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

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ABSTRACT

A rapid biodiversity assessment of insects and associated Laboulbeniales fungi was conducted over the course of five nights in August, 2018 at two central Florida lakes: Lake Eustis and the nearby protected and restored National Natural Landmark, Emeralda Marsh Conservation Area (EMCA), which encompasses a portion of Lake Griffin. These locations were selected because Lake Eustis was surveyed for Laboulbeniales in 1897 by mycologist Dr. Roland Thaxter, but has not since been investigated. Because Lake Eustis has been urbanized, with the lake perimeter almost entirely altered by human development, the site offers a look into Laboulbeniales diversity across a 121 year timeline, before and after human development. By surveying Lake Eustis and EMCA, a modern case study comparison of Laboulbeniales and insect diversity between a developed and unrestored system and a protected and restored system is made. A total of 4,022 insects were collected during the rapid assessment. Overall, insect abundance was greater at EMCA, with 3,001 insects collected, compared to 1,021 insects collected from Eustis. Though family level insect richness was comparable between sites, with 55 families present at EMCA and 56 at Eustis, 529 out of 3,001 (17.6%) of the insects collected at EMCA were hosts

to parasitic Laboulbeniales fungi whereas only 2 out of 1,021 (0.19%) collected from Eustis were infected. There were 16 species of Laboulbeniales found at EMCA compared to only one at Eustis. The current number of Laboulbeniales species documented at Eustis was incredibly depauperate compared to the 27 species recorded by Thaxter in 1897, suggesting the possibility of utilizing Laboulbeniales as indicators of ecosystem health. A figure displaying host-parasite records and a species list of Laboulbeniales fungi is compiled and updated occurrence records for species of *Ceratomyces* and *Hydrophilomyces* are provided.

INTRODUCTION

Insect Diversity

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

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

Regarding estimates of living species, both described and undescribed, estimates of insect richness are even more variable. The lowest estimate is about 2 million species (Grimaldi and Engel, 2004) and the largest is a staggering 30 million tropical insect species (Erwin 1982). Erwin's estimate was based on using fogging techniques on tree canopies in neotropical forests, upon which extrapolations were made for total insect diversity. Erwin recorded trees as having

unique species of insects in their canopies and used the total tropical tree diversity of approximately 50,000 species to extrapolate. Most researchers now agree this estimate is much too high, largely because the assumption that the insects found in tree canopies would be highly host specific is likely erroneous (Grimaldi and Engel, 2004). Grimaldi and Engel endorse Gaston's (1991) estimate of about 5 million total living insect species. This estimate was based on surveying collections held by systematists around the world. Despite this method having some potential shortcomings, e.g., collection biases of individual collectors, presence of unexamined or unknown duplicates held across collections, the authors believe it is currently the most accurate estimate of global insect diversity. If this estimation is accepted, then the aforementioned figure of 925,000 named insects would represent 20% of extant insect diversity.

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

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

Fungal Diversity

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

bodies, lichen-dwelling fungi, cryptic species, and through environmental (eDNA) sequencing (Hawksworth & Lücking, 2017).

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

Laboulbeniales

Laboulbeniales (Ascomycota, Fungi) are microscopic obligate parasites on arthropods, primarily infecting insects. Laboulbeniales are considered the most diverse lineage of insect-associated fungi with ~ 2,200 described species in 142 genera, but current estimates indicate there are at least 40,000 species awaiting description (Weir and Hammond, 1997). The impact of

Laboulbeniales fungi on their insect hosts are not fully understood and basic studies of their biology are still limited. Only a handful of scientists in the world specialize in the study of Laboulbeniales, and yet, because of the size, diversity, and uniqueness of this lineage, it is undoubtedly a cradle of novel taxonomic and ecological information.

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

Foundation of Study

One of Thaxter's collection sites for Laboulbeniales was in Eustis, a small central Florida city on the east shore of Lake Eustis. Beginning in the early 1800s, colonial Europeans forcefully established Eustis on Seminole land (Preserving Eustis History, n.d.). The city was named after General Abraham Eustis, who was known for his role in wars against the Seminole people (Preserving Eustis History, n.d.). The numerous connected waterways in the region allowed for Eustis to become a hub for steamboats, and the construction of the railroad that connected many Floridian towns in 1880 led to an increase in settlement from < 500 in 1900 to 21,3000 in present day Eustis.

According to his travel records, Thaxter was in Eustis during the very early days of the city, from September 25th to October 10th, 1897 (Pfister, 1982), before most of the present urbanization. Because the methods employed by Thaxter during this trip are unpublished and

unrecorded, it is unclear precisely how much time was spent collecting or the precise locations from which he collected (Pfister, personal communication). Throughout Thaxter's work, 27 species and three varieties of Laboulbeniales were recorded and/or described from Eustis, Florida: *Autoicomyces acuminatus, Ceratomyces ansatus, C. camptosporus, C. cladophorus, C. confusus, C. filiformis, C. floridanus, C. longicornis, C. minisculus, C. mirabilus, Chitonomyces affinis, Ch. dentiferus, Ch. distortus, Ch. floridanus, Ch. hydropori, Ch. lichanophorus, Ch. occultus, Ch. psittacopsis, Ch. uncigerus, Hydraeomyces cnemidoti, H. halipli, Hydrophilomyces reflexus, Hy. rhynchophorus, Laboulbenia texana var. retusa, L. texana var. rostellata, L. texana var. tibialis, Rhynchophoromyces elephantinus, Teratomyces mirificus,* and *Zodiomyces vorticellarius.*

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

The overall goal of this study was to return to Eustis in an attempt to re-collect species recorded by Thaxter, which could provide insight into shifts in biodiversity of Laboulbeniales and their associated insects since 1897 (121 years ago). Because Eustis is now impacted by urbanization, Emeralda Marsh Conservation Area (EMCA), was also sampled as a control. EMCA includes a portion of Lake Griffin and surrounding habitat and is located ~ 14 km from the east shore of Lake Eustis. Due to its designation as a National Natural Landmark since 1974 and subsequent and ongoing restoration efforts, EMCA provides a closer approximation of the habitat in which Thaxter collected 121 years ago (Fig. 3). Two biodiversity assessments were made: one over time (between Eustis in 1897 and Eustis in 2018), and one between habitats

(between Eustis and EMCA in 2018). The working hypothesis for this study is that EMCA, the restored site, would contain greater insect and fungal diversity than Eustis, the unprotected and unrestored site. I further hypothesized EMCA would be more likely to harbor species recorded by Thaxter, as compared to Eustis. These data provide a baseline for understanding how urban development around lake systems may affect biodiversity of insects and their accompanying Laboulbeniales parasites, and to begin to explore if and how Laboulbeniales may serve as a proxy for biodiversity and an indicator for ecosystem health.

METHODS

Site Description

Lake Eustis and EMCA (including Lake Griffin) are part of the Central Valley Region (Region 7508) of Florida (Fig. 1). Lakes in this subtropical region are categorized by being large, shallow, and eutrophic (Lake County Water Atlas, Emeralda Marsh, n.d.). Lake Eustis and Lake Griffin are part of the Ocklawaha Chain of Lakes, which includes a total of 10 connected lakes. The headwaters of this chain is Lake Apopka, which is fed by a natural spring and by rain. Lake Griffin is the most downstream of the 10 lakes and Lake Eustis is directly upstream from Lake Griffin. Lake Griffin empties northward into the Ocklawaha River, which ultimately connects to the St. Johns River (St. Johns River Water Management District, Lake Apopka Basin, n.d.).
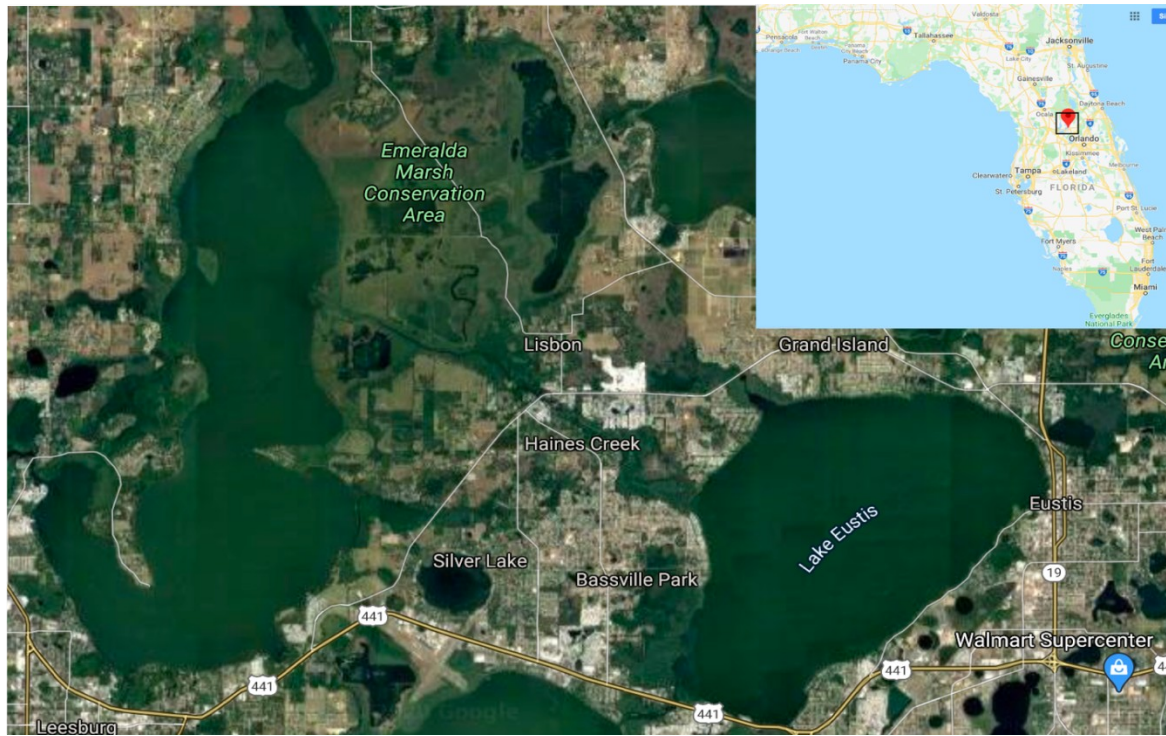
Fig. 1. Map of Lake Eustis and Lake Griffin and surrounding area of of Lake County, FL.

The surface area of Lake Griffin is ~ 38 km^2; Lake Eustis is ~ 31 km^2. The average depth of Lake Griffin is ~ 2 m and Lake Eustis is ~ 3 m. The bottom of the Lake Griffin is composed of soft organic matter measuring an average of 1.7 m thick (Fulton et al, 2015). Equivalent measurements for Lake Eustis were not available. Over the past ~ 150 years, the Ocklawaha Chain of Lakes have experienced a barrage of human manipulations including, draining, dredging, levying, agricultural conversion, waste dumping, and nutrient loading. In the 1950s, the eastern area of Lake Griffin was levied and drained and converted into agricultural land and muck farms. These farms became an external source of nutrient loading into Lake Griffin (Fulton, 2015).

As of 2004, pervious and impervious percentages of the Lake Griffin Basin was 65% and 35% respectively, whereas the basin containing Lake Eustis (Burrell Basin) was 50% pervious/impervious (Fulton et al, 2004). Surface area coverage by emergent and floating-leaved vegetation decreased from ~ 50% in the 1940's to < 2% in the 1970's (Fulton et al, 2015). Similar data and exact mapping of wetland and vegetation loss is not available for Lake Eustis,

however mention is made in an issue of Engineering News and American Railway Journal (1884) of Apopka Drainage Company draining ~ 0.4 km^2 between Lake Eustis and Lake Dora for farming. Additionally, > 405 km^2 of wetlands and forested uplands were destroyed by human development in Lake County as a whole (Lake County's Comprehensive Plan EAR – Conservation Element, 1997).

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

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

due to the upstream restoration efforts at Lake Apopka, whereas Lake Griffin is likely benefit

from the extensive restoration efforts at EMCA in addition to the upstream efforts (Fulton,

2015).

Insect Collection

A rapid biodiversity assessment was conducted over five days, August 14th–18th, 2018.

Fig. 2. Lake Eustis collection site with altered littoral zone with impervious wall. Photosource: pinterest.com

Fig. 3. EMCA collection site with a natural littoral zone. Photo by author.

These dates

were chosen to be in the same subtropical season as Thaxter's visit. Again, while Thaxter's

precise methodology is not known, many of the species recorded from Eustis, e.g., members of

Autoicomyces, Ceratomyces, Hydrophilomyces, Rhynchophoromyces, and *Zodiomyces* are found

on nocturnal aquatic beetles indicating he likely used a light-based trap as part of his collection

methods. Insects were therefore collected using an ultraviolet trapping method. This popular

entomological collection method was chosen in order to collect a broad range of taxa

(Szentkirályi, 2002; van Wielink and Spijkers, 2013). Given that many insects are nocturnal and

given the risk of alligator encounter in and near the perimeter of the lakes, this method was

deemed to be both effective and safe. A black light, (2805 DC Light Night Collecting Light, DC,

12 Volt, 15 Watt BL) was set against a white sheet, which was placed approximately 5 m from

the water's edge. Insects were collected via aspirator and transferred to 70% ethanol for storage.

Three collectors spent three hours at each of the two sites per night, totaling 90 effort hours of

collecting. Equal collection effort was made at both sites and the starting location alternated each

night between Eustis or EMCA.

Fungal Collection

All collected insects were scanned for infections of Laboulbeniales under a Nikon

stereomicroscope at 20–40×. Presence/absence data were recorded for each insect. All insect

specimens were identified to family level using Marshall (2006) and are housed at at SUNY

College of Environmental Science & Forestry (SUNY-ESF) in Syracuse, New York.Fungal thalli

were removed with a micropin and mounted in glycerin using previously described methods

(Benjamin, 1971, 1986, 1993). All fungi were identified to species using Thaxter (1896, 1908,

1924, 1926, 1931) and Majewski (1994). Voucher specimens are housed at SUNY-ESF

Data and Data Analyses

Abundance, species richness, and species diversity (Simpson's and Shannon-Weiner, H')

were calculated for insects and Laboulbeniales. Comparisons are presented from the two time

periods for collections in Eustis (1897 and 2018) as well as between Eustis and EMCA. As this

was a case study, actual statistical comparisons could not made as it would have been

psuedoreplication.

RESULTS

A total of 4,022 insects were collected during the rapid assessment (Fig. 6). Overall insect

abundance was greater at EMCA, with 3,001 insects collected, compared to 1,021 insects

collected from Eustis (Table 1). Insect richness at the family level was comparable between lakes with species from 55 families trapped at EMCA and 56 at Eustis. Species diversity indices were comparable between the two sites, with H' = 0.44 and D = 0.09 at EMCA and H' = 0.40 and D = 0.09 at Eustis. The different insect assemblages are presented in Figs. 4.6–8. The most noteworthy contrast between the lakes was the relative abundance of the family Hydrophilidae (water scavenger beetles), with 1,923 individuals collected from EMCA and only 13 from Eustis.

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

Table 1. Species of Laboulbeniales recorded from Eustis in 1897 and 2018 and from EMCA in 2018

Species	Eustis 1897	Eustis 2018	EMCA 2018

Autoicomyces acuminatus	X		
Ceratomyces ansatus	X		X
Ceratomyces camptosporus	X		
Ceratomyces cladophorus	X		
Ceratomyces confusus	X		X
Ceratomyces filiformis	X		X
Ceratomyces floridanus	X		
Ceratomyces longicornis	X		X
Ceratomyces minisculus	X		
Ceratomyces mirabilus	X		X
Chitonomyces affinis	X		
Chitonomyces dentiferus	X		
Chitonomyces distortus	X		
Chitonomyces hydropori	X		
Chitonomyces lichanophorus	X		
Chitonomyces occultus	X		
Chitonomyces psittacopsis	X		
Chitonomyces uncigerus	X		
Chitonomyces sp.			X
Hesperomyces virescens		X	X
Hydraeomyces cnemidoti	X		
Hydraeomyces halipli	X		
Hydrophilomyces gracilis			X
Hydrophilomyces hamatus			X
Hydrophilomyces reflexus	X		
Laboulbenia texana var. *retusa*	X		
Laboulbenia texana var. *rostellata*	X		
Laboulbenia texana var. *tibialis*	X		
Laboulbenia sp. 1			X

Laboulbenia sp. 2			X
Rhynchophoromyces elephantinus	X		
Teratomyces mirificus	X		
Zodiomyces vorticellarius.	X		X

OCCURRENCE RECORDS

Ceratomyces Thaxt.

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

Ceratomyces ansatus Thaxt.

Specimens examined: EMCA, FL, USA, August 14–18, 2018 on *Tropisternus sp* (Hydrophilidae, Coleoptera) (Fig. 4E).

This species has not been formally recorded in North America since its original publication by Thaxter (1908), where he reported the type from Brazil, and a specimen from Eustis, FL. Weir and Rossi (2001) report this species from Santa Cruz Dept., Bolivia. Within the collection of Dr. Richard Benjamin, currently housed at SYRF, there are heretofore unreported specimens

of *Ceratomyces ansatus* collected (in chronological order) from: Jackson, IL, USA, 1909 (RKB 436A, RKB 495A); Morelos, Mexico, 1948 (RKB 1956, RKB 1756A); Alachua, FL, USA, 1954 (RKB 1773A); Musuas, Nicaragua, 1945 (RKB 1987); Turrialba, Costa Rica, 1955 (RKB 1990A); San Antonio, El Salvador, 1957 (RKB 3708E); Porto Alegre, Brazil, Date N/A (RKB 1314).

Ceratomyces confusus Thaxt.

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on *Tropisternus sp* (Hydrophilidae, Coleoptera) (Fig. 4B).

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

Ceratoymyces filiformis Thaxt.

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on *Tropisternus sp* (Hydrophilidae, Coleoptera) (Fig. 4D).

This widespread species was first recorded from *Tropisternus glaber* and *T. nimbatus* in Milford, CT, Kittery Point, ME, and Arlington, MA, USA by Thaxter (1896). It was later

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

Ceratoymyces longicornis Thaxt.

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on *Tropisternus sp.* (Hydrophilidae, Coleoptera) (Fig. 4A).

This species was described from Eustis, FL, USA on *Tropisternus sp.* and has not been reported since (1931) in or outside the original locality.

Ceratoymyces mirabilis Thaxt.

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on *Tropisternus sp* (Hydrophilidae, Coleoptera) (Fig. 4C).

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

2256D, RKB 2256E); San Diego, CA, USA, 1960 (RKB 3515A, RKB 3515B, RKB 3515C);

Chiapas, Mexico, 1964 (RKB 2303, RKB 2304A); Yellowstone National Park, WY, USA, 1971

(RKB 2810A, RKB 2810B, RKB 2810C); OR, USA, Date N/A (RKB 1958A)

Fig. 4. A) *Ceratomyxa brevisornis* B) *Ceratomyxa confusa* C) *Ceratomyxa mirabilis* D)

Fig. 5. A) *Hydrophilomyces hamatus*; B) a cluster of *Hydrophilomyces gracilis*.

Hydrophilomyces Thaxt.

The genus *Hydrophilomyces* was erected by Thaxter (1908) and now contains 17 species. Subsequent contributions to this genus have been made by Picard (1910), Spegazzini (1915),

Thaxter (1931), Sarna and Milewska (1977), Majewski (1974, 1983, 1994), Hulden (1983), Tavares (1985), Rossi (1990), Santamaria (2006). Two species of the genus – *H. reflexus* and *H. rhynchophorus* – were originally described from Eustis, FL by Thaxter and were first placed within the genus *Ceratomyces* (1900) before moved to *Hydrophilomyces* in 1908. These species were not re-collected in this study, however, two other members of the genus, *H. gracilis* and *H. hamatus* were collected.

Hydrophilomyces hamatus **Majewski**

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on *Cercyon sp.* (Hydrophilidae, Coleoptera) (Fig. 5A).

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

Hydrophilomyces gracilis **Majewski**

Specimens Examined: EMCA, FL, USA, August 14–18, 2018 on *Cercyon sp.* (Hydrophilidae, Coleoptera) (Fig. 5B).

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

DISCUSSION

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

combination of all these factors uniquely positions the Laboulbeniales as a focal group for fungal diversity studies.

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

In this study, the contrast of the abundance of Hydrophilidae, more specifically the subfamily Hydrophilinae, between sites is of particular note, given that Hydrophilinae are host to most of the fungal infections recorded at EMCA. Because Hydrophilinae are nocturnal and attracted to light (Thorp and Rogers, 2015), it is possible that ambient artificial light from the town of Eustis diminished the relative attraction of the UV light, contributing to the difference in Hydrophilidae abundance between sites. However, given the well-established and devastating effect of artificial light at night on many nocturnal insects, it is not unreasonable to posit that artificial light

introduced with urban development coupled with outright habitat destruction around the perimeter of Lake Eustis and surrounding wetlands disrupted normal biological activities of the Hydrophilinae population, leading to population decline (Owens et al, 2019). The loss of emergent and floating-leaved vegetation may also play a role in the insect population declines. Such vegetation was relatively abundant at the EMCA collection site as it had been purposefully re-introduced (Fulton, 2015) compared to Eustis where no such efforts have been reported. If the insect population declined, then the associated fungal population may have also declined. At EMCA, the infection rate of the Hydrophilidae population was 1 in 5, but 0 of the 13 Hydrophilinae collected from Eustis were infected. This would perhaps suggest insect population dwindled to levels no longer facilitative of social transmission of Laboulbeniales, which is thought to be the primary mode of transmission for this group (De Kesel, 1993, Weir and Beakes, 1995). In other words, Laboulbeniales require a certain host population density, meaning that Laboulbeniales decline may be a harbinger for insect population decline. The presence or absence of Laboulbeniales may be a proxy for the robustness of the insect population, which in turn may be a proxy for the overall health of the habitat. This raises the question: is there a quantifiable threshold at which insect population decline leads to extirpation of Laboulbeniales species?

Future studies should seek to address the above question and build upon and replicate the methodology of this study in numerous habitats in order to garner evidence for the utility of Laboulbeniales as indicators of ecosystem health. When considering Laboulbeniales, collection of insects offers a two-for-one assessment of insects and fungi, providing a more textured, multi-kingdom understanding of biodiversity at a given site. In this study, similar richness of insect families was found at both sites, while the presence of fungi was markedly different, with a

17.6% infection rate at EMCA and a 0.19% infection rate at Eustis. In this case, without

attention paid to the fungal dimension, incorrect conclusions may be drawn about population

trends or robustness, and overall biodiversity of these two habitats. A multi-kingdom assessment

consolidates resources and effort, which increases the feasibility of conducting biodiversity

monitoring. Making such work more feasible is attractive given the mounting pressure of climate

change and the related impacts on biodiversity. These findings also highlight that specialist

organisms, such as the highly host specific and obligately associated members of the

Laboulbeniales, may be of particular risk for population decline, range restriction, loss of genetic

diversity, extirpation, and total extinction in a changing climate (Thomas, 2000; Warren et al,

2001). For such organisms, establishing protected areas and carrying out focused monitoring
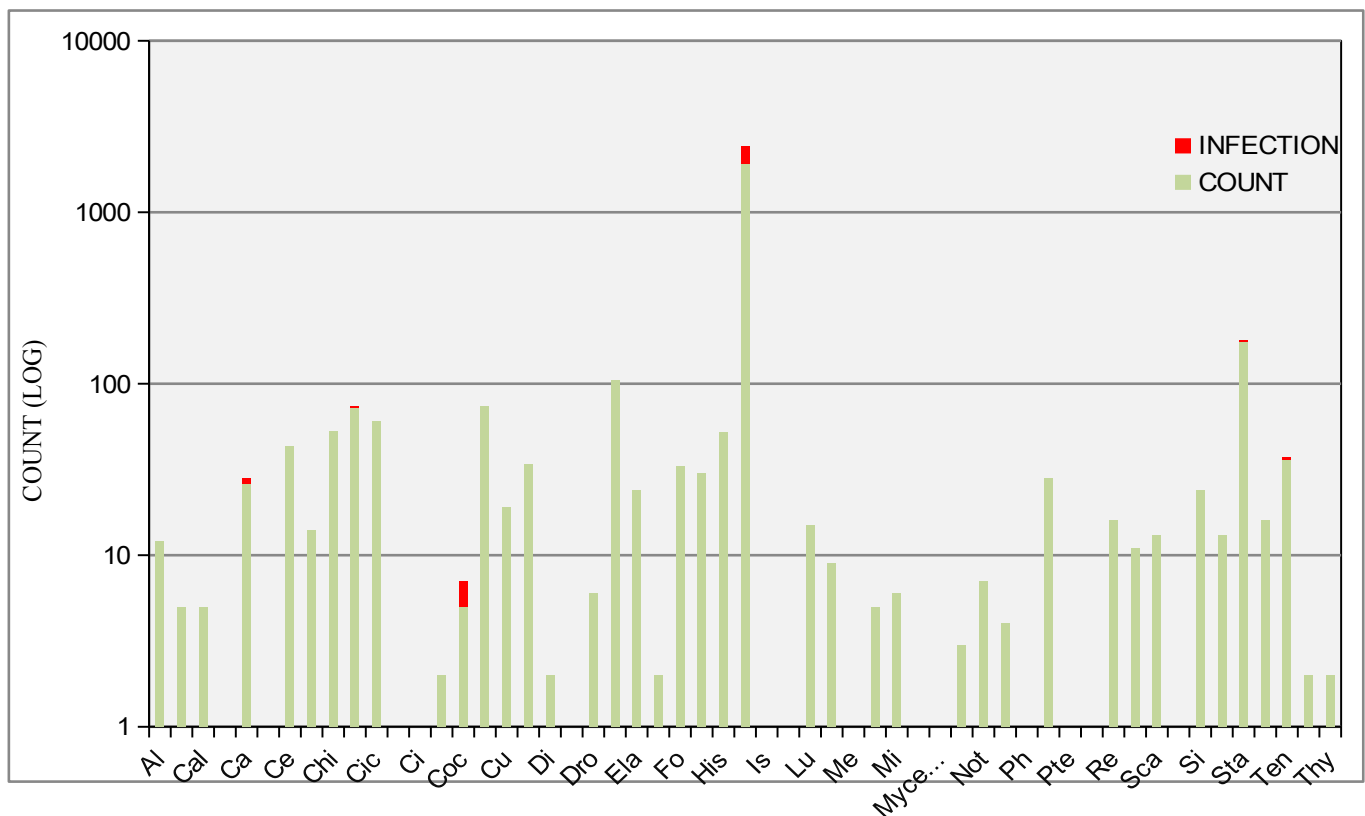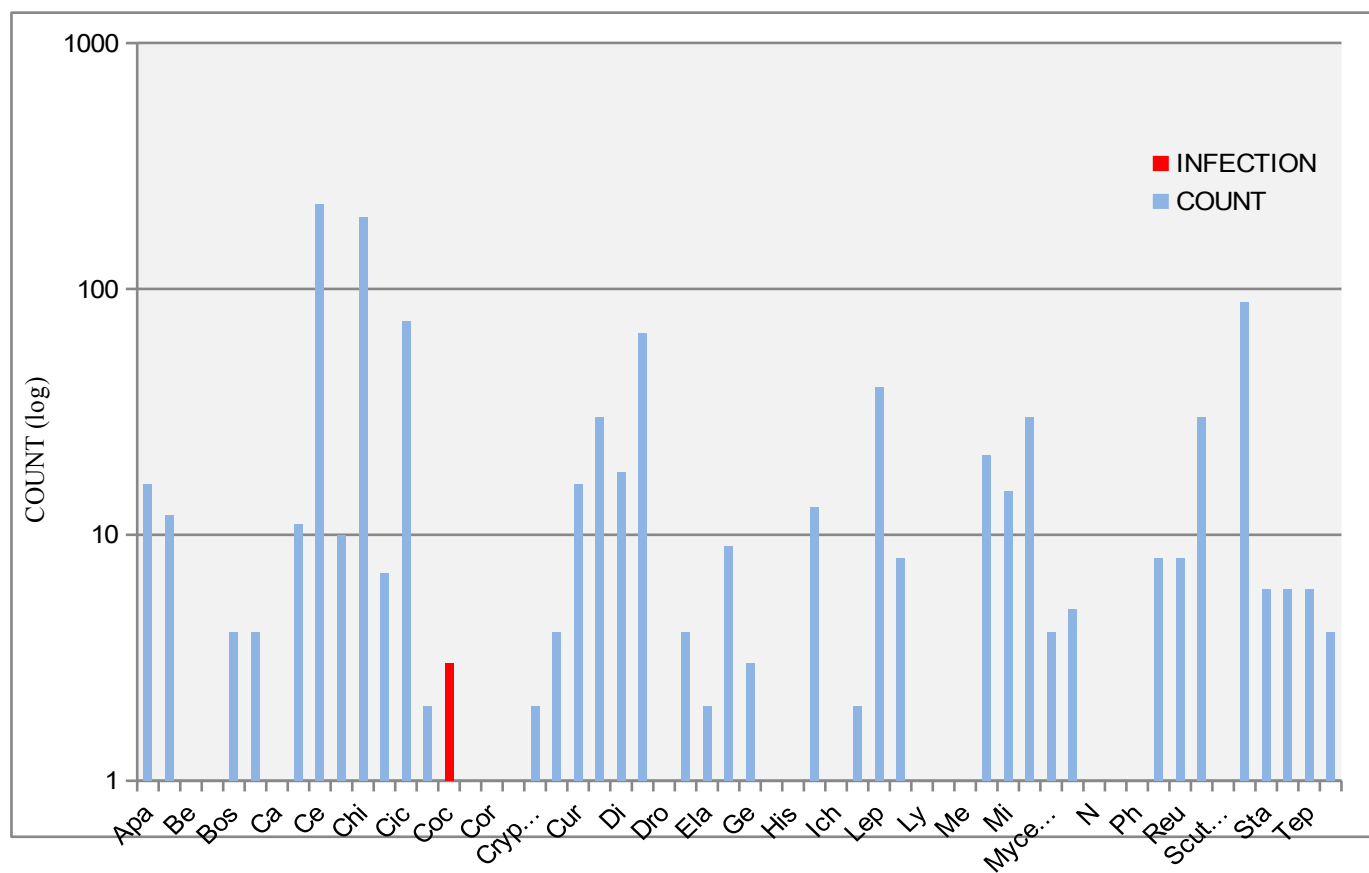

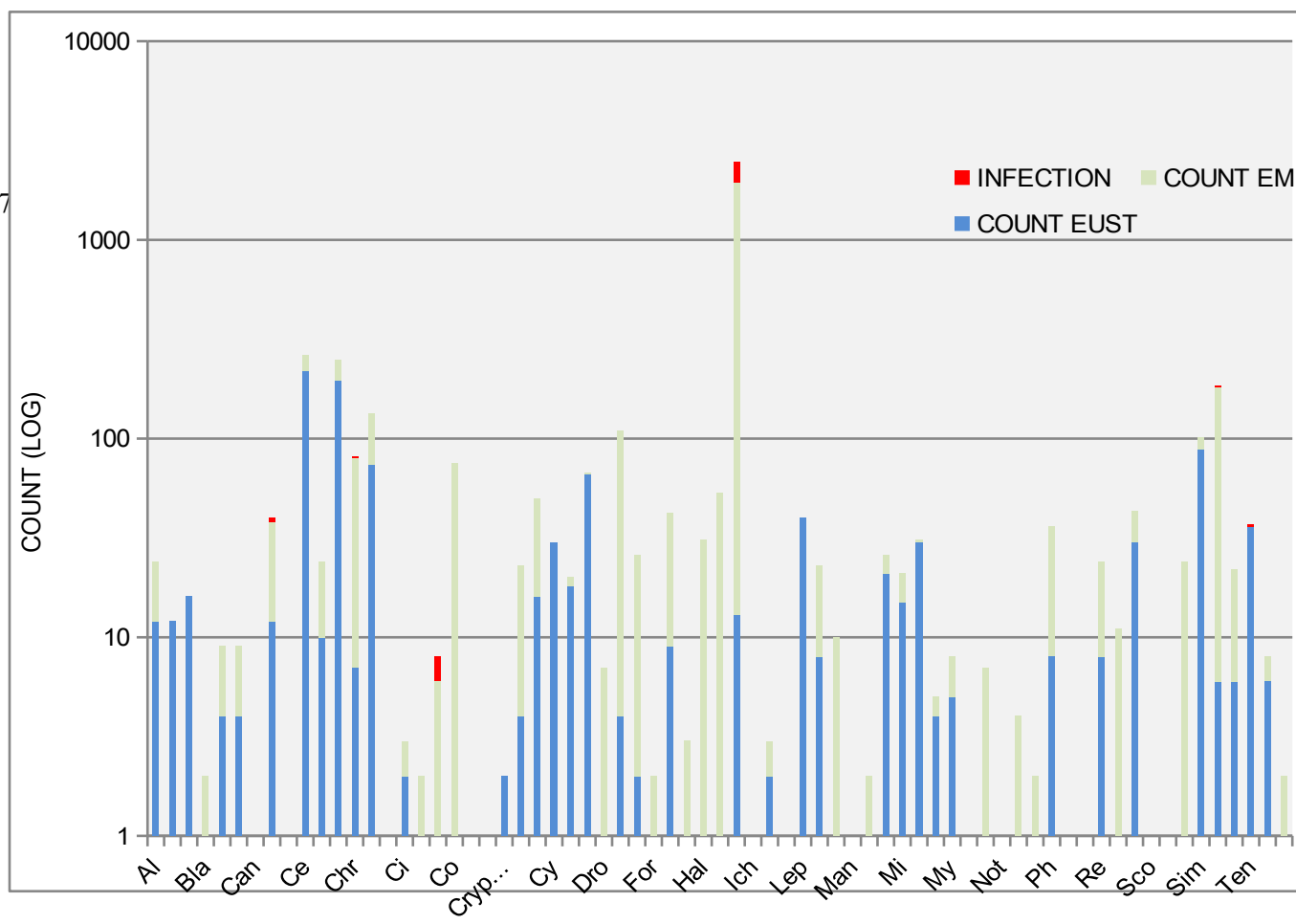protocols is of great importance (Chape, 2005).

Fig. 7

Fig. 8. Insect family richness, abundance and infection at EMCA and Lake Eustis. Note that both of the two Coccinellidae individulas collected at Lake Eustis were infected, thus appearing red and not blue.
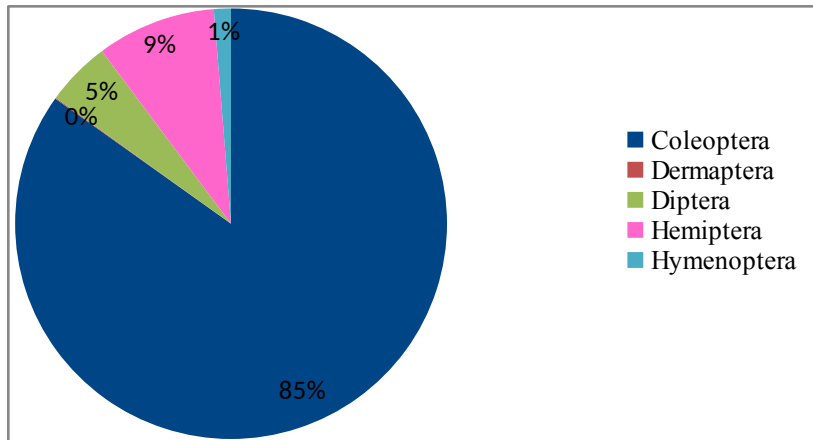
Fig. 9. Relative abundance of insects by order from Eustis. Hemiptera and Diptera were the two dominant orders at Eustis.
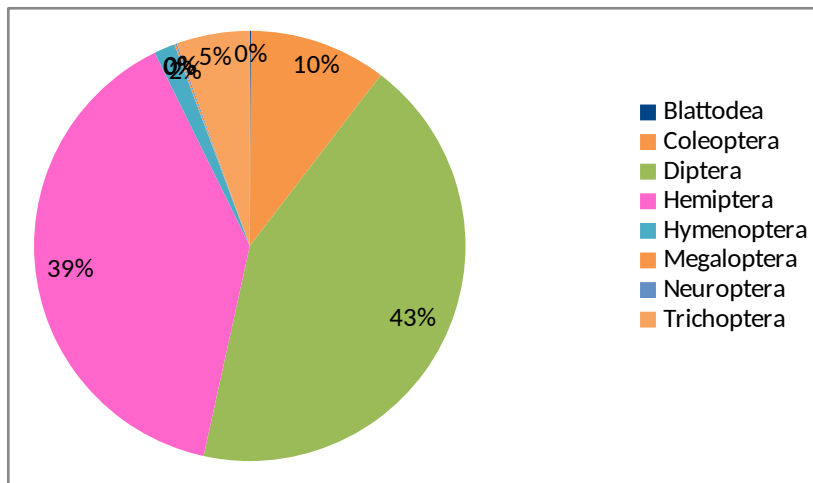

Fig. 10. Relative abundance of insects by order from EMCA. The dominant order at EMCA was Coleoptera, hosting all detected species of Laboulbeniales.

APPENDIX

Table. 1A. Insect family count and infection data from Eustis.

	EUSTIS				EMCA		
ORDER	FAMILY	COUNT	INFECTION	ORDER	FAMILY	COUNT	INFECTION
Blattodea	Blattidae	1	0	Coleoptera	Bostrichidae	5	0
Coleoptera	Bostrichidae	4	0	Coleoptera	Cantheridae	1	0
Coleoptera	Carabidae	11	0	Coleoptera	Carabidae	26	2
Coleoptera	Chrysomelidae	7	0	Coleoptera	Chrysomelidae	73	1
Coleoptera	Coccindelidae	1	2	Coleoptera	Cicindelidae	1	0
Coleoptera	Curculionidae	16	0	Coleoptera	Cleridae	2	0
Coleoptera	Dytiscidae	4	0	Coleoptera	Coccinellidae	5	2
Coleoptera	Elateridae	2	0	Coleoptera	Curculionidae	34	0
Coleoptera	Haliplidae	1	0	Coleoptera	Dytiscidae	105	0
Coleoptera	Histeridae	1	0	Coleoptera	Elateridae	24	0
Coleoptera	Hydrophilidae	13	0	Coleoptera	Haliplidae	30	0
Coleoptera	Lucanidae	8	0	Coleoptera	Histeridae	52	0
Coleoptera	Meliodae	1	0	Coleoptera	Hydrophilidae	1923	519
Coleoptera	Scarabaeidae	30	0	Coleoptera	Lampryidae	1	0

Order	Family	Count	Count2	Order	Family	Count	Count2
Coleoptera	Staphylinidae	6	0	Coleoptera	Lucanidae	15	0
Diptera	Caliphoridae	4	0	Coleoptera	Meloidae	1	0
Diptera	Chaoboridae	10	0	Coleoptera	Scarabaeidae	13	0
Diptera	Chironomidae	195	0	Coleoptera	Scolytinae	1	0
Diptera	Cryptochetidae	2	0	Coleoptera	Silphidae	24	0
Diptera	Culicidae	4	0	Coleoptera	Staphylinidae	175	3
Diptera	Dixidae	18	0	Coleoptera	Tenebrionidae	36	1
Diptera	Dolichopodidae	66	0	Dermaptera	Forficulidae	2	0
Diptera	Drosophilidae	1	0	Diptera	Calliphoridae	5	0
Diptera	Muscidae	30	0	Diptera	Carnidae	1	0
Diptera	Mycetophilidae	4	0	Diptera	Chaoboridae	14	0
Diptera	Phoridae	1	0	Diptera	Chironomidae	53	0
Diptera	Simuliidae	88	0	Diptera	Culicidae	19	0
Diptera	Tachinidae	6	0	Diptera	Dixidae	2	0
Diptera	Tephritidae	6	0	Diptera	Dolichopodidae	1	0
Diptera	Tipulidae	4	0	Diptera	Drosophilidae	6	0
Hemiptera	Alydidae	12	0	Diptera	Muscidae	1	0
Hemiptera	Berytidae	1	0	Diptera	Mycetophilidae	1	0
Hemiptera	Cercopidae	220	0	Diptera	Phoridae	1	0
Hemiptera	Cicadellidae	74	0	Diptera	Sarcophagidae	11	0
Hemiptera	Cimbicidae	2	0	Diptera	Simuliidae	13	0
Hemiptera	Corixidae	1	0	Diptera	Tachinidae	16	0
Hemiptera	Cydnidae	30	0	Diptera	Tephritidae	2	0

Order	Family	Count	Value	Order	Family	Count	Value
Hemiptera	Gerridae	3	0	Hemiptera	Alydidade	12	0
Hemiptera	Issidae	2	0	Hemiptera	Cercopidae	43	0
Hemiptera	Lygaeidae	1	0	Hemiptera	Cicadellidae	60	0
Hemiptera	Membracidae	21	0	Hemiptera	Corixidae	74	0
Hemiptera	Miridae	15	0	Hemiptera	Issidae	1	0
Hemiptera	Nabidae	1	0	Hemiptera	Lygaeidae	9	0
Hemiptera	Notonectidae	1	0	Hemiptera	Membracidae	5	0
Hemiptera	Phymatidae	8	0	Hemiptera	Miridae	6	0
Hemiptera	Reduviidae	8	0	Hemiptera	Notodonctidae	7	0
Hemiptera	Scutelleridae	1	0	Hemiptera	Pentatomatidae	4	0
Hymenoptera	Crabronidae	1	0	Hemiptera	Phymatidae	28	0
Hymenoptera	Formiciidae	9	0	Hemiptera	Pyrrhocoridae	1	0
Hymenoptera	Ichneumonidae	1	0	Hemiptera	Reduviidae	16	0
Hymenoptera	Mymaridae	5	0	Hemiptera	Thyreocoridae	2	0
Megaloptera	Corydalidae	1	0	Hymenoptera	Cimbicidae	1	0
Neuroptera	Mantispidae	1	0	Hymenoptera	Formicidae	33	0
Trichoptera	Apateniidae	16	0	Hymenoptera	Mymaridae	3	0
Trichoptera	Leptoceridae	40	0	Hymenoptera	Pteromalidae	1	0

Data Accessibility:
- All insect and fungal material is available for loan upon request from SUNY-ESF.

-All insect biodiversity data will be archived with Dryad upon acceptance by the journal.

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