addition, little evidence of host specificity was observed and only 28% and 27% of the variation in the arthropod community structure could be explained by the host mushroom species and host mushroom order, respectively.

Overall, Koskinen et al. obtained fewer mushroom specimens of fewer species than what was obtained in the current study (319 specimens from 12 species vs 1,017 specimens from approximately 200 species) and yet they were able to document far more interactions and a greater range of insect species. This is primarily because they were not limited by the inability to collect and extract DNA from individual insects. Interestingly, in the current study, the CO1 primers were successfully used to amplify approximately 20 CO1 sequences

from total fungal DNA. However, this technique was only used on a few select samples from the 2018 collection and it did not involve any metabarcoding.

The insect identification success rate observed by Koskinen et al. was also high: of the 310 mushroom samples included in the final analysis, at least one arthropod was identified from 295 of these samples. The authors note that on average, they identified 0.68 arthropod species per mushroom fruiting body[42]. As a rough estimate and extrapolation, if this method were used for all 1,017 fruiting bodies collected during this study, potentially close to 700 insect species could have been identified. Thus, the current study was likely only able to document a small subset of the interactions between the collected mushrooms and insects.

There are a few advantages to dissecting the mushrooms for analysis, as was done in the current study. For example, observations about the location where the insects are found (e.g. in the gills, the stipe, the volva, on the surface of the mushroom, etc.) can be recorded. If insects from different regions of the mushroom are analyzed separately, it might be possible to determine if different insect species that share each mushroom partition the fruiting bodies. Direct observations are also useful because they can provide insight into how different insects use the mushroom resource. For example, in the current study, every Coleoptera species was collected as a mature beetle from the mushroom. In contrast, the majority of Diptera species were collected as larvae. This highlights the potential differences between how these two groups of organisms use mushrooms as a resource: beetles primarily for food and flies for food but also as a place for larvae to complete their lifecycle. If mushrooms are homogenized without examining the insects

inside, it would not be possible to distinguish between species that develop inside the mushrooms versus species that primarily feed on the mushroom tissue.

4.10. Future research based on this work

This study has demonstrated that the macrofungal communities of forests within Hamilton and the Tillsonburg area are diverse and potentially contain previously undocumented or uncharacterized mushroom species. It would be interesting to document the diversity of mushrooms in some of these regions more carefully. Cootes Paradise contains a wide range of mushroom species and given the ecological significance of this area, it would be beneficial to have a stronger understanding of the diversity of mushrooms that inhabit this forest. Future studies could focus exclusively on the diversity of mushrooms within Cootes Paradise and could involve an expanded sampling area to include more regions within the forest. When collecting mushrooms, it would also be beneficial to document more details about the sampling sites. For example, each sample could be tagged with GPS coordinates and when the mushrooms are collected, information about the soil conditions and other environmental variables could be obtained to allow for analyses looking at the correlation between different environmental factors and mushroom diversity.

There are also multiple additional network analyses that were not examined in this study that could provide more information about the structure of this mushroom-insect association network. The bipartite package, for example, allows for many other network properties to be calculated such as nestedness (another measure of structure), as well as connection and participation values (which allows for the quantification of the roles that

different species have within networks)[43]. There are also multiple ways to incorporate phylogenies and taxonomic relationships into analyses of network structure besides the preliminary analyses conducted here[90]. As discussed before, limiting the number of mushroom species that are collected to a certain family could potentially allow for a more focused study.

One key conclusion from this study is that many of the same interactions and overall patterns between mushrooms and insects have been previously documented in Europe. This is interesting considering the large geographical distance between the two regions. Further studies could involve conducting a global survey of a few select mushroom species that are found on multiple continents to see if the observed interactions are consistent between locations. For example, *Lactarius* species are found throughout the Northern Hemisphere[97]. It would be interesting to collect *Lactarius* species from as many locations as possible to determine if *E. nigroscutellata* is consistently found within these mushrooms world-wide. Overall, there is a potential to examine the biogeography of these interactions to try and understand the historical and evolutionary processes that shape similar interactions between mushrooms and insects on a larger geographical scale. Finally, the metabarcoding approached described by Koskinen et al. could potentially be used to extract DNA from mushroom specimens that have already been collected, such as herbarium specimens or specimens collected from studies, such as this one, that were dried upon collection. The DNA of organisms that were collected and dried with the mushroom may still be stable within the dried mushrooms, which could allow for the identification of insects and arthropods from the multitude of samples that have already been collected.

5. Conclusion

To our knowledge, this is the first time that mushroom-insect interactions have been closely examined and documented in Canada. Overall, a diverse range of insect species were found in association with just over 1,000 mushroom specimens collected from forests in the Hamilton and Tillsonburg, Ontario regions. Consistent with previous studies, a few insect species collected here display evidence of specificity or preference in their choice of mushroom host. However, the overall network is characterized by moderately generalized interactions, with many polyphagous insect species that associate with mushrooms from different families and orders. Based on preliminary analyses, there is limited evidence that phylogenetic signal influences the structure of the network. Further analysis is required to understand the seasonal, spatial or ecological factors that might influence the observed interactions. Overall, this study contributes greatly to our current understanding of mushroom and insect diversity in this region of Southern Ontario.

Studying species interactions is an important area of ecological research. When we understand how species interact with one another, we can understand the different roles that these species play within the greater ecosystem. Mushrooms are not just decomposers or plant symbionts: these fungi are a critical resource for hundreds of insect species, as well as other organisms such as mites, springtails and nematodes. Examining and characterizing these interactions can allow us to better understand the intricacies of the nature world around us.

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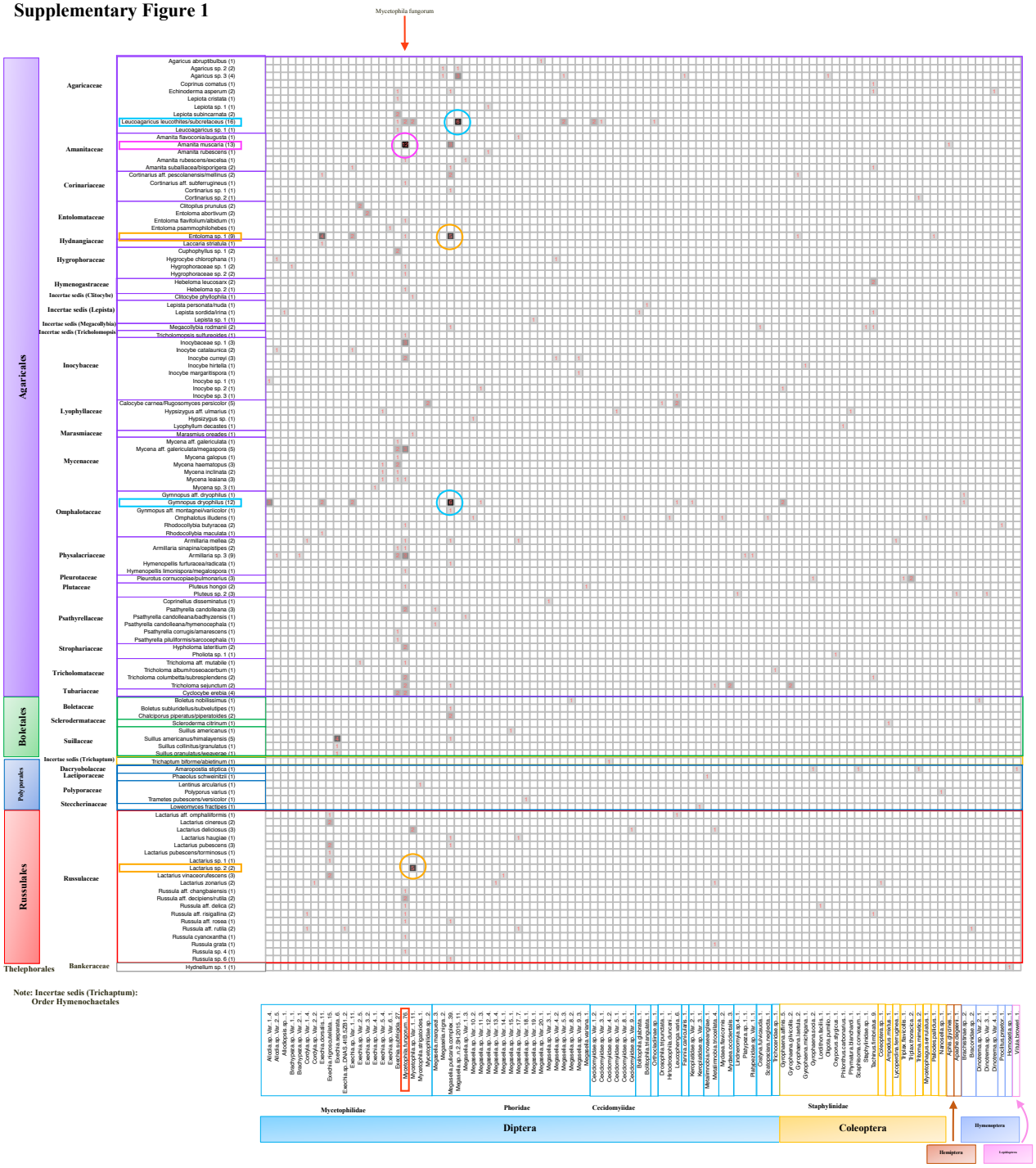
7. Supplementary Data

Supplementary Figure 1: A complete summary of the mushroom-insect associations represented as a matrix. Mushrooms are along the left-hand side arranged by family and order. Insects are along the bottom, also arranged by family and order. The numbers in the boxes indicate the number of documented interactions between those species. The most frequent interactions are circled.

Supplementary Table 1: Summary of select interactions between mushrooms and insects that have previously been documented. Genera and species in bold indicate samples collected and interactions observed during this study. The notes provide more detail about some of the observed interactions. The location where each study was conducted is listed.

Supplementary Table 2: Reduced raw dataset of all mushrooms collected during this study (including the date and location) with information about the insect and non-insect arthropods/nematodes that were identified from each specimen. Since this is a reduced dataset, the family and order classifications of each specimen are not provided. * means the mushroom has an ambiguous identification (i.e. more than two species had an equivalently high similarity match). ◉ means the mushroom was identified based on a photograph.

Supplementary Figure 1



Supplementary Table 1

Mushroom Family/Order	Mushroom Species	Documented Insect Associations	Comparison to this study	Location and Reference
Agaricales				
Agaricales (overall)		***Bolitophila*** *sp.*		*Finland, Russia, Europe (broadly)*[22]
Agaricaeae	***Agaricus sp.***	***Phoridae* sp.,** *Pegomya* sp. – rarely fungus gnats	*seven of the nine documented interactions are to ***Megaselia* sp.**, no interactions with *Mycetophila* fungus gnats	*Finland*[14]
		Megaselia nigra (prefers *Agaricus* species)	*two specimens were collected, both in association with *Agaricus* species	Czech Republic and Slovakia[23]
	Corprinus comatus	No documented interactions with Diptera	*one interaction: *Tachinus fimbriatus* (Coleoptera)	*Finland*[14]
Amanitaceae	Amanitaceae (overall)	***Mycetophila fungorum****, Exechia separata*	(see notes below)	*Europe (broadly)*[13]
	Amanita sp.	Various *Drosophila* sp.		*New York, USA*[15]
		Polyphagous Diptera		*Finland*[14]
		Mycetophila fungorum		*Europe (broadly)*[13] *Estonia*[24]
	Amanita rubescens	*Mycetophila fungorum*		*Finland*[14], Czech Republic and Slovakia[23]
		Megaselia sp.	*one interaction: *Megaselia* sp.	*Finland*[14]
	Amanita muscaria	***Mycetophila fungorum***	*12 interactions with *Mycetophila fungorum,* two with *Megaselia pulicaria complex*, both generalists	*Finland*[14], Czech Republic and Slovakia[23]
		Megaselia sp.		*Finland*[14],
		Hymenoptera sp., ***Phoridae sp.***, various *Drosophila* and ***Mycetophila*** species		*New Zealand*[66]
	Amanita vaginata	*Allodia sp., Mycetophila fungorum*	*no documented interactions for this mushroom species	*Finland*[14]

Cortinariaceae	Cortinariaceae (overall)	*Mycetophila fungorum, Exechia separata*		*Europe (broadly)*[13]
	Cortinarius sp.	Various *Drosophila* sp.		*New York, USA*[15]
		Mycetophila fungorum (most common)	*one interaction with *Mycetophila fungorum*, others with *Megaselia pulicaria complex* and *Exechia sp.*	*Finland*[14] *Europe (broadly)*[13] *Estonia*[24]
Hydnangiaceae	***Laccaria* sp.**	**Polyphagous Diptera** – fungus gnats	*one interaction with *Exechia dorsalis*	*Finland*[14]
Hygrophoraceae	**Hygrophoraceae sp.**	***Exechia sp., Mycetophila sp.**, Bolitophila sp.*		*Europe (broadly)*[13]
Hymenogastraceae	***Galerina marginata***	Fungus gnats	*no documented interactions for this mushroom species	*Finland*[14]
	Hebeloma sp.	*Exechia fusca*	*two interactions: *Mycetophila fungorum, Tachinus fimbriatus* (Coleoptera)	*Finland*[14]
Incertae sedis - Clitocybe	***Clitocybe* sp.**	*Bolitophila, Allodiopsis, Tarnania* sp., *Mycetophila fungorum* (most common)	*one interaction, not to any of these genera	*Finland*[14]
Incertae sedis - Collybia	***Collybia* sp.**	**Polyphagous Diptera** – fungus gnats	*two interactions with *Megaselia pulicaria complex*, a generalist	*Finland*[14]
Incertae sedis – Lepista	***Lepista* sp.**	***Bolitophila**, **Allodiopsis**, Tarnania* sp. *Mycetophila fungorum* (most common)	*two interactions with *Bolitophila* species and one to *Allodiopsis* sp.	*Finland*[14]
Incertae sedis - Melanoleuca	***Melanoleuca* sp.**	Polyphagous Diptera, mostly fungus gnats	*one specimen from this genus, no documented interaction	*Finland*[14]
Incertae sedis - Tricholomopsis	***Tricholomopsis* sp.**	**Polyphagous Diptera**, mostly fungus gnats	*one interaction: *Mycetophila fungorum*, a generalist	*Finland*[14]
Inocybaceae	***Inocybe sp. (likely mixtilis)***	*Drosophila transvera*	*one interaction: *Allodia* sp.	*Finland*[14]
Mycenaceae	***Mycena rubromarginata***	Various wood-inhabiting beetles	*no interactions documented with this species	*Norway*[41]
	***Mycena* sp.**	**Polyphagous Diptera** *Drosophila* sp.	*all interactions to *Mycena* species are with *Mycetophila fungorum*, *Exechia subfrigida* and *Exechia* sp. 5	*Finland*[14]
Omphalotaceae	***Rhodocollybia sp.***	*Allodiopsis rustica*	*all documented interactions are to different species	*Estonia*[24]
	Rhodocollybia butyraceae	***Mycetophila fungorm***		Czech Republic and Slovakia[23]

Physalacriaceae	***Armillaria sp.***	***Mycetophila fungorum***	(see notes below)	*Europe (broadly)*[13]
	Armillaria gallica	*Oxyporus* sp. (Coleoptera)		Discussed in Cai 2016[79]
		Mycetophila fungorum		Czech Republic and Slovakia[23]
	Armillaria mellea	**Polyphagous Diptera**, ***Mycetophila fungorum*** (most common)	*one interaction each to *Mycetophila fungorum* and *Megaselia pulicaria complex*, two generalists	*Finland*[14]
Pleurotaceae	*Pleurotus ostreatus*	*Oxyporus* sp. (Coleoptera) Staphylinidae sp. (Coleoptera)	*three Pleurotaceae species collected, interactions with one Staphylinidae and two Erotylidae species	Discussed in Cai 2016[79] *Virginia, USA*[26]
Plutaceae	***Pluteus sp.***	A few **polyphagous Diptera** – **Phoridae** species	**Mycetophila fungorum*, *Megaselia variana* (from *Pluteus hongoi*)	*Finland*[14]
	Pluteus cervinus	*Megaselia sp., Mycetophila fungorum,* **Platypezidae sp.**	*one interaction with Lindneromyia sp.4 (Platypezidae)	*Finland*[14]
Psathyrellaceae	***Psathyrella candolleana***	***Megaselia sp.***		*Finland*[14]
		Megaselia marquezi	*three *M.marquezi* specimens collected, all in association with *Psathyrella sp.* or *Psathyrella candolleana*	*Los Angeles, USA*[20]
		Mycetophila fungorum		Czech Republic and Slovakia[23]
Tricholomataceae		***Mycetophila fungorum****, Exechia separata*		*Europe (broadly)*[13]
	***Tricholoma* sp.**	**Polyphagous Diptera**, mostly fungus gnats	*most common interaction with *Mycetophila fungorum*, a generalist	*Finland*[14]
Russulales				
Russulaceae	**Russulaceae** (overall)	***Mycetophila fungorum*** (most common), ***Cordyla sp.***	*five of the six documented Cordyla species in association with the Russulaceae family *multiple interactions with *Mycetophila fungorum*	*Finland*[14], *Europe (broadly)*[13]
		Exechia nigroscutellata	*all species only found in association with Russulaceae mushrooms	*Britain*[21]

	Lactarius sp.	***Exechia nigroscutellata***	*no interactions with *Mycetophila fungorum* documented *nine interactions with *Exechia nigroscutellata*	*Finland*[14] *Europe (broadly)*[13], *Estonia*[24]
	Lactarius torminosus	***Exechia nigroscutellata**, Mycetophila fungorum, Megaselia sp.*	*one interaction with *E.nigroscutellata*	*Finland*[14]
	Russula sp.	Various *Drosophila* sp.		*New York, USA*[15]
		Mycetophila strigatoides		*Finland, Russia, Europe (broadly)*[22]
		Mycetophila fungorum		*Estonia*[24]
	Russula delica	**Polyphagous Diptera**	*one interaction with *Mycetophila fungorum*, a generalist	*Finland*[14]
	***Stereum* sp.**	Various wood-inhabiting beetles Multiple mycetophilid species (Diptera)	*no interactions documented for this genus	Norway[41] *Europe (broadly)*[13]
Boletales				
Boletaceae	Boletaceae (overall)	*Mycetophila fungorum, Mycetophila signatoides,* ***Exechia separata***	*only found *Exechia separata* in association with *Suillus* mushrooms	*Europe (broadly)*[13], *Britain*[21]
	Chalciporus piperatus	**Polyphagous Diptera**	*interactions with *Megaselia pulicaria* complex, a generalist	*Finland*[14]
	***Leccinum* sp.**	*Pegomya* sp., some fungus gnats	*no interactions documented for this genus	*Finland*[14]
	Scleroderma sp.	Few mycetophilids, generally only polyphagous Mycetophilids	*only one documented interaction with a Coleoptera species	*Europe (broadly)*[13]
Suillaceae	***Suillus* sp.**	***Exechia separata***, other Bolitophila and Exechiopsis fungus gnats		*Finland*[14], *Estonia*[24]
		Mycetophila fungorum		*Estonia*[24]
Polyporales				
Polyporales (overall)		Some *Mycetophila*, other mycetophilids	*one Limoniidae, four Mycetophilidae, three Cecidomyiidae and one each of Phoridae and Keroplatidae observed in association with Polyporales mushrooms	*Europe (broadly)*[13]
Polyporaceae	***Polyporus sp.***	***Mycetophila strigatoides***	*principally associated with Polyporus, reared from a *Lentinus* species – one documented association with a *Lentinus* species	*Finland, Russia, Europe (broadly)*[22] Czech Republic and Slovakia[23]

	Trametes sp.	*Mycetophila strigatoides*		*Finland, Russia, Europe (broadly)*[22]
	Trametes versicolor	Various wood-inhabiting beetles	*one interaction documented with a *Megaselia* (Diptera) species	*Norway*[41]
	Tyromyces chioneus	***Mycetophila mohilevensis***	*this is a rare Diptera species, previously found in association with a Polypore mushroom – one *M. mohilevensis* specimen obtained in this study, found in association with an unidentified Polypore species	Czech Republic and Slovakia[23]
Hymenochaetales				
Hymenochaetales (overall)		Few **mycetophilid** species		*Europe (broadly)*[13]
Incertae sedis (Trichaptum)	***Trichaptum abietinum***	Various wood-inhabiting beetles	*one interaction documented with a Diptera species	*Norway*[41]
Cantharellales				
Hydnaceae	***Cantharellus* sp.**	Infestation rare, Limoniinae sp.		*Finland*[14]
		Few mycetophilid species (Diptera)	*two interactions documented: *Mycodrosophila dimidiata* and ***Mycetophila*** sp.	*Europe (broadly)*[13]
Pezizales				
Pezizales (in general)		***Allodia*** are the most abundant	*one interaction documented to Helvella sp.: *Allodia* species	*Europe (broadly)*[13]

Supplementary Table 2

Date	Location	Sample Number	Mushroom Identification	Insect Identification	Non-Insect Arthropod ID
07-Sep-18	Cootes Paradise	09.07.01	Lepiota sp.	Megaselia sp. Var. 12	-
09-Sep-18	Sandor Property	09.09.01	Amanita sp.	-	-
17-Sep-18	Dundas Valley	09.17.01	Picipes badius	-	-
17-Sep-18	Dundas Valley	09.17.02	Arrhenia sp.	-	-
17-Sep-18	Dundas Valley	09.17.03	Clitopilus cf. prunulus	-	-
17-Sep-18	Dundas Valley	09.17.04	Russula aff. rutila	Exechia sp. DNAS-41B-5ZB1	-
17-Sep-18	Dundas Valley	09.17.05	Russula sp.	-	-
17-Sep-18	Dundas Valley	09.17.06	Neofavolus cf. alveolaris	-	-
17-Sep-18	Dundas Valley	09.17.07	Tetrapyrgos cf. nigripes	-	-
17-Sep-18	Dundas Valley	09.17.08	Chalciporus cf. piperatus/piperatoides	-	-
17-Sep-18	Dundas Valley	09.17.09	Russula sp.	Megaselia pulicaria complex	-
17-Sep-18	Dundas Valley	09.17.10	Clitopilus cf. prunulus	Exechia sp. Var. 2	-
23-Sep-18	Wirth Property	09.23.01	Leucoagaricus leucothites/subcretaceus	Megaselia sp. n.2 SH-2015	Trachelipus sp.
23-Sep-18	Wirth Property	09.23.03	Leucoagaricus leucothites	-	-
23-Sep-18	Wirth Property	09.23.04	Leucoagaricus leucothites/subcretaceus	Mycetophila sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.05	Leucoagaricus leucothites/subcretaceus	-	-
23-Sep-18	Wirth Property	09.23.06	Leucoagaricus leucothites/subcretaceus	Megaselia sp. n.2 SH-2015 (2)	-
23-Sep-18	Wirth Property	09.23.07	Leucoagaricus leucothites/subcretaceus	-	-
23-Sep-18	Wirth Property	09.23.08	Leucoagaricus leucothites/subcretaceus	-	-
23-Sep-18	Wirth Property	09.23.11	Clitocybe cf. squamulosa/Infundibulicybe cf. gibba	-	-
23-Sep-18	Wirth Property	09.23.12	Lactarius sp.*	Mycetophila sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.13	Leucoagaricus leucothites/subcretaceus	-	-
23-Sep-18	Wirth Property	09.23.14	Clitocybe cf. phyllophila	-	-
23-Sep-18	Wirth Property	09.23.15	Lactarius sp.*	-	-
23-Sep-18	Wirth Property	09.23.16	Leucoagaricus leucothites/subcretaceus	Megaselia sp. Var. 5	-
23-Sep-18	Wirth Property	09.23.17	Lactarius deliciosus	-	-
23-Sep-18	Wirth Property	09.23.18	Lactarius sp.*	Mycetophila sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.19	Gymnopus dryophilus/nubicola	-	-
23-Sep-18	Wirth Property	09.23.20	Lactarius cf. deliciosus	Metalimnobia triocellata (2)	-
23-Sep-18	Wirth Property	09.23.21	Lactarius cf. deliciosus	Mycetophila sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.22	Suillus americanus/himalayensis	-	-
23-Sep-18	Wirth Property	09.23.23	Leucoagaricus leucothites/subcretaceus	-	-
23-Sep-18	Wirth Property	09.23.24	-	Megaselia sp. Var. 5	-
23-Sep-18	Wirth Property	09.23.25	-	Cecidomyiidae sp. Var. 1	-
				Mycetophila sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.26	Leucoagaricus leucothites/subcretaceus	Megaselia sp. n.2 SH-2015	-

				Cecidomyiidae sp. Var. 3	-
23-Sep-18	Wirth Property	09.23.27	Leucoagaricus leucothites/subcretaceus	Megaselia sp. Var. 5	-
23-Sep-18	Wirth Property	09.23.28	Lactarius sp.*	Mycetophila sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.29	Leucoagaricus leucothites/subcretaceus	Megaselia sp. n.2 SH-2015 (2)	-
23-Sep-18	Wirth Property	09.23.31	Leucoagaricus leucothites/subcretaceus	-	-
23-Sep-18	Wirth Property	09.23.32	Lycoperdaceae sp.	-	-
23-Sep-18	Wirth Property	09.23.33	Clitocybe cf. phyllophila	Mycetophila sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.34	Gymnopus dryophilus	-	-
23-Sep-18	Wirth Property	09.23.35	Leucoagaricus leucothites/subcretaceus	Megaselia sp. n.2 SH-2015 (2)	-
23-Sep-18	Wirth Property	09.23.36	Suillus americanus/himalayensis	-	-
23-Sep-18	Wirth Property	09.23.37	Clitocybe cf. phyllophila	-	-
23-Sep-18	Wirth Property	09.23.38	Leucoagaricus leucothites/subcretaceus	Megaselia sp. n.2 SH-2015 (2)	-
23-Sep-18	Wirth Property	09.23.39	Lepista sp.*	-	-
23-Sep-18	Wirth Property	09.23.41	Thelephora caryophyllea/regularis	-	-
23-Sep-18	Wirth Property	09.23.42	Leucoagaricus leucothites/subcretaceus	Cecidomyiidae sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.43	Gymnopus aff. montagnei/variicolor	-	-
23-Sep-18	Wirth Property	09.23.44	Clitocybe cf. phyllophila	-	-
23-Sep-18	Wirth Property	09.23.45	Leucoagaricus leucothites/subcretaceus	Cecidomyiidae sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.47	Lepiota subincarnata	-	-
23-Sep-18	Wirth Property	09.23.48	Rhodocollybia maculata	-	-
23-Sep-18	Wirth Property	09.23.49	Lactarius cf. deliciosus	Mycetophila sp. Var. 1	-
				Cecidomyiidae sp. Var. 9	-
23-Sep-18	Wirth Property	09.23.53	Lactarius sp.*	Mycetophila sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.54	Rhodocollybia maculata	Exechia dorsalis	Parasitidae sp.
23-Sep-18	Wirth Property	09.23.56	Lactarius sp.*	Mycetophila sp. Var. 1	-
23-Sep-18	Wirth Property	09.23.57	Lactarius sp.*	-	-
23-Sep-18	Sandor Property	09.23.58	Agaricus cf. abruptibulbus	Megaselia sp. Var. 20	-
23-Sep-18	Sandor Property	09.23.59	Amanita muscaria	-	-
23-Sep-18	Sandor Property	09.23.60	Armillaria sp.*	Platypezidae sp. Var. 1	-
23-Sep-18	Sandor Property	09.23.61	Gymnopilus cf. spectabilis/junonius	-	-
23-Sep-18	Sandor Property	09.23.62	Coprinellus cf. radians/xanthothrix	-	-
23-Sep-18	Sandor Property	09.23.68	Entoloma strictius	-	-
25-Sep-18	McMaster, DBAC	09.25.01	Agaricus sp.*	Megaselia sp. Var. 5	-
				Megaselia nigra	-
29-Sep-18	Dundas Valley	09.29.02	-	-	Hypogastruridae sp.
29-Sep-18	Dundas Valley	09.29.03	Fomitopsis betulina	-	-
29-Sep-18	Dundas Valley	09.29.05	Mycena leaiana	-	-
29-Sep-18	Dundas Valley	09.29.07	Picipes badius	-	-

29-Sep-18	Dundas Valley	09.29.08	Gymnopus cf. dryophilus	-	-
29-Sep-18	Dundas Valley	09.29.09	Scleroderma areolatum	-	-
29-Sep-18	Dundas Valley	09.29.10	Loweomyces fractipes	-	-
29-Sep-18	Dundas Valley	09.29.12	Mycena aff. inclinata	-	-
29-Sep-18	Dundas Valley	09.29.13	Mycena cf. inclinata	-	-
29-Sep-18	Dundas Valley	09.29.14	Mycena cf. inclinata/alcalina	-	-
29-Sep-18	Cootes Paradise	09.29.15	Mycena sp.	-	-
29-Sep-18	Cootes Paradise	09.29.16	Mycena filopes	-	-
29-Sep-18	Cootes Paradise	09.29.17	Stropharia sp.	-	-
29-Sep-18	Cootes Paradise	09.29.18	Mycena cf. haematopus	-	-
29-Sep-18	Cootes Paradise	09.29.19	Stropharia sp.	-	-
29-Sep-18	Cootes Paradise	09.29.20	Tyromyces galactinus	-	-
29-Sep-18	Cootes Paradise	09.29.21	Stropharia sp.	-	-
29-Sep-18	Cootes Paradise	09.29.22	Mycena leaiana	Exechia subfrigida (2)	-
29-Sep-18	Cootes Paradise	09.29.23	Polyporus picipes	-	-
29-Sep-18	Cootes Paradise	09.29.24	Mycena aff. galericulata/megaspora	Mycetophila fungorum	Tomocerus sp.
				-	Julidae sp.
29-Sep-18	Cootes Paradise	09.29.25	Coprinus bellula/cortinata	-	-
29-Sep-18	Cootes Paradise	09.29.26	Trichaptum biforme/subchartaceum	-	-
29-Sep-18	Cootes Paradise	09.29.28	Mycena aff. galericulata/megaspora	Mycetophila fungorum	-
29-Sep-18	Cootes Paradise	09.29.29	Suillus americanus/himalayensis	-	-
29-Sep-18	Cootes Paradise	09.29.30	Suillus americanus/himalayensis	-	-
29-Sep-18	Cootes Paradise	09.29.31	Suillus americanus/himalayensis	-	-
30-Sep-18	Lowrie Property	09.30.01	Amanita muscaria	Mycetophila fungorum	-
				Megaselia pulicaria complex	-
				Aphis glycines	-
30-Sep-18	Lowrie Property	09.30.02	Amanita muscaria	Megaselia pulicaria complex	-
30-Sep-18	Lowrie Property	09.30.03	Amanita muscaria	Mycetophila fungorum	-
30-Sep-18	Lowrie Property	09.30.04	Amanita muscaria	Mycetophila fungorum	-
30-Sep-18	Lowrie Property	09.30.05	Hymenopellis furfuracea/radicata	-	-
30-Sep-18	Lowrie Property	09.30.06	Gymnopus subnudus/Marasmiellus subnudus	-	-
30-Sep-18	Lowrie Property	09.30.07	Russula aff. risigallina	-	Hypogastruridae sp.
30-Sep-18	Lowrie Property	09.30.08	Lepiota aff. erminea	-	-
30-Sep-18	Lowrie Property	09.30.09	Hebeloma cf. leucosarx	Tachinus fimbriatus	-
30-Sep-18	Lowrie Property	09.30.10	Stereum sp.*	-	-
30-Sep-18	Lowrie Property	09.30.11	Gymnopus cf. spongiosus	-	-
30-Sep-18	Lowrie Property	09.30.12	Cortinarius sp.*	Megaselia pulicaria complex	Hypogastruridae sp.
30-Sep-18	Lowrie Property	09.30.13	Russula cf. grata	Metalimnobia triocellata	Julidae sp. (2)

30-Sep-18	Lowrie Property	09.30.14	Mycena cf. haematopus	Exechia sp. Var. 5	-
30-Sep-18	Lowrie Property	09.30.15	Hericium sp.*	-	-
30-Sep-18	Lowrie Property	09.30.17	Lactarius cf. pubescens	-	-
30-Sep-18	Lowrie Property	09.30.18	Marasmius cf. siccus/fulvoferrugineus	-	-
30-Sep-18	Lowrie Property	09.30.19	Lactarius cf. pubescens	-	-
30-Sep-18	Lowrie Property	09.30.22	Lactarius cf. pubescens	-	-
30-Sep-18	Lowrie Property	09.30.24	-	-	Hypogastruridae sp.
30-Sep-18	Lowrie Property	09.30.25	Hebeloma cf. leucosarx	Tachinus fimbriatus	-
30-Sep-18	Lowrie Property	09.30.26	Hygrocybe chlorophana	-	-
30-Sep-18	Lowrie Property	09.30.27	Mycena leaiana	-	-
30-Sep-18	Lowrie Property	09.30.28	Amanita cf. vaginata	-	-
30-Sep-18	Lowrie Property	09.30.29	Amanita muscaria	-	-
30-Sep-18	Lowrie Property	09.30.31	Lactarius pubescens	Megaselia pulicaria complex	-
30-Sep-18	Lowrie Property	09.30.32	Lactifluus aff. volemus	-	-
30-Sep-18	Lowrie Property	09.30.33	Hygrocybe cf. marchii	-	-
30-Sep-18	Lowrie Property	09.30.34	Russula aff. risigallina	Mycetophila fungorum	Proctolaelaps sp.
				Cordyla sp. Var. 1	-
30-Sep-18	Lowrie Property	09.30.35	Amanita sp.	-	-
30-Sep-18	Lowrie Property	09.30.36	Hymenopellis cf. furfuracea/radicata	-	-
30-Sep-18	Lowrie Property	09.30.37	Hymenopellis furfuracea/radicata	Megaselia pulicaria complex	-
30-Sep-18	Lowrie Property	09.30.38	-	Tachinus fimbriatus	-
				Lordithon facilis	-
				Mycetophila fungorum	-
30-Sep-18	Lowrie Property	09.30.39	Amanita muscaria	Mycetophila fungorum (2)	-
30-Sep-18	Lowrie Property	09.30.44	Rhodocollybia butyracea	-	Hypogastruridae sp.
30-Sep-18	Lowrie Property	09.30.45	Daedaleopsis confragosa/Lenzites betulina	-	-
30-Sep-18	Lowrie Property	09.30.46	Russula aff. risigallina	Tachinus fimbriatus	-
30-Sep-18	Lowrie Property	09.30.47	Mycena cf. galericulata/megaspora	-	-
30-Sep-18	Lowrie Property	09.30.49	Scleroderma citrinum	-	-
01-Oct-18	Sandor Property	10.01.01	-	Metalimnobia triocellata	-
01-Oct-18	Sandor Property	10.01.02	Tapinella atrotomentosa	-	-
01-Oct-18	Sandor Property	10.01.04	Amanita muscaria	Mycetophila fungorum	Hypogastruridae sp.
				Megaselia pulicaria complex	-
01-Oct-18	Sandor Property	10.01.05	Mycena cf. leaiana	-	-
01-Oct-18	Sandor Property	10.01.06	Amanita muscaria	Mycetophila fungorum	-
01-Oct-18	Sandor Property	10.01.07	Lactarius vinaceorufescens	-	-
01-Oct-18	Sandor Property	10.01.08	Amanita muscaria	Mycetophila fungorum (2)	-
01-Oct-18	Sandor Property	10.01.09	Inocybe sp.*	-	Parasitylenchidae sp.

01-Oct-18	Sandor Property	10.01.10	Mycena haematopus	-	-
01-Oct-18	Sandor Property	10.01.11	Amanita muscaria	-	-
01-Oct-18	Sandor Property	10.01.12	Lactarius vinaceorufescens	-	-
01-Oct-18	Sandor Property	10.01.13	Lepiota cf. grangei/boudieri	-	-
01-Oct-18	Sandor Property	10.01.14	Amanita muscaria	Mycetophila fungorum	Hypogastruridae sp.
01-Oct-18	Sandor Property	10.01.15	-	Mycetophila fungorum	-
01-Oct-18	Sandor Property	10.01.16	Cyclocybe cf. erebia	Mycetophila fungorum	-
01-Oct-18	Sandor Property	10.01.17	Amanita suballiacea/bisporigera	-	-
01-Oct-18	Sandor Property	10.01.19	Amanita muscaria	Mycetophila fungorum	-
01-Oct-18	Sandor Property	10.01.20	Rhodocollybia butyracea	Proclitus praetor	-
01-Oct-18	Sandor Property	10.01.21	Amanita muscaria	-	-
01-Oct-18	Sandor Property	10.01.22	Trametes gibbosa	-	-
01-Oct-18	Sandor Property	10.01.23	Amanita sp.*	-	-
01-Oct-18	Sandor Property	10.01.24	Artomyces pyxidatus	-	-
01-Oct-18	Sandor Property	10.01.25	Cyclocybe cf. erebia	Exechia subfrigida	-
01-Oct-18	Sandor Property	10.01.26	Lactarius vinaceorufescens	Megaselia sp. Var. 14	-
01-Oct-18	Sandor Property	10.01.27	Roridomyces aff. roridus	-	-
01-Oct-18	Sandor Property	10.01.28	Inocybe sp.	-	-
10-Oct-18	Cootes Paradise	10.10.01	Amanita cf. suballiacea/bisporigera	Megaselia pulicaria complex	-
				Exechia sp. Var. 1	-
10-Oct-18	Cootes Paradise	10.10.03	Amanita suballiacea/bisporigera	Tachinus fimbriatus	-
10-Oct-18	Cootes Paradise	10.10.04	Lepista sp.*	Megaselia sp. Var. 19 (3)	Hypogastruridae sp.
10-Oct-18	Cootes Paradise	10.10.05	Leucoagaricus leucothites	-	-
10-Oct-18	Cootes Paradise	10.10.06	Suillus americanus/himalayensis	Exechia separata	-
10-Oct-18	Cootes Paradise	10.10.08	Leucoagaricus cf. leucothites/subcretaceus	Orthocladiinae sp.	-
10-Oct-18	Cootes Paradise	10.10.09	Lactarius cf. pubescens	Exechia nigroscutellata	-
10-Oct-18	Cootes Paradise	10.10.10	Amanita suballiacea/bisporigera	-	Julidae sp.
10-Oct-18	Cootes Paradise	10.10.11	Lactarius cf. pubescens/torminosus	Exechia nigroscutellata	-
10-Oct-18	Cootes Paradise	10.10.12	Amanita muscaria	Mycetophila fungorum	-
10-Oct-18	Cootes Paradise	10.10.13	Amanita suballiacea/bisporigera	-	-
10-Oct-18	Cootes Paradise	10.10.14	Lycoperdon perlatum	-	-
10-Oct-18	Cootes Paradise	10.10.15	Lactarius pubescens	Exechia nigroscutellata (2)	-
10-Oct-18	Cootes Paradise	10.10.16	Amanita suballiacea/bisporigera	-	-
10-Oct-18	Cootes Paradise	10.10.17	Cyclocybe cf. erebia	Exechia subfrigida	Trachelipus sp.
				-	Hypogastruridae sp.
10-Oct-18	Cootes Paradise	10.10.18	Lepiota cf. cristata/castanea	-	-
10-Oct-18	Cootes Paradise	10.10.19	Mycena aff. galericulata/megaspora	Exechia subfrigida	Hypogastruridae sp.
10-Oct-18	Cootes Paradise	10.10.20	Amanita suballiacea/bisporigera	-	-

10-Oct-18	Cootes Paradise	10.10.21	Mycena cf. inclinata	-	-
10-Oct-18	Cootes Paradise	10.10.22	Lycoperdon cf. perlatum	-	-
10-Oct-18	Cootes Paradise	10.10.23	Mycena sp.	-	-
10-Oct-18	Cootes Paradise	10.10.24	Amanita aff. lavendula/citrina	-	-
10-Oct-18	Cootes Paradise	10.10.25	Mycena aff. galericulata/megaspora	-	-
10-Oct-18	Cootes Paradise	10.10.26	Mycena aff. inclinata	-	-
10-Oct-18	Cootes Paradise	10.10.27	Amanita cf. lavendula/citrina	-	-
10-Oct-18	Cootes Paradise	10.10.29	Amanita aff. lavendula/citrina	-	-
10-Oct-18	Cootes Paradise	10.10.30	Amanita cf. lavendula/citrina	-	-
10-Oct-18	Cootes Paradise	10.10.31	Entoloma flavifolium/albidum	Mycetophila fungorum	Hypogastruridae sp.
10-Oct-18	Cootes Paradise	10.10.32	Lepiota cf. cristata	-	-
10-Oct-18	Cootes Paradise	10.10.33	-	-	Julidae sp.
10-Oct-18	Cootes Paradise	10.10.34	Mycena aff. inclinata	-	-
10-Oct-18	Cootes Paradise	10.10.35	Mycena aff. galericulata/megaspora	-	Choneiulus palmatus
10-Oct-18	Cootes Paradise	10.10.36	Lepiota cf. cristata	-	-
10-Oct-18	Cootes Paradise	10.10.37	Psathyrella corrugis/amarescens	-	-
10-Oct-18	Cootes Paradise	10.10.38	Mycena aff. galericulata/megaspora	Exechia subfrigida	-
10-Oct-18	Cootes Paradise	10.10.39	Inocybaceae sp.*	Mycetophila fungorum	-
10-Oct-18	Cootes Paradise	10.10.40	Inocybe lanatodisca/ Inosperma maculatum	-	-
10-Oct-18	Cootes Paradise	10.10.41	Mycena aff. galericulata/megaspora	-	Rhabditidae sp.
				-	Hypogastruridae sp.
10-Oct-18	Cootes Paradise	10.10.42	Mycena haematopus	-	-
10-Oct-18	Cootes Paradise	10.10.43	Entoloma flavifolium/albidum	-	-
10-Oct-18	Cootes Paradise	10.10.44	Galerina marginata	-	-
10-Oct-18	Cootes Paradise	10.10.45	Russula aff. changbaiensis	Mycetophila fungorum	-
10-Oct-18	Cootes Paradise	10.10.46	Mycena aff. galericulata/megaspora	Mycetophila fungorum	-
10-Oct-18	Cootes Paradise	10.10.47	Mycena aff. inclinata	-	-
10-Oct-18	Cootes Paradise	10.10.48	Inocybaceae sp.*	Mycetophila fungorum	-
10-Oct-18	Cootes Paradise	10.10.49	Russula aff. changbaiensis	-	-
10-Oct-18	Cootes Paradise	10.10.50	Mycena cf. inclinata	-	Julidae sp.
10-Oct-18	Cootes Paradise	10.10.51	Mycena leaiana	Mycetophila fungorum	-
11-Oct-18	Cootes Paradise	10.11.02	Armillaria sp.*	Mycetophila fungorum	-
11-Oct-18	Cootes Paradise	10.11.03	Armillaria cf. sinapina/cepistipes	Exechia subfrigida	-
11-Oct-18	Cootes Paradise	10.11.04	Mycena cf. inclinata	-	-
11-Oct-18	Cootes Paradise	10.11.05	Armillaria sp.*	-	-
11-Oct-18	Cootes Paradise	10.11.06	Mycena haematopus	Exechia subfrigida	-
11-Oct-18	Cootes Paradise	10.11.07	Leucoagaricus sp.	-	-
11-Oct-18	Cootes Paradise	10.11.08	Leucoagaricus sp.	Exechia subfrigida (2)	-

11-Oct-18	Cootes Paradise	10.11.09	Cyclocybe cf. erebia	Mycetophila fungorum	-
11-Oct-18	Cootes Paradise	10.11.10	Armillaria cf. sinapina/cepistipes	Mycetophila fungorum	-
11-Oct-18	Cootes Paradise	10.11.11	Mycena galopus	Exechia subfrigida	-
11-Oct-18	Cootes Paradise	10.11.12	Tricholomopsis sulfureoides	Mycetophila fungorum	-
11-Oct-18	Cootes Paradise	10.11.13	Mycena cf. inclinata	Exechia subfrigida	-
11-Oct-18	Cootes Paradise	10.11.14	Mycena aff. galericulata	Exechia subfrigida	-
11-Oct-18	Cootes Paradise	10.11.15	Armillaria sp.*	Platypeza sp. 1 (2)	-
11-Oct-18	Cootes Paradise	10.11.16	Pluteus hongoi	Mycetophila fungorum	-
11-Oct-18	Cootes Paradise	10.11.17	Mycena cf. inclinata	Exechia sp. Var. 5	-
11-Oct-18	Cootes Paradise	10.11.18	Pluteus hongoi	-	-
11-Oct-18	Cootes Paradise	10.11.19	Hypholoma lateritium	Mycetophila fungorum	-
11-Oct-18	Cootes Paradise	10.11.20	Leucoagaricus sp.	-	-
11-Oct-18	Cootes Paradise	10.11.21	Trametes versicolor	-	-
11-Oct-18	Cootes Paradise	10.11.22	Lepiota cristata	Exechia subfrigida	-
11-Oct-18	Cootes Paradise	10.11.23	Coprinus cf. comatus	Tachinus fimbriatus	-
11-Oct-18	Cootes Paradise	10.11.24		Gyrophaena socia	-
				Triplax flavicollis	-
11-Oct-18	Cootes Paradise	10.11.25	Pleurotus cornucopiae/pulmonarius	-	-
11-Oct-18	Cootes Paradise	10.11.26	Hypholoma lateritium	Mycetophila fungorum	-
11-Oct-18	Cootes Paradise	10.11.27	Mycena haematopus	Exechia subfrigida	-
11-Oct-18	McMaster, DBAC	10.11.28	Leucoagaricus leucothites/subcretaceus	-	-
11-Oct-18	McMaster, DBAC	10.11.29	Agaricus sp.*	Megaselia nigra (2)	-
11-Oct-18	McMaster, DBAC	10.11.30	Leucoagaricus leucothites/subcretaceus	-	-
11-Oct-18	McMaster, DBAC	10.11.31	Leucoagaricus leucothites/subcretaceus	Mycetophila fungorum	-
11-Oct-18	McMaster, DBAC	10.11.32	Leucoagaricus leucothites/subcretaceus	Exechia subfrigida	-
11-Oct-18	McMaster, DBAC	10.11.33	Lepiota cristata	-	-
11-Oct-18	McMaster, DBAC	10.11.34	Leucoagaricus leucothites/subcretaceus	-	-
11-Oct-18	McMaster, DBAC	10.11.35	Leucoagaricus leucothites/subcretaceus	Mycetophila fungorum	-
11-Oct-18	McMaster, DBAC	10.11.36	Leucoagaricus leucothites/subcretaceus	-	-
12-Oct-18	Dundas Valley	10.12.01	Agaricus sp.*	-	-
12-Oct-18	Dundas Valley	10.12.02	Lepista personata/nuda	Bolitophila triangulata	-
12-Oct-18	Dundas Valley	10.12.03	Leucoagaricus leucothites/subcretaceus	-	-
12-Oct-18	Dundas Valley	10.12.04	Lepiota magnispora	-	Eupodidae sp.
12-Oct-18	Dundas Valley	10.12.05	Fomitopsis betulina	-	-
12-Oct-18	Dundas Valley	10.12.06	Russula aff. decipiens/rutila	Mycetophila fungorum (2)	Julidae sp.
12-Oct-18	Dundas Valley	10.12.07	Armillaria cf. mellea	Mycetophila fungorum (2)	-
12-Oct-18	Dundas Valley	10.12.08	Leucoagaricus leucothites/subcretaceus	-	-
12-Oct-18	Dundas Valley	10.12.09	Clitopilus cf. prunulus	Exechia sp. Var. 2	-

12-Oct-18	Dundas Valley	10.12.10	Lycoperdon excipuliforme/Calvatia turneri	-	-
12-Oct-18	Dundas Valley	10.12.11	Russula aff. decipiens/rutila	Mycetophila fungorum	Dicyrtomidae sp.
12-Oct-18	Dundas Valley	10.12.12	Russula sp.*	-	Dicyrtomidae sp.
12-Oct-18	Dundas Valley	10.12.13	Entoloma aff. sericellum	-	-
12-Oct-18	Dundas Valley	10.12.14	Coprinellus xanthothrix/domesticus	-	-
12-Oct-18	Dundas Valley	10.12.15	Lepiota magnispora	-	Eupodidae sp.
12-Oct-18	Dundas Valley	10.12.16	Lepiota subincarnata	Exechia subfrigida (2)	-
12-Oct-18	Dundas Valley	10.12.17	Armillaria sp.*	-	Lepidocyrtus paradoxus
12-Oct-18	Dundas Valley	10.12.18	Clitopilus cf. prunulus	-	-
12-Oct-18	Dundas Valley	10.12.19	Lepiota subincarnata	Exechia subfrigida	-
13-Oct-18	Welland	10.13.01	Abortiporus biennis	-	Oribatulidae sp.
14-Oct-18	Barrie	10.14.01	Laccaria cf. bicolor/laccata	-	-
14-Oct-18	Barrie	10.14.02	Cantharellula cf. umbonata	-	-
14-Oct-18	Barrie	10.14.03	Amanita muscaria	Mycetophila fungorum	-
14-Oct-18	Barrie	10.14.04	Tricholoma cf. album/roseoacerbum	-	-
14-Oct-18	Barrie	10.14.05	Cortinarius aff. xanthocephalus/anomalus	-	-
14-Oct-18	Barrie	10.14.06	Lycoperdaceae sp.*	-	-
14-Oct-18	Barrie	10.14.08	Amanita aff. lavendula/citrina	-	-
14-Oct-18	Barrie	10.14.10	Amanita muscaria	Mycetophila fungorum	-
14-Oct-18	Barrie	10.14.11	Amanita muscaria	-	-
14-Oct-18	Barrie	10.14.12	Tricholoma aff. mutabile	Mycetophila fungorum	-
				Exechia sp. Var. 2*	-
14-Oct-18	Barrie	10.14.13	Tricholoma cf. album/roseoacerbum	Trichoceridae sp.	-
15-Oct-18	Sandor Property	10.15.01	Leucocybe candicans	-	-
15-Oct-18	Sandor Property	10.15.02	Lactarius vinaceorufescens	-	-
15-Oct-18	Sandor Property	10.15.03	Lactarius vinaceorufescens	-	Rhabditidae sp.
15-Oct-18	Sandor Property	10.15.04	Entoloma brunneosericeum	-	-
15-Oct-18	Sandor Property	10.15.05	Mycena robusta	-	Hypogastruridae sp.
15-Oct-18	Sandor Property	10.15.06	Lactarius vinaceorufescens	-	-
15-Oct-18	Sandor Property	10.15.07	Entoloma brunneosericeum	-	-
15-Oct-18	Sandor Property	10.15.08	-	-	Hypogastruridae sp.
15-Oct-18	Sandor Property	10.15.09	Russula betularum	-	-
15-Oct-18	Sandor Property	10.15.10	Hygrophoraceae sp.	-	-
15-Oct-18	Sandor Property	10.15.11	Lepiota magnispora	-	-
15-Oct-18	Sandor Property	10.15.13	Hygrophoraceae sp.	Brachypeza sp. Var. 1	-
15-Oct-18	Sandor Property	10.15.14	Lactarius vinaceorufescens	-	-
15-Oct-18	Sandor Property	10.15.15	Scleroderma citrinum	-	-
15-Oct-18	Sandor Property	10.15.16	Lactarius cf. vinaceorufescens/chrysorrheus	-	Hypogastruridae sp.

15-Oct-18	Sandor Property	10.15.17	Russula cf. puellaris	-	Tomoceridae sp.
				-	Hypogastruridae sp.
15-Oct-18	Sandor Property	10.15.18	Russula betularum	-	-
15-Oct-18	Sandor Property	10.15.19	Leucocybe candicans	-	Entomobryidae sp.
15-Oct-18	Sandor Property	10.15.20	Scleroderma citrinum	-	-
15-Oct-18	Sandor Property	10.15.21	Hygrophoraceae sp.	Mycetophila fungorum	-
15-Oct-18	Sandor Property	10.15.22	Gerhardtia aff. borealis	-	-
15-Oct-18	Sandor Property	10.15.23	Gymnopilus cf. underwoodii/validipes	-	-
15-Oct-18	Sandor Property	10.15.24	Lactarius vinaceorufescens	-	Rhabditidae sp.
15-Oct-18	Sandor Property	10.15.25	Hygrophoraceae sp.	-	Eupodidae sp.
15-Oct-18	Sandor Property	10.15.26	Leucoagaricus leucothites/subcretaceus	Mycetophila sp. Var. 1 (2)	-
15-Oct-18	Sandor Property	10.15.27	Leucoagaricus leucothites/subcretaceus	-	-
15-Oct-18	Sandor Property	10.15.29	Hypsizygus sp.*	Megaselia sp. Var. 10 (2)	-
15-Oct-18	Sandor Property	10.15.30	Lycoperdon sp.*	-	-
15-Oct-18	Sandor Property	10.15.31	Lactarius cf. chrysorrheus/vinaceorufescens	-	-
15-Oct-18	Sandor Property	10.15.32	Armillaria sp.*	Mycetophila fungorum (2)	-
15-Oct-18	Sandor Property	10.15.33	Armillaria sp.*	Brachypeza sp. Var. 2	-
15-Oct-18	Sandor Property	10.15.34	Amanita muscaria	-	-
15-Oct-18	Sandor Property	10.15.36	Lactarius vinaceorufescens	-	Schendyla nemorensis
				-	Isotomidae sp.
15-Oct-18	Sandor Property	10.15.37	Amanita muscaria	-	-
15-Oct-18	Sandor Property	10.15.38	Roridomyces aff. roridus	-	-
15-Oct-18	Sandor Property	10.15.39	Lactarius vinaceorufescens	-	Eupodidae sp. (2)
15-Oct-18	Sandor Property	10.15.40	Armillaria sp.*	Mycetophila fungorum	-
15-Oct-18	Sandor Property	10.15.41	Scleroderma citrinum	-	-
15-Oct-18	Sandor Property	10.15.42	Hygrophoraceae sp.	-	Parajulidae sp.
				-	Hypogastruridae sp.
15-Oct-18	Sandor Property	10.15.43	Lactarius vinaceorufescens/chrysorrheus	-	-
15-Oct-18	Sandor Property	10.15.44	Agaricales sp.*	-	Rhabditidae sp.
15-Oct-18	Sandor Property	10.15.45	Russula sp.*	Mycetophila fungorum	-
15-Oct-18	Sandor Property	10.15.46	Lactarius vinaceorufescens	Exechia nigroscutellata	-
15-Oct-18	Sandor Property	10.15.47	Pholiota sp.*	Oxyporus stygicus	-
15-Oct-18	Sandor Property	10.15.48	Mycetinis scorodonius	-	-
18-Oct-18	Sandor Property	10.18.01	Lepista cf. sordida/irina	Tachinus fimbriatus	Hypogastruridae sp.
				Bolitophila glabrata*	-
				Allodiopsis sp.	-
18-Oct-18	Sandor Property	10.18.02	Entoloma abortivum	-	-
18-Oct-18	Sandor Property	10.18.03	Entoloma abortivum	-	-

18-Oct-18	Sandor Property	10.18.04	Gymnopilus underwoodii/validipes	-	-
18-Oct-18	Sandor Property	10.18.05	Gymnopilus underwoodii/validipes	-	-
18-Oct-18	Sandor Property	10.18.06	Pholiota squarrosoides/jahnii	-	Hypogastruridae sp.
18-Oct-18	Sandor Property	10.18.07	Armillaria sp.*	-	Hypogastruridae sp.
				-	Eupodidae sp.
18-Oct-18	Sandor Property	10.18.08	Pluteus aurantiorugosus	-	-
18-Oct-18	Sandor Property	10.18.09	Phlebia tremellosa	-	-
18-Oct-18	Sandor Property	10.18.10	Marasmius oreades	Mycetophila sp. Var. 1	-
18-Oct-18	Sandor Property	10.18.11	Inocybe sp.	-	-
18-Oct-18	Sandor Property	10.18.12	Hygrocybe chlorophana	-	-
18-Oct-18	Sandor Property	10.18.13	Armillaria sp.*	-	-
18-Oct-18	Sandor Property	10.18.14	Galerina marginata	-	Hypogastruridae sp.
18-Oct-18	Sandor Property	10.18.15	Crepidotus sp.	-	-
18-Oct-18	Sandor Property	10.18.16	Inocybaceae sp.	-	-
18-Oct-18	Sandor Property	10.18.17	Armillaria sp.*	Allodia sp. Var. 2	-
18-Oct-18	Sandor Property	10.18.18	Entoloma abortivum	-	-
18-Oct-18	Sandor Property	10.18.19	Lactarius vinaceorufescens	Exechia nigroscutellata	-
18-Oct-18	Sandor Property	10.18.20	Armillaria sp.*	-	-
18-Oct-18	Sandor Property	10.18.21	Hebeloma sp.*	Mycetophila fungorum	-
30-May-19	Cootes Paradise	05.30.01	Lentinus cf. arcularius	Mycetophila strigatoides	-
18-Jun-19	McMaster, DBAC	06.18.01	Agaricus sp.*	Megaselia sp. n.2 SH-2015 (3)	-
				Oligota pumilio	-
18-Jun-19	McMaster, DBAC	06.18.02	Agaricus sp.*	Megaselia sp. n.2 SH-2015 (2)	-
					-
18-Jun-19	McMaster, DBAC	06.18.03	-	Megaselia sp. n.2 SH-2015	-
28-Jun-19	Cootes Paradise	06.28.01	Psathyrella candolleana/hymenocephala	-	-
28-Jun-19	Cootes Paradise	06.28.02	Psathyrella candolleana	-	-
28-Jun-19	Cootes Paradise	06.28.03	Psathyrella candolleana	-	-
28-Jun-19	Cootes Paradise	06.28.04	Psathyrella candolleana/hymenocephala	-	-
28-Jun-19	Cootes Paradise	06.28.07	Psathyrella candolleana	Megaselia marquezi	-
28-Jun-19	Cootes Paradise	06.28.08	Psathyrella candolleana	-	-
28-Jun-19	Cootes Paradise	06.28.09	Pleurotus cornucopiae/pulmonarius	Triplax thoracica	-
28-Jun-19	Cootes Paradise	06.28.10	Psathyrella cf. candolleana/badhyzensis	-	-
28-Jun-19	Cootes Paradise	06.28.11	Trichaptum cf. biforme	-	-
28-Jun-19	Cootes Paradise	06.28.12	Bolbitius reticulatus/bisporus	-	-
28-Jun-19	Cootes Paradise	06.28.14	Psathyrella candolleana	Mycetophila fungorum	Balaustium sp.
28-Jun-19	Cootes Paradise	06.28.16	Agaricales sp.◉	Mycetophila fungorum (2)	-
01-Jul-19	Dundas Valley	07.01.01	Amanita sp.◉	Allodia sp. Var. 2	-

				Mycetophila fungorum	-
01-Jul-19	Dundas Valley	07.01.02	Amanita sp.◉	Megaselia pulicaria complex	-
01-Jul-19	Dundas Valley	07.01.03	Amanita sp.◉	Megaselia sp. Var. 17	-
01-Jul-19	Dundas Valley	07.01.09	Agaricales sp.◉	Dinotrema sp. jft01 (Var. 1)	-
04-Jul-19	Subdivision, Hamilton	07.04.01	Agaricales sp.◉	Sciaridae sp. Var. 1	-
10-Jul-19	Cootes Paradise	07.10.01	Agaricales sp.◉	Mycetophila fungorum (2)	-
				Megaselia sp. Var. 1	-
11-Jul-19	Rail Trail	07.11.02	-	Megaselia sp. Var. 1	-
11-Jul-19	Rail Trail	07.11.03	-	Megaselia sp. Var. 1	-
				Megaselia sp. Var. 2	-
11-Jul-19	Rail Trail	07.11.04	-	Megaselia sp. Var. 12	-
11-Jul-19	Rail Trail	07.11.05	-	Megaselia sp. Var. 2	-
12-Jul-19	Cootes Paradise	07.12.01	Entoloma sp.◉	Megaselia pulicaria complex (2)	-
12-Jul-19	Cootes Paradise	07.12.02	Psathyrella sp.◉	Megaselia sp. Var. 2	-
12-Jul-19	Cootes Paradise	07.12.03	Agaricales sp.◉	Megaselia sp. Var. 12	-
12-Jul-19	Cootes Paradise	07.12.07	Psathyrella sp.◉	-	Hypogastruridae sp.
12-Jul-19	Cootes Paradise	07.12.08	Psathyrella sp.◉	-	Hypogastruridae sp.
12-Jul-19	Cootes Paradise	07.12.09	Psathyrella sp.◉	-	Hypogastruridae sp.
12-Jul-19	Cootes Paradise	07.12.10	Psathyrella sp.◉	Megaselia marquezi	-
12-Jul-19	Cootes Paradise	07.12.11	Entoloma sp.◉	Exechia sp. Var. 2 (2)	-
12-Jul-19	Cootes Paradise	07.12.16	Loweomyces fractipes	-	-
12-Jul-19	Cootes Paradise	07.12.17	Psathyrella candolleana/badhyzensis	Megaselia sp. Var. 1	Parasitidae sp.
12-Jul-19	Cootes Paradise	07.12.18	Psathyrella candolleana/badhyzensis	-	-
12-Jul-19	Cootes Paradise	07.12.19	Megacollybia rodmanii	Tachinus fimbriatus (2)	-
				Megaselia pulicaria complex	-
				Cratyna fulvicauda	-
12-Jul-19	Cootes Paradise	07.12.20	Megacollybia rodmanii	Staphylinidae sp.*	-
13-Jul-19	Sandor Property	07.13.01	-	Megaselia pulicaria complex	-
				Megaselia sp. Var. 12	-
				Phytocoris eximius	-
13-Jul-19	Sandor Property	07.13.02	-	Mycetophila fungorum	-
15-Jul-19	Private property, Hamilton	07.15.01	Psathyrella candolleana	Mycetophila fungorum	-
15-Jul-19	Private property, Hamilton	07.15.03	Coprinellus disseminatus	Megaselia sp. Var. 3	-
15-Jul-19	Subdivision, Hamilton	07.15.04	Agaricus bitorquis	-	-
19-Jul-19	Rail Trail	07.19.01	-	Megaselia sp. Var. 17	-
23-Jul-19	Cootes Paradise	07.23.03	Lactarius cf. haugiae	Megaselia pulicaria complex	-
				Megaselia sp. Var. 17	-
23-Jul-19	Cootes Paradise	07.23.04	Amanita sp.◉	Mycetophila fungorum	-

23-Jul-19	Cootes Paradise	07.23.06	Agaricales sp.◉	-	Parasitidae sp.
				-	Hypogastruridae sp.
23-Jul-19	Cootes Paradise	07.23.07	Amanita flavoconia/augusta	Megaselia sp. Var. 17 (2)	-
23-Jul-19	Cootes Paradise	07.23.08	Amanita rubescens	Megaselia sp. Var. 12	-
23-Jul-19	Cootes Paradise	07.23.09	Boletus nobilissimus	Megaselia sp. Var. 8	-
				Dinotrema sp. Var. 2	-
23-Jul-19	Cootes Paradise	07.23.12	Amanita rubescens/excelsa	Mycetophila fungorum (2)	Hypogastruridae sp.
				Megaselia sp. Var. 1	-
23-Jul-19	Cootes Paradise	07.23.15	Agaricales sp.◉	Mycetophila fungorum	-
				Megaselia sp. Var. 12	-
23-Jul-19	Cootes Paradise	07.23.16	Lactarius sp.◉	Megaselia sp. Var. 13	Trachelipus sp.
				Mydaea occidentalis	-
23-Jul-19	Cootes Paradise	07.23.17	Russulaceae sp.*	Keroplatidae sp. Var. 1	-
23-Jul-19	Cootes Paradise	07.23.24	Boletus sp.◉	Megaselia sp. Var. 8 (4)	-
23-Jul-19	Cootes Paradise	07.23.28	Amanita sp.◉	Megaselia sp. Var. 7	-
23-Jul-19	Cootes Paradise	07.23.29	Russula sp.◉	Exechia sp. D - S-41B-5ZB1	-
				Mycetophila fungorum	-
23-Jul-19	Cootes Paradise	07.23.31	Amanita sp.◉	Megaselia sp. Var. 17	-
23-Jul-19	Cootes Paradise	07.23.33	Amanita sp.◉	Megaselia sp. Var. 16 (3)	Parasitidae sp.
23-Jul-19	Cootes Paradise	07.23.34	Lactarius sp.◉	Megaselia sp. Var. 13	-
				Fulvius slateri	-
23-Jul-19	Cootes Paradise	07.23.36	Inocybe sp.◉	Megaselia sp. Var. 9	-
				Megaselia sp. Var. 13	-
23-Jul-19	Cootes Paradise	07.23.38	Russulaceae sp.*	Megaselia sp. Var. 17	-
23-Jul-19	Cootes Paradise	07.23.41	Trichaptum biforme	-	-
23-Jul-19	Cootes Paradise	07.23.44	Stereum sp.*	-	-
23-Jul-19	Cootes Paradise	07.23.45	Trichaptum biforme	-	-
23-Jul-19	Cootes Paradise	07.23.46	Pluteus sp.*	Lindneromyia sp.4	-
23-Jul-19	Cootes Paradise	07.23.48	Russula aff. risigallina	-	-
23-Jul-19	Cootes Paradise	07.23.49	Russula aff. aurea	-	-
23-Jul-19	Cootes Paradise	07.23.50	Russula aff. changbaiensis	-	-
23-Jul-19	Cootes Paradise	07.23.53	Russula sp.◉	Megaselia pulicaria complex	Trachelipus sp.
				Mycetophila fungorum	-
23-Jul-19	Cootes Paradise	07.23.54	Lactarius argillaceifolius	-	-
23-Jul-19	Cootes Paradise	07.23.55	-	-	-
23-Jul-19	Cootes Paradise	07.23.56	Cantharellus sp.◉	Mycodrosophila dimidiata	-
				Mycetophila sp. Var. 2	-
23-Jul-19	Cootes Paradise	07.23.57	Hygrocybe sp.	-	-

23-Jul-19	Cootes Paradise	07.23.58	Russula aff. risigallina/postiana	-	Hypogastruridae sp.
23-Jul-19	Cootes Paradise	07.23.59	Russula sp.◉	Mycetophila fungorum	-
23-Jul-19	Cootes Paradise	07.23.60	Pluteus hongoi	-	-
23-Jul-19	Cootes Paradise	07.23.61	Inocybe margaritispora	Megaselia sp. Var. 9	-
23-Jul-19	Cootes Paradise	07.23.62	Russula aff. changbaiensis	-	-
23-Jul-19	Cootes Paradise	07.23.63	Russula sp.	-	-
25-Jul-19	McMaster, field	07.25.01	Boletaceae sp.*	-	-
25-Jul-19	McMaster, field	07.25.02	Amanita sp.	-	-
31-Jul-19	Sandor Property	07.31.01	-	Fulvius slateri	-
31-Jul-19	Sandor Property	07.31.05	Tapinella atrotomentosa	-	-
31-Jul-19	Sandor Property	07.31.06	Tapinella atrotomentosa	-	-
31-Jul-19	Sandor Property	07.31.07	Tapinella atrotomentosa	-	-
31-Jul-19	Sandor Property	07.31.08	Crepidotus mollis/calolepis	-	Tomoceridae sp.
				-	Sarcoptiformes sp.
31-Jul-19	Sandor Property	07.31.09	Scleroderma citrinum	-	-
31-Jul-19	Sandor Property	07.31.10	Roridomyces aff. roridus	-	-
31-Jul-19	Sandor Property	07.31.11	Tapinella atrotomentosa	-	-
31-Jul-19	Sandor Property	07.31.12	Scleroderma citrinum	Ampedus mixtus	-
31-Jul-19	Sandor Property	07.31.14	Tapinella atrotomentosa	-	-
31-Jul-19	Sandor Property	07.31.15	Tapinella atrotomentosa	-	-
31-Jul-19	Sandor Property	07.31.16	Tapinella atrotomentosa	-	-
31-Jul-19	Sandor Property	07.31.17	Tapinella atrotomentosa	-	-
31-Jul-19	Sandor Property	07.31.18	Chalciporus piperatus	-	Tomoceridae sp.
31-Jul-19	Sandor Property	07.31.19	Roridomyces aff. roridus	-	-
31-Jul-19	Wirth Property	07.31.20	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.21	Gymnopus aff. montagnei/variicolor	-	-
31-Jul-19	Wirth Property	07.31.22	Calocybe carnea/Rugosomyces persicolor	Mycetophilidae sp.*	Dicyrtomidae sp.
31-Jul-19	Wirth Property	07.31.23	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.24	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.25	Roridomyces aff. roridus	-	-
31-Jul-19	Wirth Property	07.31.27	Calocybe carnea/Rugosomyces persicolor	Mycetophilidae sp.*	Parasitidae sp.
				-	Pachymerium ferrugineum
31-Jul-19	Wirth Property	07.31.28	Calocybe carnea/Rugosomyces persicolor	Leucophenga varia	-
31-Jul-19	Wirth Property	07.31.29	Gymnopus dryophilus	Megaselia sp. Var. 11	-
31-Jul-19	Wirth Property	07.31.30	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.31	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.32	Gymnopus aff. montagnei/variicolor	-	-
31-Jul-19	Wirth Property	07.31.33	Inocybe sp.◉	Megaselia sp. Var. 11	-

				Leucophenga varia	-
31-Jul-19	Wirth Property	07.31.34	Inocybe aff. oblectabilis	-	-
31-Jul-19	Wirth Property	07.31.35	Gymnopus dryophilus	-	-
31-Jul-19	Wirth Property	07.31.36	Gymnopus dryophilus	-	-
31-Jul-19	Wirth Property	07.31.37	Thelephora caryophyllea/regularis	-	-
31-Jul-19	Wirth Property	07.31.38	Inocybe sp.*	Gyrophaena affinis Megaselia sp. Var. 11	Dicyrtomidae sp. Rhabditidae sp.
31-Jul-19	Wirth Property	07.31.39	Gymnopus aff. dryophilus	-	-
31-Jul-19	Wirth Property	07.31.40	Gymnopus dryophilus	Allodia sp. Var. 1	-
31-Jul-19	Wirth Property	07.31.41	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.42	Gynmopus aff. montagnei/variicolor	Megaselia pulicaria complex (3)	-
31-Jul-19	Wirth Property	07.31.44	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.45	Tapinella atrotomentosa	-	-
31-Jul-19	Wirth Property	07.31.46	Gymnopus cf. dryophilus	Leucophenga varia	-
31-Jul-19	Wirth Property	07.31.47	Tapinella atrotomentosa	-	-
31-Jul-19	Wirth Property	07.31.48	Tapinella atrotomentosa	-	-
31-Jul-19	Wirth Property	07.31.49	Calocybe carnea/Rugosomyces persicolor	Drosophila tripunctata	-
31-Jul-19	Wirth Property	07.31.50	Gymnopus dryophilus	Gyrophaena affinis	Dicyrtomidae sp.
31-Jul-19	Wirth Property	07.31.51	Gymnopus dryophilus	Allodia sp. Var. 1 (2)	Dicyrtomidae sp.
31-Jul-19	Wirth Property	07.31.52	Gymnopus dryophilus	Brachistinae sp.	Dicyrtomidae sp.
31-Jul-19	Wirth Property	07.31.53	Gymnopus dryophilus	-	-
31-Jul-19	Wirth Property	07.31.54	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.55	Gymnopus aff. dryophilus	Brachistinae sp.	-
31-Jul-19	Wirth Property	07.31.56	Calocybe carnea/Rugosomyces persicolor	Leucophenga varia	-
31-Jul-19	Wirth Property	07.31.57	Gymnopus dryophilus	-	-
31-Jul-19	Wirth Property	07.31.58	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.59	Tapinella atrotomentosa	-	-
31-Jul-19	Wirth Property	07.31.60	Inocybe flocculosa/stuntzii	-	-
31-Jul-19	Wirth Property	07.31.61	Inocybe sp.*	Leucophenga varia	-
31-Jul-19	Wirth Property	07.31.62	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.63	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.64	Gymnopus dryophilus	Megaselia pulicaria complex	Dicyrtomidae sp.
31-Jul-19	Wirth Property	07.31.65	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.66	Gymnopus dryophilus	-	-
31-Jul-19	Wirth Property	07.31.67	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.70	Calocybe carnea/Rugosomyces persicolor	-	Oligolophus tridens
31-Jul-19	Wirth Property	07.31.71	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.72	Thelephora sp.*	-	-

31-Jul-19	Wirth Property	07.31.73	Calocybe carnea/Rugosomyces persicolor	-	-
31-Jul-19	Wirth Property	07.31.74	Inocybe sp.*	-	-
31-Jul-19	Wirth Property	07.31.76	Gymnopus cf. dryophilus	-	-
09-Aug-19	Dundas Valley	08.09.01	Gymnopus dryophilus	Exechia sp. Var. 1	Hypogastruridae sp.
				Allodia sp. Var. 1	-
				Exechia dorsalis	-
				Megaselia pulicaria complex	-
09-Aug-19	Dundas Valley	08.09.02	-	Megaselia sp. Var. 11 (3)	-
				Megaselia pulicaria complex (2)	-
09-Aug-19	Dundas Valley	08.09.03	Lactarius cf. zonarius	Cordyla sp. Var. 2 (2)	-
09-Aug-19	Dundas Valley	08.09.04	-	Metalimnobia triocellata	-
09-Aug-19	Dundas Valley	08.09.05	-	Tricimba melancholica	-
09-Aug-19	Dundas Valley	08.09.07	Russulaceae sp.*	Cecidomyiidae sp. Var. 2	-
09-Aug-19	Dundas Valley	08.09.08	Russulaceae sp.*	-	-
09-Aug-19	Dundas Valley	08.09.09	Pluteus sp.*	Apache degeerii	-
09-Aug-19	Dundas Valley	08.09.10	-	Megaselia sp. Var. 13	-
				Limoniidae sp.	-
09-Aug-19	Dundas Valley	08.09.11	Lactarius cf. zonarius	Metalimnobia triocellata	-
				Megaselia sp. Var. 13	-
				Coleoptera sp.	-
09-Aug-19	Dundas Valley	08.09.12	Suillus americanus	Megaselia sp. Var. 15 (2)	-
09-Aug-19	Dundas Valley	08.09.13	Gymnopus dryophilus	Megaselia pulicaria complex (3)	Hypogastruridae sp.
				Keroplatidae sp. Var. 2	-
				Exechia sp. Var. 1 (2)	-
				Exechia dorsalis	-
09-Aug-19	Dundas Valley	08.09.14	Gymnopus dryophilus	Megaselia pulicaria complex	Hypogastruridae sp.
26-Aug-19	Cootes Paradise	08.26.01	Omphalotus cf. illudens	Megaselia sp. Var. 10 (2)	-
				Mycetophagus serrulatus	-
				Cecidomyiidae sp. Var. 8	-
				Mydaea flavicornis (3)	-
				Hirtodrosophila duncani	-
				Scatopsciara neglecta	-
26-Aug-19	Cootes Paradise	08.26.02	Chalciporus cf. piperatus/piperatoides	Megaselia pulicaria complex	-
26-Aug-19	Cootes Paradise	08.26.03	Russula aff. rutila	Megaselia sp. Var. 17	-
				Cordyla sp. Var. 1	-
				Braconidae sp.	-
26-Aug-19	Cootes Paradise	08.26.04	Chalciporus cf. piperatus/piperatoides	Megaselia pulicaria complex	-
26-Aug-19	Cootes Paradise	08.26.05	-	Megaselia pulicaria complex	-

26-Aug-19	Cootes Paradise	08.26.08	Neofavolus cf. alveolaris	-	-
26-Aug-19	Cootes Paradise	08.26.09	Trichaptum biforme/subchartaceum	-	-
26-Aug-19	Cootes Paradise	08.26.11	Lycoperdon sp.◉	Megaselia sp. Var. 6	-
26-Aug-19	Cootes Paradise	08.26.12	Lycoperdon sp.◉	Megaselia sp. Var. 6	Parasitidae sp.
				-	Trachelipus sp.
				-	Hypogastruridae sp.
26-Aug-19	Cootes Paradise	08.26.13	Boletus subluridellus/subvelutipes	Megaselia pulicaria complex (3)	-
26-Aug-19	Cootes Paradise	08.26.14	Callistosporium luteo-olivaceum	-	-
26-Aug-19	Cootes Paradise	08.26.15	Pluteus sp.*	Dinotrema sp. Var. 3	-
26-Aug-19	Cootes Paradise	08.26.16	Hygrocybe sp.	-	Dicyrtomina sp.
26-Aug-19	Cootes Paradise	08.26.17	Boletus subluridellus/subvelutipes	-	-
26-Aug-19	Cootes Paradise	08.26.18	Mycena haematopus	-	-
26-Aug-19	Cootes Paradise	08.26.19	Amanita solaniolens	-	-
26-Aug-19	Cootes Paradise	08.26.20	Callistosporium luteo-olivaceum	-	-
26-Aug-19	Cootes Paradise	08.26.21	Amaropostia cf. stiptica	Scaphisoma convexum	-
				Gyrophaena socia	-
				Vitula broweri	-
				Tritoma mimetica	-
26-Aug-19	Cootes Paradise	08.26.22	Loweomyces fractipes	Keroplatidae sp. Var. 3	-
26-Aug-19	Cootes Paradise	08.26.23	Trametes versicolor	-	-
28-Aug-19	Cootes Paradise	08.28.01	Lepiota cf. boudieri	-	-
28-Aug-19	Cootes Paradise	08.28.03	Marasmius rotula	-	-
28-Aug-19	Cootes Paradise	08.28.04	Agaricales sp.◉	Megaselia sp. Var. 4 (2)	Sminthurinus bimaculatus
28-Aug-19	Cootes Paradise	08.28.05	Biannulariaceae sp.	-	-
28-Aug-19	Cootes Paradise	08.28.06	Cortinarius aff. pescolanensis/mellinus	Megaselia pulicaria complex (4)	-
28-Aug-19	Cootes Paradise	08.28.07	Scleroderma sp.	-	-
30-Aug-19	Dundas Valley	08.30.01	Cortinarius aff. pescolanensis/mellinus	Gyrophaena laetula	Hypogastruridae sp.
				Megaselia pulicaria complex (2)	Julus scandinavius
				Exechia dorsalis (3)	-
30-Aug-19	Dundas Valley	08.30.02	Entoloma sp.	-	Hypogastruridae sp.
30-Aug-19	Dundas Valley	08.30.03	Entoloma sp.	Megaselia pulicaria complex	-
30-Aug-19	Dundas Valley	08.30.04	Entoloma sp.	Exechia dorsalis (2)	-
				Megaselia pulicaria complex (2)	-
				Mycetophila fungorum	-
30-Aug-19	Dundas Valley	08.30.05	Entoloma sp.	Pallodes pallidus	-
				Gyrophaena laetula	-
30-Aug-19	Dundas Valley	08.30.06	Entoloma sp.	Exechia dorsalis	-
				Megaselia pulicaria complex	-

30-Aug-19	Dundas Valley	08.30.07	Entoloma sp.	-	-
30-Aug-19	Dundas Valley	08.30.08	Agaricales sp.◉	Exechia sp. Var. 1	-
30-Aug-19	Dundas Valley	08.30.09	Agaricales sp.◉	Exechia dorsalis	-
30-Aug-19	Dundas Valley	08.30.10	Entoloma sp.	Exechia sp. Var. 1 (2)	-
30-Aug-19	Dundas Valley	08.30.11	Agaricales sp.◉	Scardiella approximatella	-
				Exechia sp. Var. 1	-
30-Aug-19	Dundas Valley	08.30.13	Entoloma sp.	-	Hypogastruridae sp.
30-Aug-19	Dundas Valley	08.30.14	Entoloma sp.	Exechia dorsalis	-
30-Aug-19	Dundas Valley	08.30.15	Entoloma sp.	Exechia sp. Var. 1	-
30-Aug-19	Dundas Valley	08.30.16	Entoloma sp.	Megaselia pulicaria complex	-
30-Aug-19	Dundas Valley	08.30.17	Entoloma sp.	Exechia dorsalis (7)	-
				Megaselia pulicaria complex	-
30-Aug-19	Dundas Valley	08.30.18	Agaricales sp.◉	Exechia sp. Var. 2	-
30-Aug-19	Dundas Valley	08.30.19	Agaricales sp.◉	Exechia dorsalis	Oppiidae sp.
				Exechia sp. Var. 1	-
30-Aug-19	Dundas Valley	08.30.20	Entoloma sp.	-	-
30-Aug-19	Dundas Valley	08.30.21	-	-	Hypogastruridae sp.
30-Aug-19	Dundas Valley	08.30.22	-	Exechia dorsalis	-
30-Aug-19	Dundas Valley	08.30.23	Scleroderma areolatum	-	-
02-Sep-19	Sandor Property	09.02.01	Agaricales sp.◉	Metalectra discalis	Tomoceridae sp.
02-Sep-19	Sandor Property	09.02.02	Inocybe cf. curreyi	Mycetophila fungorum	-
02-Sep-19	Sandor Property	09.02.03	Lactarius aff. omphaliiformis	Exechia nigroscutellata (3)	-
				Leucophenga varia	-
02-Sep-19	Sandor Property	09.02.05	Lactarius aff. omphaliiformis	-	-
02-Sep-19	Sandor Property	09.02.06	-	Homosetia marginimaculella	-
02-Sep-19	Sandor Property	09.02.07	Laccaria striatula	Exechia dorsalis	-
02-Sep-19	Sandor Property	09.02.08	Inocybe sp.◉	Mycetophila fungorum	-
02-Sep-19	Sandor Property	09.02.09	Tapinella atrotomentosa	-	-
02-Sep-19	Sandor Property	09.02.10	Hygrocybe chlorophana	Megaselia sp. Var. 4 (3)	-
02-Sep-19	Sandor Property			Allodia sp. Var. 2	-
02-Sep-19	Sandor Property	09.02.11	Inocybe cf. curreyi	Megaselia sp. Var. 4 (2)	-
02-Sep-19	Sandor Property			Megaselia sp. Var. 9 (2)	-
02-Sep-19	Sandor Property	09.02.12	Inocybe cf. curreyi	Mycetophila fungorum (2)	-
02-Sep-19	Sandor Property	09.02.13	Inocybe cf. curreyi	-	-
02-Sep-19	Sandor Property	09.02.15	Pleurotus cornucopiae/pulmonarius	Triplax thoracica	-
02-Sep-19	Sandor Property	09.02.16	Gyroporus aff. castaneus	-	-
02-Sep-19	Sandor Property	09.02.18	Laccaria striatula	-	-
02-Sep-19	Sandor Property	09.02.19	Hygrocybe sp.	-	-

02-Sep-19	Sandor Property	09.02.20	Inocybe aff. subfulva	-	-
02-Sep-19	Sandor Property	09.02.21	Scleroderma citrinum	-	-
02-Sep-19	Sandor Property	09.02.23	Inocybaceae sp.*	Mycetophila fungorum	-
02-Sep-19	Sandor Property	09.02.24	Polyporus varius	Nanosella sp.	-
02-Sep-19	Sandor Property	09.02.25	Tapinella atrotomentosa	-	-
02-Sep-19	Sandor Property	09.02.26	Hygrocybe sp.	-	Dicyrtomidae sp.
02-Sep-19	Sandor Property	09.02.27	Trametes gibbosa	-	-
02-Sep-19	Sandor Property	09.02.28	Scleroderma citrinum	-	-
02-Sep-19	Sandor Property	09.02.29	Inocybe lanatodisca/Inosperma maculatum	-	-
02-Sep-19	Sandor Property	09.02.30	Inocybe cf. geophylla	-	-
02-Sep-19	Sandor Property	09.02.31	Inocybe aff. salicis	-	-
06-Sep-19	Cootes Paradise	09.06.01	Suillus americanus/himalayensis	Megaselia pulicaria complex	Trachelipus sp.
06-Sep-19	Cootes Paradise	09.06.02	Hydnellum sp.	Homosetia sp.	-
06-Sep-19	Cootes Paradise	09.06.03	Suillus americanus/himalayensis	-	-
06-Sep-19	Cootes Paradise	09.06.04	Entoloma sp.	-	-
06-Sep-19	Cootes Paradise	09.06.05	Lycoperdon perlatum	-	-
06-Sep-19	Cootes Paradise	09.06.06	Armillaria mellea	Cordyla sp. Var. 1	-
				Megaselia sp. Var. 17	-
				Megaselia pulicaria complex (2)	-
				Lycoperdina ferruginea	-
06-Sep-19	Cootes Paradise	09.06.07	Russula sp.*	-	-
06-Sep-19	Cootes Paradise	09.06.08	Stereum hirsutum	-	-
06-Sep-19	Cootes Paradise	09.06.09	Russula aff. delica	Mycetophila fungorum	Trachelipus sp.
06-Sep-19	Cootes Paradise	09.06.12	Amaropostia cf. stiptica	-	-
06-Sep-19	Cootes Paradise	09.06.13	Amanita muscaria	-	-
06-Sep-19	Cootes Paradise	09.06.14	Inocybe aff. curreyi	-	-
06-Sep-19	Cootes Paradise	09.06.15	Suillus sp.◉	Scaphisoma rubens	-
06-Sep-19	Cootes Paradise	09.06.16	Inocybe sp.	-	-
06-Sep-19	Cootes Paradise	09.06.17	Russula aff. delica	-	-
07-Sep-19	Norfolk County	09.07.01	Melanoleuca sp.*	-	-
09-Sep-19	Dundas Valley	09.09.02	Suillus americanus/himalayensis	-	-
09-Sep-19	Dundas Valley	09.09.03	Russula aff. olivacea	-	-
09-Sep-19	Dundas Valley	09.09.04	Loweomyces fractipes	-	-
09-Sep-19	Dundas Valley	09.09.08	Russula sp.*	-	-
09-Sep-19	Dundas Valley	09.09.09	Stereum sp.*	-	-
09-Sep-19	Dundas Valley	09.09.11	Russula aff. romellii/rubroalba	-	-
09-Sep-19	Dundas Valley	09.09.12	Daedaleopsis confragosa/Lenzites betulina	-	-
09-Sep-19	Dundas Valley	09.09.13	Mycena leaiana	-	-

09-Sep-19	Dundas Valley	09.09.14	Mycena cf. leaiana	-	-
09-Sep-19	Dundas Valley	09.09.15	Lentinellus cf. ursinus	-	-
09-Sep-19	Dundas Valley	09.09.16	Russula aff. delica	Lordithon facilis	-
09-Sep-19	Dundas Valley	09.09.17	Mycena sp.	-	-
09-Sep-19	Dundas Valley	09.09.18	Mycena leaiana	-	-
09-Sep-19	Dundas Valley	09.09.19	Mycena leaiana	-	-
11-Sep-19	McMaster, LSB	09.11.01	Agaricus bitorquis	-	Entomobrya unostrigata
18-Sep-19	Cootes Paradise	09.18.01	Lycoperdon perlatum	-	-
18-Sep-19	Cootes Paradise	09.18.02	Inocybe aff. rimosa/melliolens	-	-
18-Sep-19	Cootes Paradise	09.18.03	Callistosporium luteo-olivaceum	-	Entomobryomorpha sp.
18-Sep-19	Cootes Paradise	09.18.04	Pluteus hongoi	-	-
18-Sep-19	Cootes Paradise	09.18.05	Pluteus hongoi	-	-
20-Sep-19	McMaster, DBAC	09.20.01	Agaricus sp.*	Megaselia sp. n.2 SH-2015	-
20-Sep-19	McMaster, DBAC	09.20.02	Bovista cf. plumbea	-	-
20-Sep-19	McMaster, DBAC	09.20.04	Phaeolus cf. schweinitzii	Metalimnobia novaeangliae	-
20-Sep-19	Cootes Paradise	09.20.05	Echinoderma cf. asperum	Megaselia pulicaria complex (2)	-
				Dinotrema sp. Var. 4	-
				Tachinus fimbriatus	-
24-Sep-19	McMaster, DBAC	09.24.01	Agaricus sp.◉	Megaselia sp. n.2 SH-2015 (4)	-
24-Sep-19	McMaster, DBAC	09.24.02	Agaricus sp.*	Megaselia sp. n.2 SH-2015	-
				Fannia canicularis	-
25-Sep-19	Cootes Paradise	09.25.01	Russula sp.◉	Cecidomyiidae sp. Var. 6	-
25-Sep-19	Cootes Paradise	09.25.02	Cortinarius aff. pescolanensis/mellinus	-	-
25-Sep-19	Cootes Paradise	09.25.04	Suillus americanus/himalayensis	Exechia separata (2)	-
25-Sep-19	Cootes Paradise	09.25.05	Crepidotus sp.◉	Platypezidae sp. Var. 2	Trichoniscus pusillus
25-Sep-19	Cootes Paradise	09.25.06	-	Mycetophila fungorum	-
				Blastobasis glandulella	-
25-Sep-19	Cootes Paradise	09.25.07	Russula cf. cremeirosea	-	-
25-Sep-19	Cootes Paradise	09.25.09	Russula aff. pelargonia/clariana	-	-
25-Sep-19	Cootes Paradise	09.25.10	Suillus americanus/himalayensis	Exechia separata	-
25-Sep-19	Cootes Paradise	09.25.11	Suillus americanus/himalayensis	Exechia separata (3)	-
25-Sep-19	Cootes Paradise	09.25.12	Russula aff. pelargonia/clariana	-	-
26-Sep-19	McMaster, DBAC	09.26.01	Hymenopellis limonispora	-	-
27-Sep-19	McMaster, DBAC	09.27.02	Hymenopellis limonispora	-	-
30-Sep-19	Cootes Paradise	09.30.01	Amanita suballiacea/bisporigera	-	Hypogastruridae sp.
01-Oct-19	McMaster, DBAC	10.01.01	Hypsizygus aff. ulmarius	Exechia sp. Var. 5	-
				Cecidomyiidae sp. Var. 5	-
				Phymatura blanchardi	-

01-Oct-19	McMaster, DBAC	10.01.03	Hymenopellis cf. limonispora	-	-
01-Oct-19	McMaster, DBAC	10.01.04	Hymenopellis cf. limonispora/megalospora	Mycetophila fungorum (2)	-
01-Oct-19	McMaster, DBAC	10.01.05	Psathyrella cf. candolleana/hymenocephala	Megaselia marquezi (2)	-
01-Oct-19	McMaster, DBAC	10.01.06	Lyophyllum aff. decastes	-	-
05-Oct-19	Wirth Property	10.05.01	Gymnopus cf. dryophilus	Megaselia pulicaria complex	Parasitidae sp.
05-Oct-19	Wirth Property	10.05.03	Calocybe carnea/Rugosomyces persicolor	-	Orchesella cincta
05-Oct-19	Wirth Property	10.05.04	Calocybe carnea/Rugosomyces persicolor	-	-
05-Oct-19	Wirth Property	10.05.05	Clitocybe cf. squamulosa/gibbosa	-	-
05-Oct-19	Wirth Property	10.05.06	Mycena sp.*	-	-
05-Oct-19	Wirth Property	10.05.07	Calocybe carnea/Rugosomyces persicolor	-	-
05-Oct-19	Wirth Property	10.05.08	Tricholoma sp.◉	Pegomya sp. (2)	-
05-Oct-19	Wirth Property	10.05.10	Inocybe aff. catalaunica	-	-
05-Oct-19	Wirth Property	10.05.11	Gymnopus dryophilus	-	-
05-Oct-19	Wirth Property	10.05.12	-	-	-
05-Oct-19	Wirth Property	10.05.13	Collybia sp.◉	Braconidae sp. Megaselia pulicaria complex	- -
05-Oct-19	Wirth Property	10.05.14	Collybia sp.◉	Megaselia pulicaria complex (2) Gyrophaena affinis	- -
05-Oct-19	Wirth Property	10.05.15	Calocybe carnea/Rugosomyces persicolor	-	-
05-Oct-19	Wirth Property	10.05.16	Strobilurus esculentus	-	-
05-Oct-19	Wirth Property	10.05.17	Agaricales sp.◉	Allodia sp. Var. 2 -	Dicyrtomidae sp. Parasitidae sp.
05-Oct-19	Wirth Property	10.05.18	Strobilurus sp.*	-	-
05-Oct-19	Wirth Property	10.05.20	Clitocybe aff. phyllophila	-	-
05-Oct-19	Wirth Property	10.05.22	Tricholoma argyraceum	-	-
05-Oct-19	Wirth Property	10.05.23	Rhodocollybia butyracea	Mycetophila fungorum	-
05-Oct-19	Wirth Property	10.05.26	Lepista sp.◉	Gyrophaena antennalis/insolens	-
05-Oct-19	Wirth Property	10.05.27	Inocybe ochroalba/phaeoleuca	-	-
05-Oct-19	Wirth Property	10.05.28	Gymnopus cf. dryophilus	Megaselia pulicaria complex Gyrophaena affinis	Choneiulus palmatus -
05-Oct-19	Wirth Property	10.05.29	Inocybe catalaunica	Exechia sp. Var. 1	-
05-Oct-19	Wirth Property	10.05.30	Baeospora cf. myosura	-	-
05-Oct-19	Wirth Property	10.05.32	Inocybe flocculosa/stuntzii	-	-
05-Oct-19	Wirth Property	10.05.33	Inocybe cf. catalaunica	Allodia sp. Var. 2	-
05-Oct-19	Wirth Property	10.05.34	Baeospora cf. myosura	-	-
05-Oct-19	Wirth Property	10.05.35	Calocybe carnea/Rugosomyces persicolor	-	-
05-Oct-19	Wirth Property	10.05.36	Lepista sp.*	-	Tylenchida sp.
05-Oct-19	Wirth Property	10.05.37	Clitocybe cf. phyllophila	-	-

05-Oct-19	Wirth Property	10.05.38	Strobilurus sp.*	-	-
05-Oct-19	Wirth Property	10.05.39	Gymnopus dryophilus	-	-
05-Oct-19	Wirth Property	10.05.40	Inocybe flocculosa/stuntzii	-	-
05-Oct-19	Wirth Property	10.05.41	Mycena aff. rubromarginata	-	-
05-Oct-19	Wirth Property	10.05.42	Collybia sp.◉	Gyrophaena affinis	-
05-Oct-19	Wirth Property	10.05.43	Lepiota subincarnata	-	-
05-Oct-19	Wirth Property	10.05.45	Mycena aff. rubromarginata	-	-
05-Oct-19	Wirth Property	10.05.46	Mycena sp.*	-	-
05-Oct-19	Wirth Property	10.05.47	Inocybe sp.*	-	-
05-Oct-19	Wirth Property	10.05.48	Inocybe sp.*	-	-
05-Oct-19	Wirth Property	10.05.49	Clitocybe cf. phyllophila	-	-
05-Oct-19	Wirth Property	10.05.51	Inocybe flocculosa/stuntzii	-	-
05-Oct-19	Wirth Property	10.05.52	Calocybe carnea/Rugosomyces persicolor	-	-
05-Oct-19	Wirth Property	10.05.53	Inocybe sp.*	-	-
05-Oct-19	Wirth Property	10.05.54	Inocybe sp.*	-	-
05-Oct-19	Wirth Property	10.05.58	Lepiota subincarnata	-	-
05-Oct-19	Wirth Property	10.05.59	-	-	Tylenchida sp.
05-Oct-19	Wirth Property	10.05.60	Inocybe cf. flocculosa	-	-
05-Oct-19	Wirth Property	10.05.61	Mycena pura	-	-
05-Oct-19	Lowrie Property	10.05.62	-	Exechia nigroscutellata	-
05-Oct-19	Lowrie Property	10.05.63	Lactifluus sp.	-	-
05-Oct-19	Lowrie Property	10.05.64	Russula cyanoxantha	Mycetophila fungorum (2)	Hypogastruridae sp.
05-Oct-19	Lowrie Property	10.05.65	Russula aff. rosea	Mycetophila fungorum (2)	Hypogastruridae sp.
05-Oct-19	Lowrie Property			Megaselia pulicaria complex	-
05-Oct-19	Lowrie Property	10.05.66	Inocybe hirtella	Gyrophaena michigana	-
05-Oct-19	Lowrie Property	10.05.67	Trametes pubescens/versicolor	Megaselia sp. Var. 18	-
05-Oct-19	Lowrie Property	10.05.68	Russula sp.◉	Mycetophila fungorum (2)	Hypogastruridae sp.
				Orchesia castanea	-
				Mydaea flavicornis	-
05-Oct-19	Lowrie Property	10.05.69	Tricholoma sejunctum	Mycetophila fungorum (2)	-
				Megaselia pulicaria complex	-
				Mydaea occidentalis	-
				Gyrophaena gilvicollis	-
05-Oct-19	Lowrie Property	10.05.70	Tricholoma sp.◉	Mycetophila fungorum	-
05-Oct-19	Lowrie Property	10.05.71	Entoloma sp.◉	Mycetophila fungorum	-
				Exechia sp. Var. 1	-
05-Oct-19	Lowrie Property	10.05.72	Cuphophyllus pratensis	-	Hypogastruridae sp. (2)
05-Oct-19	Lowrie Property	10.05.73	Russula sp.◉	-	Hypogastruridae sp.

05-Oct-19	Lowrie Property	10.05.74	Russula sp.◉	Cordyla sp. Var. 1	-
				Mycetophila fungorum	-
05-Oct-19	Lowrie Property	10.05.75	-	Exechia sp. Var. 1 (2)	Hypogastruridae sp.
05-Oct-19	Lowrie Property	10.05.76	Russula sp.◉	Megaselia pulicaria complex (3)	-
05-Oct-19	Lowrie Property	10.05.77	Tricholoma sejunctum	Mycetophila fungorum	-
				Gyrophaena gilvicollis (2)	-
				Metalimnobia triocellata	-
				Mydaea occidentalis	-
05-Oct-19	Lowrie Property	10.05.78	Leccinum sp.	-	Hypogastruridae sp.
05-Oct-19	Lowrie Property	10.05.79	-	Exechia subfrigida (2)	-
				Allodia sp. Var.1	-
				Exechia nigroscutellata	-
				Atheta modesta	-
05-Oct-19	Lowrie Property	10.05.80	-	Exechia subfrigida	-
05-Oct-19	Lowrie Property	10.05.81	Tricholoma cf. columbetta/subresplendens	Mycetophila fungorum	-
				Tachinus fimbriatus	-
05-Oct-19	Lowrie Property	10.05.82	Russula sp.◉	Mycetophila fungorum	-
05-Oct-19	Lowrie Property	10.05.83	Russula sp.◉	Exechia nigroscutellata	-
				Mycetophila fungorum	-
05-Oct-19	Lowrie Property	10.05.84	-	Exechia nigroscutellata (3)	-
05-Oct-19	Lowrie Property	10.05.85	Russula sp.◉	Exechia nigroscutellata	-
05-Oct-19	Lowrie Property	10.05.86	Galerina marginata	-	-
05-Oct-19	Lowrie Property	10.05.87	-	Megaselia pulicaria complex	-
05-Oct-19	Lowrie Property	10.05.88	Russula sp.◉	Mycetophila fungorum (3)	-
				Megaselia pulicaria complex	-
05-Oct-19	Lowrie Property	10.05.90	Russula sp.◉	Exechia nigroscutellata	-
05-Oct-19	Lowrie Property	10.05.93	Russula sp.◉	Exechia subfrigida	-
05-Oct-19	Lowrie Property	10.05.96	-	Exechia subfrigida	-
05-Oct-19	Lowrie Property	10.05.98	Scleroderma citrinum	-	-
05-Oct-19	Lowrie Property	10.05.99	Cuphophyllus cf. pratensis	-	Hypogastruridae sp.
05-Oct-19	Lowrie Property	10.05.100	-	Coleoptera sp. (2)	-
05-Oct-19	Lowrie Property	10.05.101	Tricholoma cf. columbetta/subresplendens	Mycetophila fungorum	-
06-Oct-19	Sandor Property	10.06.01	Leucoagaricus cf. leucothites/subcretaceus	-	-
06-Oct-19	Sandor Property	10.06.02	Lactarius sp.◉	Exechia nigroscutellata	-
06-Oct-19	Sandor Property	10.06.04	Melanophyllum sp.	-	-
08-Oct-19	McMaster, DBAC	10.08.04	Lyophyllum decastes	Philonthus carbonarius	-
09-Oct-19	Cootes Paradise	10.09.01	Psathyrella candolleana	-	-
09-Oct-19	Cootes Paradise	10.09.02	Trichaptum cf. biforme/abietinum	-	-

09-Oct-19	Cootes Paradise	10.09.03	Echinoderma asperum	Exechia subfrigida	Trachelipus sp.
09-Oct-19	Cootes Paradise	10.09.04	Conocybe sp.◉	Sciaridae sp. Var. 2	-
09-Oct-19	Cootes Paradise	10.09.06	Psathyrella candolleana	-	-
09-Oct-19	Cootes Paradise	10.09.07	Cortinarius aff. subferrugineus	Mycetophila fungorum	Hypogastruridae sp.
09-Oct-19	Cootes Paradise	10.09.08	Cortinarius sp.	Tritoma mimetica	-
09-Oct-19	Cootes Paradise	10.09.09	Mycena cf. leaiana	Exechia sp. Var. 5	-
09-Oct-19	Cootes Paradise	10.09.11	Amaropostia cf. stiptica	-	-
09-Oct-19	Cootes Paradise	10.09.14	-	Bolitotherus cornutus	Oxidus gracilis
09-Oct-19	Cootes Paradise	10.09.16	Trichaptum biforme/abietinum	-	-
09-Oct-19	Cootes Paradise	10.09.17	Psathyrella sp.*	-	-
09-Oct-19	Cootes Paradise	10.09.18	Psathyrella sp.*	-	-
11-Oct-19	Sandor Property	10.11.01	Entoloma psammophilohebes	Exechia sp. Var. 6 (3)	-
11-Oct-19	Sandor Property	10.11.02	Russula aff. risigallina	-	-
11-Oct-19	Sandor Property	10.11.04	Lactarius sp.◉	Exechia nigroscutellata	-
11-Oct-19	Sandor Property	10.11.05	Scleroderma citrinum	-	-
11-Oct-19	Lowrie Property	10.11.06	Armillaria sp.*	Exechia subfrigida	-
11-Oct-19	Lowrie Property	10.11.07	Hygrocybe sp.◉	Mycetophila fungorum	-
11-Oct-19	Lowrie Property	10.11.09	-	Exechia subfrigida	Dicyrtomidae sp.
				-	Entomobryomorpha sp.
11-Oct-19	Lowrie Property	10.11.10	Lactarius sp.*	Exechia nigroscutellata (3)	-
11-Oct-19	Lowrie Property	10.11.11	Hebeloma sp.*	-	-
11-Oct-19	Lowrie Property	10.11.12	Mycena filopes	-	-
11-Oct-19	Lowrie Property	10.11.13	Lactarius cf. cinereus	Exechia nigroscutellata	-
11-Oct-19	Lowrie Property	10.11.16	Hygrophoraceae sp.*	Mycetophila fungorum (2)	Dicyrtomidae sp.
				Mycetophila fungorum	-
11-Oct-19	Lowrie Property	10.11.17	Lactarius cf. cinereus	-	-
11-Oct-19	Lowrie Property	10.11.18	Helvella sp.◉	Allodia sp. Var. 3	-
11-Oct-19	Lowrie Property	10.11.19	Pluteus cf. hongoi	Megaselia variana (2)	-
11-Oct-19	Lowrie Property	10.11.21	Mycena sp.	Exechia sp. Var. 4	-
11-Oct-19	Lowrie Property	10.11.22	-	Exechia sp. Var. 6	-
11-Oct-19	Lowrie Property	10.11.23	-	Exechia sp. Var. 1	Dicyrtomidae sp.
11-Oct-19	Lowrie Property	10.11.24	Trametes versicolor/ochracea	-	-
11-Oct-19	Lowrie Property	10.11.25	-	-	-
11-Oct-19	Lowrie Property	10.11.26	Psathyrella piluliformis/sarcocephala	Exechia subfrigida	-
11-Oct-19	Lowrie Property	10.11.27	Entoloma cuspidiferum	-	-
11-Oct-19	Lowrie Property	10.11.28	Mycena leaiana	-	-
11-Oct-19	Lowrie Property	10.11.29	Entoloma aff. sericellum	-	-
11-Oct-19	Lowrie Property	10.11.30	Armillaria sp.*	Exechia subfrigida	-

11-Oct-19	Lowrie Property	10.11.31	Entoloma abortivum	Exechia sp. Var. 3	-
11-Oct-19	Lowrie Property	10.11.33	Lactarius cf. cinereus	Exechia nigroscutellata (2)	-
11-Oct-19	Lowrie Property	10.11.34	Entoloma psammophilohebes	-	-
11-Oct-19	Lowrie Property	10.11.35	Hygrocybe sp.	-	Dicyrtomidae sp.
				-	Hyloniscus riparius
11-Oct-19	Lowrie Property	10.11.36	Russula sp.	-	-
11-Oct-19	Lowrie Property	10.11.37	-	-	Entomobryomorpha sp.
11-Oct-19	Lowrie Property	10.11.40	Cuphophyllus sp.	Exechia subfrigida	-
11-Oct-19	Lowrie Property	10.11.41	Psathyrella corrugis/amarescens	Exechia subfrigida	-
11-Oct-19	Lowrie Property	10.11.42	Lactarius aff. cinereus	-	-
11-Oct-19	Lowrie Property	10.11.43	Neofavolus aff. alveolaris	-	-
11-Oct-19	Lowrie Property	10.11.46	-	Exechia nigroscutellata	-
11-Oct-19	Lowrie Property	10.11.47	Hygrophoraceae sp.*	Exechia sp. Var. 1	-
11-Oct-19	Lowrie Property	10.11.48	Marasmius sp.◉	Exechia subfrigida	-
11-Oct-19	Lowrie Property	10.11.49	Entoloma abortivum	Exechia sp. Var. 3	-
11-Oct-19	Lowrie Property	10.11.50	Pluteus sp.*	-	-
11-Oct-19	Lowrie Property	10.11.51	Agaricales sp.◉	Pseudexechia sp.	-
11-Oct-19	Lowrie Property	10.11.53	Agaricales sp.◉	Exechia subfrigida	-
11-Oct-19	Lowrie Property	10.11.54	Cuphophyllus sp.	Exechia subfrigida	-
11-Oct-19	Lowrie Property	10.11.55	Hygrocybe sp.◉	Exechia subfrigida	-
11-Oct-19	Lowrie Property	10.11.57	Inocybe sp.	Allodia sp. Var. 1	-
20-Oct-19	Sandor Property	10.20.01	Boletaceae sp.◉	Mycetophila fungorum (2)	-
20-Oct-19	Sandor Property	10.20.02	Hymenopellis limonispora/megalospora	-	-
21-Oct-19	McMaster, DBAC	10.21.01	Leucoagaricus leucothites/subcretaceus	-	-
21-Oct-19	McMaster, DBAC	10.21.02	Hymenopellis limonispora/megalospora	-	-
21-Oct-19	McMaster, DBAC	10.21.03	Agaricus sp.*	-	-
21-Oct-19	Cootes Paradise	10.21.05	Russulaceae sp.*	Exechia nigroscutellata	-
				Cordyla sp. Var. 2	-
21-Oct-19	Cootes Paradise	10.21.06	Tricholoma cf. aurantium	-	-
21-Oct-19	Cootes Paradise	10.21.07	Galerina marginata	-	Hypogastruridae sp.
21-Oct-19	Cootes Paradise	10.21.08	Hebeloma sp.*	-	Hypogastruridae sp.
21-Oct-19	Cootes Paradise	10.21.09	Trichaptum biforme/abietinum	Cecidomyiidae sp. Var. 4	-
21-Oct-19	McMaster, Other	10.21.10	Suillus granulatus/weaverae	Exechia separata	-
21-Oct-19	McMaster, Other	10.21.11	Parasola lactea/leiocephala	-	-
29-Oct-19	Dundas Valley	10.29.01	Hymenopellis limonispora/megalospora	-	Scheloribates clavilanceolatus
29-Oct-19	Dundas Valley	10.29.03	Suillus collinitus/granulatus	Exechia separata	-
29-Oct-19	Dundas Valley	10.29.04	Suillus collinitus/granulatus	-	-
29-Oct-19	Dundas Valley	10.29.05	Suillus collinitus/granulatus	-	-

29-Oct-19	Dundas Valley	10.29.07	Agaricales sp.◉	-	Hypogastruridae sp.
29-Oct-19	Dundas Valley	10.29.08	Hypsizygus aff. ulmarius	-	-
29-Oct-19	Dundas Valley	10.29.09	Inocybe semifulva	-	-
29-Oct-19	Dundas Valley	10.29.10	Daedaleopsis confragosa/tricolor/Lenzites betulina	-	-
29-Oct-19	Dundas Valley	10.29.11	Agaricales sp.	-	-
06-Nov-19	Wirth Property	11.06.01	Tricholoma sp.◉	-	Isotomidae sp.
06-Nov-19	Wirth Property	11.06.02	Clitocybe vibecina	-	-
06-Nov-19	Wirth Property	11.06.03	Hebeloma mesophaeum	-	-
26-Nov-19	McMaster, DBAC	11.26.05	-	Bolitophila glabrata	-
30-Nov-19	Dundas Valley	11.30.04	Polyporaceae sp.◉	Thymalus marginicollis	-
				Mycetophila mohilevensis (2)	-
30-Nov-19	Dundas Valley	11.30.07	Polyporaceae sp.◉	Sciophila plurisetosa	-
				Thymalus marginicollis	-
				Mycetophila mohilevensis	-
30-Nov-19	Dundas Valley	11.30.08	Polyporaceae sp.◉	Thymalus marginicollis	-
30-Nov-19	Dundas Valley	11.30.10	Polyporaceae sp.◉	Cecidomyiidae sp. Var. 7 (2)	-
30-Nov-19	Dundas Valley	11.30.11	Polyporaceae sp.◉	Cecidomyiidae sp. Var. 7	-
30-Nov-19	Dundas Valley	11.30.12	Polyporaceae sp.◉	Cecidomyiidae sp. Var. 4	-
30-Nov-19	Dundas Valley	11.30.13	Polyporaceae sp.◉	Cis levettei	-