Systematics, Phylogeography and Ecology of Elaphomycetaceae

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor of Philosophy in the Department of Biology in the Graduate School
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ABSTRACT

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Abstract

This dissertation is an investigation of the systematics, phylogeography, and ecology of a globally distributed fungal family, the Elaphomycetaceae. In Chapter 1, we assess the literature on fungal phylogeography, reviewing large-scale phylogenetics studies and performing a meta-data analysis of fungal population genetics. In particular, we examined the possible effects of asexuality, trophic niche, dispersal method, and ocean barriers on population structure. In Chapter 2, we examine the systematics and phylogeography of the Elaphomycetaceae, a family consisting of the truffle genus *Elaphomyces* and the stalked genus *Pseudotulostoma*, hypothesizing that the mammal-dispersed truffle would show evidence of dispersal limitation. Using DNA sequence data, we determined that *Pseudotulostoma* is derived from a lineage of *Elaphomyces*, indicating that *Elaphomyces* as currently defined is paraphyletic. The distribution of each subgenus of *Elaphomyces* is nearly global; representative species have been found on every continent save Africa and Antarctica. This biogeographic pattern does not follow the pattern expected by a scenario of continental vicariance. Dating analysis in BEAST confirmed that broadly distributed clades are, in most cases, too young for this pattern to be explained by continental vicariance, indicating that occasional long-distance dispersal has been a significant component in the biogeographic history of the Elaphomycetaceae. This finding contradicts our initial hypothesis that the mammal-

dispersed truffles would be dispersal- limited. In Chapter 3, we investigate the role of *Elaphomyces* as a host for the fungal parasite *Elaphocordyceps*, a parasite derived from insect pathogens that attacks both insect larvae and *Elaphomyces*, its only fungal host. We examined the biogeography of *Elaphocordyceps* isolated from *Elaphomyces* specimens in order to test whether it, like its host, showed recent connections between the Southern and Northern Hemispheres. We also evaluated the pathogenicity of infection as determined by a visual rubric for the truffle gleba, the phylogenetic distribution of *Elaphocordyceps* species on its host, testing for seasonal, climate, and host-parasite effects. In Chapter 4, based on the phylogeographic pattern seen in *Elaphomyces* that resembles that of some air-dispersed fungi, we used theoretical and experimental methods to test whether *Elaphomyces* could be dispersed by air. We tested the capacity for air dispersal with an experimental test of passive air dispersal on the powdery spores of *Elaphomyces morettii* and found that these large spores could disperse over a short distance (10 m) in comparable numbers with the spores of the giant puffball *Calvatia cyathiformis*, which is known to be air-dispersed. The major findings of this thesis are that 1) fungi in general show high dispersal ability, but that trophic niche and dispersal mode may affect population structure, 2) that *Pseudotulostoma*, a stalked genus, is derived from the truffle *Elaphomyces*, 3) that the Elaphomycetaceae have experienced frequent long-distance dispersal despite 4) that the fitness of *Elaphomyces* as indicated by glebal development varies with host-parasite interactions based on species identity, but not with climate or

season, and that 5) *Elaphomyces* spores, should they be released into the air, can remain in the air long enough to be dispersed long distances by the wind. The overall conclusion of this thesis is that, while *Elaphomyces* is clearly reliant on animal vectors for excavation and dispersal, its past history of long-distance dispersal and current spore trajectories indicate it can be passively air-dispersed as well.

Dedication

I dedicate this dissertation to my parents, Matthew and Susan Reynolds.

Contents

List of Tables

List of Figures

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Chapter 1. The effect of life history traits and geography on fungal population structure

The field of phylogeography examines the evolutionary history of organisms in a spatial framework: how and where organisms have evolved, the frequency and direction of dispersal, and possible barriers to dispersal **[1]**. The fungi are the least studied group of organisms for phylogeography **[2]**, but some key species of ecologically diverse fungi have been recently investigated. As more fungi continue to be discovered, and as molecular phylogenetics continues to illuminate their history and biology, it is crucial to have a good basis for formulating hypothesis for fungal ecology and evolution. We review phylogenetic patterns and present a metadata analysis of population genetics summary statistics from several studies on fungi in order to evaluate how life history traits have impacted fungal phylogeographic history.

There are two major, historical hypotheses for fungal phylogeography. The Bass-Becking hypothesis for microbial biogeography postulated that "Everything is everywhere; the environment selects **[3]**." The propagative power of fungi could indicate support for the hypothesis of ubiquity; a single *Calvatia gigantea* fruiting body can produce 7×10^{13} airborne spores **[4]**. However, many species of mushrooms are known to be endemic to single continents or even smaller areas, and are evidence against the hypothesis of ubiquity. Oceans were proposed as major geographical barriers for fungi **[5]**, and continental vicariance was invoked to explain the patterns of

fungal biogeography. This hypothesis is supported by the Gondwanan distribution of several fungal species restricted to the Southern Hemisphere, and by the observation of Holarctic species that were only recently separated post-Pleistocene. However, the continental vicariance hypothesis has been challenged by at least a few recent studies that, using molecular phylogenetics, indicated that the evolutionary time for several putative Gondwanan fungi was too recent to have actually been established by continental vicariance alone.

Evidence from both phylogenetics and population genetics indicates that the fungi have diverse phylogeographic histories and range from global, panmictic species to highly structured populations. We review the trends indicated by phylogenetic analysis and use summary statistics from population genetics studies to test how major life history traits in the fungi may have affected their distribution. The fungi differ in dispersal strategy, life cycles that may include an asexual stage, and symbiosis. These life history traits are hypothesized to influence effective dispersal, the ability of an organism to disperse to and populate a new area. We hypothesized that fungi with different suites of life history traits would tend to show different phylogeographic patterns due to differences in historical dispersal ability. We first begin by reviewing sequenced-based phylogenetic and phylogeographic studies, then move to examining evidence from population genetics.

1.1 Understanding fungal phylogeography through tree topology

Broadly speaking, fungal phylogenetic studies and phylogeographic studies can be subdivided into three categories based on ecological niche: pathogen, mutualists, and decomposers. These groups include specialists and generalists. For example, fungi that decompose wood may be more adapted to break down conifers, while others may be generalist soil fungi. Specialization may impose a constraint on effective dispersal. We therefore hypothesize that generalists have fewer geographically defined lineages than specialists, and that decomposers may, in general, have fewer such lineages than symbiotic organisms.

1.1.1 Pathogens

Both continental lineages and long-distance dispersal have been indicated for plant pathogens. The plant pathogen *Gibberella fujikuroi*/*Fusarium* complex, consisting of several dozen species, produces airborne sexual and asexual spores, and was shown to have three major continental lineages, for America, Asia, and Africa **[6]**. The phylogeographic pattern appears to have arisen with the breakup of Pangaea; this idea is further supported by the estimated date of the origin of *Fusarium*, 100 mya. The species in the *G. fujikuroi* complex are specialized to their hosts and in many cases, have spread with them. *Gibberella fujikuroi* appears to be one of the few cases of fungi that have been supported as having phylogeographic patterns truly derived from continental vicariance. However, it depends heavily on the dating system used, which was not able

to be calibrated independently for this group. A study of the wheat scab fungus *Fusarium graminearum*, found worldwide, revealed a complex pattern of several geographically restricted lineages **[7]**. In this case, at least three instances of long distance dispersal were thought to have occurred prior to any possible human-mediated dispersal. Because the major pattern was of Northern Hemisphere strains arising from several Southern Hemisphere lineages, the authors again invoked a Gondwanan breakup as the explanation for the overall pattern of diversity. For both studies, the Gondwanan origin may be problematic, in that the estimated age for the entire *Gibberella*/*Fusarium* genus is old enough for the ancestral *Fusarium* to have been present on Gondwana, but the sub-generic groups in question may not have been. However, it is also possible that even if the ancestral clades of *Gibberella fujikuroi* and *Fusarium graminearum* originated after the continents had separated, that they were still in close enough proximity for dispersal to occur.

Evidence of long-distance dispersal is commonly observed in pathogens. A four-gene study of the animal pathogen *Histoplasma capsulatum* found continentally restricted lineages, with the highest genetic diversity in Latin America **[8]** with a recent connection between Europe and Australia. The authors suggested a radiation from Latin America. *Phragmidium violaceum*, a rust introduced to Australia 20 years ago to control the invasive European blackberry, was found to have diverged from the original introduced strain **[9]**. The rusts present in New Zealand were from multiple transfers from

Australia. This study was based on AFLP markers, and shows how quickly fungi may evolve in a new habitat, either through mutation or recombination.

1.1.2 Mutualists

Studies on mutualist fungi include lichens, mycorrhizae, and insect symbionts. In both global and Northern Hemisphere studies, mutualists have shown a blend of continental lineages and long-distance dispersal. In some cases, the patterns for highly structured lineages could be explained by invoking continental vicariance, but other fungi had developed too recently and must have obtained their current distribution through ancient dispersal events.

The lichenized ascomycete *Pleopsidium* shows phylogeographic structure for each of three clades of closely related species and subspecies **[10]**. *Pleopsidium chlorophanum* is divided into a European and a South Africa/Antarctica clade. The second clade contains several European and North American species, while the third clade spans the Middle East and Central Asia. Each of these clades is further subdivided into individual geographic variants. Even apparently dispersal limited lichens are known to disperse over 200 m, and it has been suggested that habitat requirements and competition are more important constraints on effective dispersal than dispersal ability alone **[11]**.

A complex of closely related lichen species in the genus *Leptogium* shows a pattern of continental lineages for European, North American, South American and African lichens **[12]**. The topology of the phylogeny correlates with the continental

vicariance pattern, but the authors rejected the hypothesis that continental vicariance had led to this highly structured phylogeography. The origin of the *Leptogium* species complex was estimated at approximately 16.2 million years using a relaxed molecular clock, too recent to be explained by continental vicariance. However, the estimated rate of molecular evolution was taken from an estimate for a species of ascomycete in a different class – that of the Erysiphales in the class Leotiomycetes **[13]**. Molecular rates of evolution are almost certainly different for *Leptogium*. It is possible that, if the ribosomal genes for *Leptogium* evolve an order of magnitude more slowly than in the Erysiphales, the clade is in fact far older than estimated, and that the breakup of supercontinents explains its distribution.

There have been two published phylogeographic studies of lichens in the Northern Hemisphere. The lichen *Letharia vulpina* was found to have dispersed from North America to Europe based on the relatively high diversity of North American specimens and the placement of the European clade in one of the North American lineages **[14]**. The Arctic lichen *Porpidia flavicunda* was studied in Scandinavia, North Atlantic islands, and Quebec, and was found to have five major lineages that were not restricted geographically, with the exception of a clade found only on Baffin Island **[15]**. The most likely explanation is long-distance dispersal among North Atlantic landmasses **[16]**.

Ectomycorrhizal species also show a mix of geographic structure punctuated by episodes of long-distance dispersal. An ectomycorrhizal species with an amphi-Pacific distribution, *Tylopilus ballouii*, was found to consist of several geographically distinct lineages, but the possibility of long-distance dispersal could not be ruled out **[17]**. The genus *Amanita*, famous for its numerous toxic species, is found worldwide, and is thought to be mainly ectomycorrhizal. *Amanita muscaria*, the red and white-spotted fly agaric, and *A. pantherina* are two Northern Hemisphere species with continental lineages **[18]**. Both species can be broken into a Eurasian and a North American clade. The Eurasian clades can be further subdivided, first by ecology (subalpine and low altitude forests) and then by geography, Japanese and European populations being distinct. The authors proposed that, for both species, there had been migration across the North American-Eurasian land bridge during the Pleistocene. The false truffle family Hysterangiales is distributed worldwide and shows a phylogeographic pattern supporting a mixed history of vicariance and long-distance dispersal **[19]**. Most clades are restricted to the Southern Hemisphere and replicate the geographic lineages of the Gondwanan breakup, with multiple dispersal events into the Northern Hemisphere. Additionally, several sister groups are of Australia and New Zealand taxa, which can best be explained by long-distance dispersal. The authors concluded that their initial hypothesis that truffles would be more limited in dispersal than their airborne relatives was contradicted by their data.

Attine ants in the New World cultivate a basidiomycete for their food. In North America, *Trachymyrmex septentrionalis* ants were found to have an eastern and western lineage, while their fungal cultivar did not share this east-west divide, but had a phylogeographic pattern marked by a decrease in genetic diversity northward **[20]**. The basidiomycete, a relative of *Lepiota*, has not been found outside of the ant colonies, but does form fruiting bodies in the laboratory that produce airborne spores. The lack of correspondence between the ant and cultivar fungus phylogeographic patterns indicates an escape from vertical transmission for the fungus and greater connectivity among fungal populations than ant populations.

The gourmet truffle genus *Tuber* is ectomycorrhizal on angiosperms and conifers and has a Holarctic distribution, with some species found in North Africa. It develops fruiting bodies underground, and is unearthed by mammals that eat it and may spread its spores in their feces. It may also spread by vegetative growth underground on the root network **[21, 22]**. Though one could hypothesize that it would have limited dispersal ability, a recent phylogeographic study of the genus found that there have been at least nine inter-continental dispersal events, mainly between Europe and Asia **[23]**. These dispersal events, however, could have coincided with the presence of land bridges, and thus are in accordance with the hypothesis that *Tuber*, and other truffles, are limited to land-based dispersal.

1.1.3 Saprotrophs

The saprobic mold *Aspergillus fumigatus* is one of the only known cases of a globally distributed fungal species **[24]**. A sampling of 63 individuals from five continents uncovered two cryptic species, each with a global distribution and little to no further geographic subdivision. This fungus, a rare example supporting the classic hypothesis of microbial ubiquity, has several ecological features that were proposed to contribute to its phylogeography. First, it is dispersed by air, and has small spores (2-3 u) **[25]** that may be allow more long distance dispersal than larger-spored fungi. Pringle et al also emphasized the ability of this fungus to grow clonally – in fact, until their research indicated that may have the capacity for recombination, it was thought to be exclusively clonal. Most importantly, it is a generalist decomposer found in soil, and is thus relatively unconstrained in its opportunities for growth. *Aspergillus fumigatus*, then, is marked by a phylogeography that reflects its freedom from mating and habitat constraints and exemplary dispersal abilities. However, other decomposer fungi do not show this pattern.

Oceans have been implicated as barriers to dispersal for several wood-rotting fungi. *Schizophyllum commune* ***[26]*** and *Lentinula* ***[27]*** each had continentally endemic species complexes. *Schizophyllum* and *Lentinula* exhibited a separation between New World and Old World taxa. *Lentinula* also showed long-distance dispersal between New Zealand and Australia **[27]**. The oyster mushroom *Pleurotus* was found to have two

major clades, one Old World clade, and another that was predominately New World and was subdivided between Mexico and the rest of North America **[28]**. The North American clade also included some apparently recently dispersed samples from South Africa and Japan.

One of the most important hypotheses for Northern Hemisphere plant and fungal phylogeography is the East Asian – Eastern North America disjunct **[29]**, which notes a closer similarity between the flora of these areas than within continents. Recent molecular studies have challenged the view of this long held pattern. Evidence for geographically defined lineages has been found for Northern Hemisphere wood decomposers with airborne spores. The maitake mushroom *Grifola frondosa* was found to be subdivided into a North American and an Asian clade, indicating that the classically observed East Asia-Eastern North America disjunct actually represents two molecularly distinct lineages **[30]**. The saprobes *Coprinus comatus* and *Coprinellus* species were found to exhibit geographical lineages, with separation between Hawaiian, East Asian, European & North American taxa. Hawaiian taxa were most closely related to Asian taxa. Sampling in Asia was from Korea and Japan, and for *Coprinellus desiminatus* and *Coprinus comatus*, there was a clade exclusive to Japan & a clade found in both Japan and Korea **[31]**. The wood decomposer *Artomyces pyxidatus* could be subdivided into Eurasian and North American lineages **[32]**. A study of the wood rotting fungus *Coniophora puteana* uncovered three cryptic species: two widespread through the

Northern Hemisphere and one endemic to North America **[33]**. The authors suggested a North American origin for the complex. In North America, one species is widespread while the other two are found in the northeast and northwest respectively. This destructive fungus, also known as the wet rot or cellar fungus, is a major problem destroying households around the world, and was also likely one of the fungi that destroyed ship timbers in the 19th century **[34]**. Studies on a similarly destructive decomposer, *Serpula lacrymans*, found a similar pattern, but with an Asian origin rather than American **[35]**. Multiple instances of contact between mainland Asia and Japan, and apparently recent dispersal to the rest of the Northern Hemisphere, support the authors' conclusion that shipping lanes may have aided this fungus in its spread. The authors also discovered a cryptic species endemic to California that does not invade human habitats but is less aggressive and apparently is restricted to forests **[36]**. It is interesting to note that few *Serpula lacrymans* var. *lacrymans* are found in the wild; perhaps having specialized to aggressive growth on timbers has made this variety less competitive in a natural setting.

The evidence from topology-based studies on fungal phylogeography indicates a mix of phylogeographic patterns, dominated by continental lineages with instances of long-distance dispersal. These patterns indicate that, for most fungi, the Bass-Becking hypothesis of "everything is everywhere" does not hold; rather, to establish these geographically structured lineages, there have been long-term barriers to fungal

dispersal. The only known case of panmixia was an asexual, widely dispersed saprotroph. Perhaps fungal life history influences the effective dispersal and therefore the phylogeography of fungi. In order to test this hypothesis with a quantitative analysis, we conducted a survey of population genetics literature and compare the published summary statistics for ecologically diverse fungi.

1.2 Life History Trait Influence on Population Structure

Much of the literature on fungal phylogeography has reported summary statistics from population genetic analyses. The widespread use of summary statistics to describe population structure permits a quantitative comparison of disparate studies. It is important to note that many fungal life history traits, such as the presence of sexual reproduction or clonality, are poorly known. In many cases, population genetics studies have been used to uncover recombination in fungal populations that were not known to have a sexual form. The lack of definite knowledge of these characters complicates any attempt to understand their influence on phylogeography. However, as population genetics research has illuminated the life cycles of numerous fungal species, it has become more feasible to examine the role of life history traits on fungal population structure. Such an analysis could indicate avenues of further exploration and offer a better framework for future research.

Reviews and metadata analyses in other systems have found varied effects of life history traits on population genetics. A study of 205 fish species found that F_{ST} was

significantly predicted by whether the fish laid benthic or planktonic eggs and whether the study spanned a biogeographic transition **[37]**. Other possible influences, such as adult fish size, were not found to significantly contribute to population structure. Prior reviews of fish population genetics had found varied evidence for an effect of life history traits on population structure. An analysis of life history trait influence on plant population genetics found G_{st} to vary with taxonomic identity, mating system, seed dispersal mode, geographic range, and plant life form , though the contribution of the life history trait to genetic variation was low **[38]**. We have conducted a metadata analysis of the population genetics of 104 fungal species to better understand how geography and life history trait affect population structure.

1.2.1 Literature Search and Analysis Methods

We conducted a search of phylogenetic, phylogeographic, and population genetics literature for published statistics on fungal population structure using Wright's fixation index for haplotype data (F_{ST}) and other related statistics: Nei's G_{ST}, Weir and Cockerham's θ, ϕ_{ST}, and R_{ST}. F_{ST} was first developed for diallelic loci by Sewall Wright and compares the genetic variation in a local population to the variation in the total sample **[39, 40]**. G_{ST}, a related statistic that can be used to analyze multiallelic loci, is calculated from the within and among population heterozygosities (H_T-H_S/H_T) **[41]**. A similar statistic, θ, estimates population structure with an ANOVA approach comparing within and among population genetic variance **[42]**. Other statistics were developed to

handle newly developed molecular markers: ϕst for sequence data **[43]**, and R_{ST} for markers that have stepwise mutation, such as microsatellites **[44]**. Each of these population statistics ranges from 0, indicating no population differentiation, to 1, indicating fixation of the examined allele within the population. Natural populations are thought to have high gene flow if the F_{ST} (or equivalent) is under 0.05, moderate gene flow if between 0.05 and 0.10, and restricted gene flow if 0.15 or higher. We will refer to the combined population statistics as Pop-st.

For each study, we recorded the geographic study span to the nearest 10km as the maximum distance between study sites. When geographic distances were not included in the manuscripts, we calculated the distance using GoogleEarth. Precise GPS coordinates were used when given in the papers, but frequently only the nearest city and country were available. A small number of papers provided only province, state or country names, and these cases we used the center of the areas provided when measuring the study span. We also recorded H_S, Nei's measure of gene diversity. When studies provided the H_e for each locus or geographic site, we calculated H_S from the average of the H_e used in the study to calculate the summary statistic.

We recorded the following population statistics for each study if provided: the global Pop-st, the lowest and highest pairwise Pop-st, and the pairwise Pop-st for the two populations that were farthest apart. We then designated a best estimate of Pop-st for each population: global Pop-st if available, and the pairwise Pop-st for the two

farthest populations if not. Using the pairwise Pop-st for the two farthest populations imitates a case in which these were the only two populations. The best estimate Pop-st was used to calculate genetic distance: Pop-st/(1-Pop-st). The studies with their population statistics, geographic distances, and life history traits are listed in (Table 1).

We used JMP version 9 for linear regression analysis **[45]**. Genetic distance was plotted over the $\log_{10}$ of the study span to test the effects of distance, several life history traits, and the combined effect of life history trait and distance. The study sampling was biased toward some phylogenetic clades, so we also compared the Pop-st among the popular taxa, testing at the division, order, and family levels, and restricting analysis to clades represented by at least 5 samples. Because oceans have been suggested as major barriers to fungal gene flow, we tested whether studies spanning an ocean would differ in population structure than entirely land-based studies. We examined the following life history traits: dispersal mode, asexual propagules, trophic niche (defined as plant pathogen, ectomycorrhiza, or saprobe) and specialization to host or substrate. We conducted three sets of analyses: one on the full dataset, another on the studies using rapidly evolving markers such as microsatellites, AFLP, and protein-coding genes, and another that included only studies reporting Pop-st lower than 0.5. Some life history categories did not have sample points for short geographic distances. In such cases, the regressions could differ from the well-sampled categories simply because of sampling

error. We therefore performed a set of analyses rooting the regression for these categories at 0 m, Pop-st 0.

One of the difficulties comparing population statistics from different studies using different markers is that the different mutation rates affect the heterozygosity within the sampled populations, and thus may lead to an underestimation of F_{ST}, G_{ST}, and related markers **[46-48]**. While F_{ST} and related statistics range from 0 to 1, the true maximum value in an empirical study will be limited by the local heterozygosity, H_S. For G_{ST}, the maximum value will be $1-H_S$, and while the calculations are more complicated for θ and ϕ_{ST}, their values are generally similar enough to G_{ST} that $1-H_S$ can be used to estimate their maximum values as well **[49]**. We tested how making this correction would alter our calculations for genetic distance by dividing each population statistic by $1-H_S$ and plotting the standardized genetic distances over the genetic distances calculated from the reported statistics.

1.2.2 Life History Traits and Hypotheses

We examined four major potential barriers to effective dispersal: physical geographic barriers, dispersal mode, mating barriers, and specific ecological nutrient requirements. Based on previous phylogeny-based studies, an emerging hypothesis suggests that oceans form a major barrier for fungal dispersal. We hypothesized that populations separated by an ocean would have significantly higher Pop-st than studies in a contiguous land mass, even if at a similar distance. The majority of published

studies have focused on air-dispersed fungi, but some studies have assessed the patterns of fungi that disperse via water or animals. We hypothesized that these fungi would have lower Pop-st than air-dispersed fungi, particularly if they have no known ability for air dispesral. The truffle genus *Tuber* is dispersed by mammals in its sexual state, and had been thought to be limited in long-distance dispersal, though animals may be effective dispersers over shorter distances. A recent discovery of an air-borne anamorphic state in several *Tuber* species, however, led us to hypothesize that *Tuber* may have higher gene flow than other truffles that are truly dependent on animal dispersers (Healy, pers. comm).

The hypotheses for the influence of reproductive cycle were more complicated because of the complexity, diversity, and obscurity of fungal life cycles. Fungi typically have two reproductive forms: the asexual anamorph and the sexual teleomorph, either of which may be undiscovered for a particular species. The life cycles of many fungi are unknown, complicating analysis of the effect of sexuality or clonal propagation on the population structure. Population genetics has been used to better understand the life cycles of fungal species, particularly in plant pathogens. Plant pathogens such as rusts may require more than one host to complete their full life cycle: one host for the asexual stage and one for the sexual stage. When these pathogens are introduced to a new area that lacks one of the hosts, they may be limited to asexual reproduction. We simplified the analysis of life cycle by focusing on the presence or absence of an asexual stage. The

ability to grow clonally could both increase gene flow between established, sexually reproducing populations, and allow the fungus to invade new environments as clonal populations. Alternatively, clonal propagation of diverse asexual strains from a sexually reproducing population could cause frequent founder effects, raising the F_{ST} of lineages with asexual reproduction by limiting local diversity in a globally diverse metapopulation. We hypothesized that the former effect of asexuality would be most prevalent, and that populations of fungi that can reproduce asexually would tend to have lower F_{ST}.

We examined the effect of trophic niche and specialization on the population structure. We hypothesized that symbiotic fungi would have evidence of more restricted gene flow than saprobic fungi. The case of wood-rot fungi was difficult to classify, as several wood rot fungi are bark or root pathogens on living trees but continue to grow on dead wood. Because these facultative pathogens can grow as saprotrophs, and would thus have no host limitation in dispersal, we classified all wood-rotters as saprotrophs. The fungal population genetics literature is dominated by studies on agricultural pathogens that have been spread with their crop hosts and by studies on ectomycorrhizal fungi that are mutualists on tree roots. We studied whether these two major modes of symbiosis differed from each other, hypothesizing that the crop pathogens may have lower Pop-st than established ectomycorrhizal populations. We hypothesized that pathogens of agricultural crops could have artificially lowered F_{ST}

values compared to wild pathogen systems. Many symbiotic fungi, both mutualists and pathogens, exhibit a range of specialization to their hosts, while saprobes may be specialized to certain substrates. We hypothesized that specialized fungi would show more population structure than generalists, regardless of the trophic niche.

Finally, we examined whether specialist fungi would have less population structure than generalists. Few of the fungi in our study are known to depend on only one host species, so we classified fungi restricted to one host genus as specialists. In this analysis, we grouped specialized saprobes and symbionts together. There are different levels of specialization; for instance some fungi might never grow associated with conifers, but only with hardwoods, but as these encompass at times over three tree families, we could not think that their habitat was limited enough to impose a true restriction on dispersal. In addition to examining this trait for all of the fungi surveyed, we tested this hypothesis in two phylogenetic subgroups, the Agaricales and Hypocreales (n=11 for each), which were the best sampled orders in the study and had similar sampling of generalists and specialists. We also tested whether specialists had more population structure in the following ecological guilds: Ectomycorrhizae, Plant Pathogens, and ECM+Insect Pathogens.

1.2.3 Results

The studies examined used a variety of summary statistics, of which F_{ST}, G_{ST}, and ϕ_{ST} were the most common. Population structure ranged from non-existent to quite high

(Figure 1). The average value for studies reporting global Pop-st was 0.1771±0.1610 (mean ± standard deviation, n=81). For the 51 studies reporting pairwise Pop-st, the lowest value averaged 0.0556±0.0851, the highest averaged 0.2437±0.196, and the Pop-st for the two most distant populations in each study averaged 0.1996±0.1640. When the best estimates for Pop-st from each study were combined for one analysis, the average was 0.1763±0.1632. The average gene diversity, H_S, was 0.3359±0.2066 across all studies.

We examined the effect of standardizing the summary statistic using the rough calculation 1-Hs as the maximum value for Pop-st. The corrected genetic distance was significantly higher than the uncorrected distance (mean ± standard deviation = 0.4992 ± 0.8774, 0.2548 ± 0.3835, respectively). A regression of the corrected genetic distance over the original genetic distance indicated that adjusting the population statistic for heterozygosity has a minimal effect when the genetic distance is low (Figure 2). The regression was highly significant (R^2=0.7213, $p<0.0001$), but at high genetic distances the corrected genetic distance was more variable. There were 3 fungi above the regression, and they had high heterozygosity and high F_{ST} values.

The slope of Pop-st over geographic distance did not vary significantly with the type of summary statistic used (R^2 = 0.09139, ANOVA = 0.287, p-value of combined = 0.5693) (Figure 3). Additionally, the molecular marker chosen did not significantly influence the Pop-st over geographic distance (R^2 = 0.134039, ANOVA = 0.1585, p-value

of combined = 0.6985). Outliers from the general regression were: *Rhizopogon occidentalis*, *Mycosphaerella fijiensis*, *Cordyceps sinensis* and *Heterobasidion annosum*.

Fungal Pop-st varied significantly according to phylogeny when considering the phylum level (R^2 = 0.282259, ANOVA = 0.0001, p-value of combined = 0.0003) (Figure 4). The sampling was biased toward the Ascomycota and Basidiomycota, which overlapped on the regression analysis, differing from the Oomycota. Because the Oomycota were under-sampled, particularly at low distances between populations, we added a representative at 0 m and 0 Pop-st; when this root point was considered, the combined effect of geographic distance and phylum became non-significant (R^2 = 0.167411, ANOVA = 0.0034, p-value combined = 0.3066).

Fungi in studies separated by an ocean barrier were found to have a strong difference in population structure compared to fungi in a contiguous land-mass (R^2=0.13992, ANOVA = 0.0242, p-value combined = 0.083) (Figure 5). This strong effect was seen in the analysis of rapidly-evolving markers without the addition of the sample at the origin, but disappeared when this example was included and when the outliers were excluded (R2=0.2102, ANOVA= 0.0004, p-value combined = 0.4731).

The effects of most examined life history traits were found to be non-significant when geographic distance between populations was considered (Table 2). For each life history trait, there was high variance in the Pop-st values reported, particularly between the distant populations. The trends are described in (Figure 6). There were very few

studies on fungi that do not disperse by air. Of those that were included in this analysis, the unconstrained analysis showed a near horizontal regression for Pop-st over geographic distance, while the constrained analysis showed a slightly steeper regression than for the numerous air-dispersed fungi. The saprotrophic fungi showed evidence of higher dispersibility with less isolation-by-distance than the symbiotic fungi. The symbiotic fungi showed little difference between ectomycorrhizal fungi and plant pathogens, but there was a slight trend for higher population structure in the ectomycorrhizae. There was a very slight trend for the fungi with an asexual stage to have lower Pop-st at the same geographic distance than fungi lacking a known asexual stage.

The effect of host specialization was examined for all of the fungal samples and for the Hypocreales and Agaricales subsets. For the total fungal samples that had used highly variable markers, there was no significant difference in population structure, but when the plant pathogens were excluded from the analysis, the specialists had significantly higher Pop-st over geographic distance ($R^2 = 0.404$, ANOVA = 0.0111, p-value combined = 0.0021, n = 25). When ectomycorrhizae and plant pathogens were examined in isolation, neither showed significant difference between specialists and generalists (ECM: $R^2 = 0.4049$, ANOVA = 0.036, n = 20, p-value combined = 0.0727; PP: R2 = 0.1152, ANOVA = 0.0569, p-value combined = 0.819, n = 65). In the Hypocreales and Agaricales (n=11), there was a trend for specialists to have higher Pop-st than

generalists. In neither case was the trend significant, but it was much stronger in the Hypocreales (Hypocreales: R2 = 0.5044, ANOVA =0.156, p-value combined = 0.0929; Agaricales: 0.2978, ANOVA = 0.3413, p-value combined = 0.7894). Restricting our analysis to the Hypocreales plant pathogens gave a significant trend (R^2 = 0.9038, ANOVA = 0.0492, p-value combined = 0.0282, n = 7).

1.2.4 Discussion

The average F_{ST} estimator indicated high population differentiation, but the variation was quite high (0.1763±0.1632). It has been suggested that F_{ST} levels from 0-0.05 indicate panmixia, 0.05-0.15 moderate gene flow, 0.15-0.25 restricted gene flow, and >0.25 very restricted gene flow **[50]**. These general interpretations of F_{ST} indicate that the reviewed fungal population studies found, on average, substantial population structure. Of the 102 studies we examined, 50 reported either pairwise or global Pop-st over 0.15, and of these, 30 were over 0.25 (Figure 1).

We examined the effect of local gene diversity on our samples and found that, for the majority of the samples, adjusting for gene diversity yielded a similar measure of genetic distance. When heterozygosity is high in populations, the maximum G_{ST} is lowered **[46-48]**. In such cases, a simplistic interpretation of the summary statistic would be misleading, particularly in interpreting low G_{ST} values. The R_{ST} is the only common summary statistic not affected by H_S **[44, 49]**, though for biallelic markers, such as most SNPs, F_{ST} does not need to be standardized **[49]**. Ideally, each population statistic would

be standardized by dividing it by its maximum value in a given study. As many of our reviewed studies did not report the Hs, and as we wanted to include multiple types of calculations, we tested whether the correction was necessary. The risk of not standardizing the statistics is that they will be underestimating population structure. As the statistics reported in our reviewed studies range fairly evenly from quite low to very high, with approximately half of the studies showing high to very high population differentiation, we consider the risk of having underestimated population structure is low enough to justify proceeding with our analyses. The risk of underestimating G_{ST} is increased when k, the number of sampled populations, is small, and thus is greatest when examining pairwise G_{ST} **[51]**; **[49]**. We found an average increase in genetic distance and three cases where there was a sizable increase. These three studies, on *Mycosphaerella fijiensis*, *Puccinia striiformis*, and *Ophiocordyceps sinensis*, had reported high F_{ST} and high H_S.

We found that rooting an analysis could alter the conclusions of how a given trait affects population structure. For unconstrained analyses, which used only the published data, there were significant differences found between fungal classes and among orders in the Agaricomycetes, but that these differences disappeared when the analysis was constrained. This bias stems from the fact that most of the fungal studies were conducted at geographic distances large enough to uncover population differentiation and therefore were poorly represented at lesser distances. For groups that, at high

distances, had relatively high Pop-st and high variance, the regressions of unconstrained samples could show no isolation-by-distance or a negative slope. Rooting the samples was therefore an important step in correcting the sampling bias in this metadata analysis. Ideally, we would not have used only one statistic from each study, but could have included each pairwise population divergence estimate and compared the isolation-by-distance slopes of several species. By rooting the metadata sample, though, we were able to correct the most severe consequence of sample bias.

There were 4 outliers from the general regression for fungi. *Rhizopogon occidentalis* is a sticky-spored truffle found to have high population differentiation at intermediate distances, and showed high population structure in a Californian study that included populations on two different California islands, probably because of historic geographic barriers **[52]**. *Mycosphaerella fijiensis* is a pathogen of bananas and was introduced to Africa and Latin America in the 1970's, probably from Asia, and was found to have high F_{ST} between African and Latin American populations, probably due to bottleneck effects and low transmission rates relative to other crop pathogens **[53]**. *Heterobasidion abietini* a pathogen of *Abies* in Italy, the Pyrenees, central Europe, and the Balkan peninsula showed high population structure (ϕ_{ST} = 0.68), and it was suggested this was due to long-standing barriers to gene flow between these regions **[54]**. The insect pathogen *Cordyceps sinensis* showed high population structure across China,

possibly because of geographic barriers such as mountains, lakes, and rivers, and possibly also because of differences in host identity **[55]**.

We examined whether population structure varied with taxonomic identity, and found that the Ascomycota and Basidiomycota were indistinguishable, while the Oomycota had apparently lower gene flow among populations. The Oomycota were undersampled (n = 5), represented by only 4 *Phytophthora* and 1 *Pythium* species. Increasing the sampling of Oomycota would increase the variance and possibly erase the apparent difference in population structure between the Oomycota and the higher fungi. On the other hand, further studies in the Oomycota could give more support to the trend of lower population structure. The Oomycota have a different dispersal because of their motile spores, and rely on splash and soil dispersal, though some species, notably *P. ramorum*, produce airborne spores. Additionally, they can experience passive air dispersal, as the powdery mildew spores accumulate on host leaves, dry, and are borne away by the wind.

Because phylogenetic studies have found that oceans are a significant barrier to gene flow for several fungal species, we had hypothesized that fungal studies spanning an ocean would show limited gene flow compared to fungi in contiguous areas. The Pop-st across oceans was generally higher. Within a contiguous land area, population structure increases with geographic distance, but the regression for populations separated by oceans showed little isolation by distance. This can be explained by three

factors. First, studies spanning an ocean consequently cover a large geographic distance, and at these distances, Pop-st tends to be more variable. Secondly, several of the fungal species in question have populations on different continents thanks to recent, human-mediated introductions that have not had as much time to diverge as population found on the same continent by separated for longer periods. Third, populations of the same species separated by an ocean have generally high Pop-st, but the geographic distance of these two populations may not matter because any ocean itself is a sufficient barrier that 1) the size of the ocean and 2) additional distance by land does not increase the population differentiation.

Specialization on a host, for the general set of reviewed studies, was not found to significantly affect population structure, whether in the overall fungal sample or in the small sample for Agaricales and Hypocreales. In the Agaricales, the trend for specialists to show more population structure was very weak, because the variance for generalists at large distances was quite high. The Hypocreales sampling included plant and insect pathogens. When we restricted the sample to the Hypocreales plant pathogens, the specialists showed significantly more isolation-by-distance than the generalists (Figure 7). This result cannot be accepted with confidence because of the limited sampling. Generalists were represented by the cereals pathogens *Fusarium culmorum* and the grass pathogen *Epichloë festucae* and specialists were represented by *Atkinsonella hypoxylon* (on *Danthonia* grass), *Fusarium pseudograminearum* (on wheat), and *Gibberella circinata* (on

Pinus). Further research would be necessary to confirm the trend for specialists to have higher population structure than generalists, and the strength of the trend may vary according to other attributes of the fungal sample. It would be most interesting to look within single lineages containing generalists and moderately to highly specialized fungi. In our study, fungi specialized to a host genus were classified as specialists, but it could be possible that if the host genus is widespread and diverse, the fungus is not truly hampered by its host requirement because it would have a high chance of finding a host in a new area. It would therefore be ideal for fungal population genetics studies to include fungi associated with relatively rare substrates.

Of the life history traits we examined, none were found to be significant contributors to population structure when the geographic distance was considered. The non-significant trends did support most of our initial hypotheses to varying degrees. The difference between fungi with and without a known asexual stage in the life cycle was particularly weak. Ectomycorrhizal fungi and plant pathogens were well represented in the analysis of the influence of ecology, but saprobes were poorly represented, and insect pathogens had so little representation they could not be analysed. Further studies on saprobes and on insect pathogens would be most useful in exploring the trend for saprobes to have lower population structure than symbionts. Air dispersed fungi dominated the study. The fungi not producing air-dispersed spores were: the ectomycorrhizal truffle genus *Rhizopogon*, the aquatic saprobe *Tetracladium*, the

tree pathogens *Ceratocystis polonica*, *Septoria passerinii*, *Cenococcum geophilum*, *Phytophthora capsici* and the beetle bark symbiont *Ophiostoma claverigium*. Of these, the *Rhizopogon* populations appeared most limited in dispersal, showing population divergence at less than 100 km. *Rhizopogon* is a sticky-spored truffle dispersed by mammal mycophagists. The ectomycorrhizal fungus *Cenococcum geophilum* reproduces only by sclerotia that are water or animal-dispersed **[56]**, but population genetics evidence indicates recombination also occurs in this apparently asexual fungus **[57]**. The other fungi have varying modes of dispersal and retain the capacity for some long-range dispersal. *Tetracladium* could be dispersed over long distances by streams, and *Ophiostoma* is transported by a flying beetle. *Phytophthora*, a powdery mildew, may be passively dispersed by wind. More studies of fungi that do not produce air-dispersed spores are needed to test the effect of dispersal mode on population genetics. Particularly, it would be interesting to explore whether fungi dispersed by flying insects, such as the fly-dispersed Phallales stinkhorns and mutualists of leaf-cutter ants and bark beetles differ in population structure than 1) fungi that make air-dispersed spores and 2) the insect vectors. Ideal studies would examine population genetics over the same geographic barriers in order to determine not only general population structure, but whether certain geographic features restrict fungi with different dispersal methods.

Conclusion

The fungi vary widely in their phylogeographic history and patterns. Evidence from phylogenetic and population studies indicate that life history traits may play a role in fungal dispersal and phylogeography, but not at a statistically significant level. As more fungal population studies accumulate, it may be possible to better explore the role of multiple life history traits. As ocean barriers appeared to have the strongest influence on fungal population structure, we suggest it could be beneficial to explore other geographic features that may be barriers to fungal gene flow. Additionally, increasing studies within the same fungal family could allow a better comparison of life history traits. There are several life history traits that we did not examine in this analysis that could be potential influences on effective dispersal, such as spore pigmentation, obligate outcrossing, and seasonality. Ultimately, we have found that both the Bass-Becking and continental vicariance hypotheses fail to explain most fungal phylogeographic patterns, which show complex isolation-by-distance, continental lineages, and long-distance dispersal capabilities, and cannot be readily explained by either classic hypothesis. Currently, it also appears that knowing the general life history of a fungus will not allow a prediction of its population structure, particularly at large distances, where population statistics are highly variable.

Table 1. Fungal population summary statistics and life history traits. Taxonomy codes: Division: A — Ascomycota, B — Basidiomycota, H — Heterokontophyta; Class: Ag — Agaricomycetes, Do — Dothideomycetes, So — Sordiariomycetes, Le — Leotiomycetes, Ur — Uredineomycetes, En — Entomophthorales, Us — Ustilaginales, Eu — Eurotiomycetes, Oo — Oomycetes. Order: Ag — Agaricales, Bo —Boletales, Cn — Cantherellales, Cp — Capnodiales, Ce — Ceratobasidiales, Di — Diaporthales, Do — Dothideales, En — Entomophthorales, Er — Erisyphales, He — Helotiales, Hm — Hymenochaetales, Hp — Hypocreales, Op — Ophiostomatales, Pe — Peronosporales, Ph — Phyllachorales, Pl — Pleosporales, Po — Polyporales, Py — Pythiales, Ru — Russulales, Ur — Urediniales, Us — Ustilaginales, Xy — Xylariales. Ecology codes: M — mutualist, P — pathogen, S — saprobe, Specialist codes: G — generalist, S — specialist

species	Reference	#pop	marker	Hs	Ht	Stat.	Pop-st	Study Span (km)	Div.	Class	Order	Ocean?	dispersal	asexual stage	ecology	Specialist	*specialized to*	host category
Agaricus bisporus	**[58]**	4	RFLPs	-	-	Fst	0.315	9350	B	Ag	Ag	y	air	n	S (soil)	G	*G*	soil
Agaricus bisporus	**[59]**		RFLPs	0.485	0.561	Fst	0.016	12200	B	Ag	Ag	y	air	n	S (soil)	G	*G*	soil
Alternaria tenuissima	**[60]**	3	AFLP	0.15	0.16	Fst	0.283^{P}	6500	A	Do	Pl	n	air	y	P (plant)	G	*G*	crop
Armillaria ostoyae	**[61]**	9	microsat	0.566	-	Fst	0.12	160	B	Ag	Ag	n	air	n	P (plant)	S	*Pinus pinaster*	tree
Armillaria mellea **(west)**	**[62]**	4	microsat	0.47	-	θ	0.009^{P}	620	B	Ag	Ag	n	air	n	P (plant)	G	G	tree
Armillaria mellea (east)	**[62]**		microsat	0.1825	-	θ	-0.013^{P}	1320	B	Ag	Ag	n	air	n	P (plant)	G	G	tree
Atkinsonella hypoxylon	**[63]**	2	isozyme	0.103	0.262	Gst	0.299	940	A	So	Hy	n	air	y	P (plant)	S	*Danthonia*	grasses
Beauveria bassiana	**[64]**	5	minisat	-	-	θ	0.292	12400	A	So	Hy	y	air	y	P (insect)	G	G	insect
Blumeria graminis **f. sp. *tritici***	**[65]**	3	SNPs	-	-	ϕ-ct	0.442	10650	A	Le	Er	y	air	y	P (plant)	S	*Triticum aestivum*	crop
Cenococcum geophilum	**[57]**	2	SNPs	-	-	θ	0.2557	2000	A	Do	*?*	n	soil	y	M (ECM)	G	*G*	tree
Ceratocystis polonica	**[66]**	4	microsat	0.48	0.58	Gst	0.16	8840	B	Ag	Ces	y	insect	y	P (plant)	S	*Picea abies*	tree

species	Reference	#pop	marker	Hs	Ht	Stat.	Pop-st	Study Span (km)	Div.	Class	Order	Ocean?	dispersal	asexual stage	ecology	Specialist	specialized to	host category
Ceratocystis resinifera	**[67]**	9	RAPD	0.045	-	Fst	0.208	4440	B	Ag	Ce	n	air	y	S (wood) facultative P	G	*conifers*	tree
Cercospora sorghi	**[68]**	2	AFLP	0.335	-	ϕst	0.01	240	A	Do	Do	n	air	y	P (plant)	S	*Sorghum bicolor*	crop
Colletotrichum gloeosporioides	**[69]**	3	MP-CR	0.26	-	Gst	0.04	550	A	So	Ph	n	air	y	P (plant)	G	G	crop
Cordyceps sinensis	**[55]**	18	microsat	0.071	0.17	Gst	0.583	1140	A	So	Hy	n	air	y	P (insect)	S	Hepialidae	insect
Cronartium ribicola	**[70]**	9	RAPD	0.37	0.386	Fst	0.062	2200	B	Ur	Ur	n	air	y	P (plant)	S	*Pinus Ribes*	tree
Cryphonectria parasitica	**[71]**	15	VIA	0.217	0.234	Gst	0.35	1070	A	So	Di	n	air	y	P (plant)	S	*Castanea*	tree
Cryphonectria parasitica	**[72]**	17	RAPD	0.1073	0.1463	Gst	0.26	2090	A	So	Di	n	air	y	P (plant)	S	*Castanea*	tree
Cryphonectria parasitica	**[73]**	4	RFLPs	0.2713	0.42	Gst	0.11	13000	A	So	Di	y	air	y	P (plant)	S	*Castanea*	tree
Cryphonectria parasitica	**[74]**	4	microsat	0.3475	-	Gst	0.27	320	A	So	Di	n	air	y	P (plant)	S	*Castanea*	tree
Daldinia loculata	**[75]**	7	nuclear	0.37	-	Fst	0.07	7000	A	So	Xy	n	air	y	S (wood)	G	G	wood
Datronia caperata	**[76]**	5	AFLP, ITS	0.1947	-	ϕst	0.21	300	B	Ag	Po	n	air	n	S (wood)	S	*Laguncularia racemosa*	wood
Entomophthora muscae	**[77]**	5	microsat	-	0.388	θ	-0.0347	30	E	En	En	n	air	y	P (insect)	S	*Musca domestica*	insect
Epichloë festucae	**[78]**	2	AFLP	0.273	-	Fst	0.197	41	A	So	Hy	n	air	y	P (plant)	G	*Festuca Lolium Koeleria*	grasses
Epichloë festucae	**[79]**	12	microsat	-	-	Fst	0.0814	1180	A	So	Hy	n	air	y	P (plant)	G	*Festuca Lolium Koeleria*	grasses
Erisyphe necator	**[80]**	2	microsat	-	-	θ	0.15	960	A	Le	Er	n	air, water	y	P (plant)	S	*Vitus*	crop

species	Reference	#pop	marker	Hs	Ht	Stat.	Pop-st	Study Span (km)	Div.	Class	Order	Ocean?	dispersal	asexual stage	ecology	Specialist	*specialized to*	host category
Fomitopsis pinicola	**[81]**	3	RAPD	0.4133	-	Fst	0	1780	B	Ag	Po	n	air	n	S (wood)	G	G	wood
Fomitopsis rosea	**[82]**	5	nuclear	0.3	-	Fst	0.013	1200	Ba	Ag	Po	n	air	n	S (wood)	G	G	wood
Fusarium culmorum	**[83]**	7	RAPD	0.308	0.318	phi-st	0.038	80	A	So	Hy	n	air	y	P (plant)	G	G	crop
Fusarium pseudograminearum	**[84]**	2	AFLP	0.223	0.238	Gst	0.03	250	A	So	Hy	n	air	y	P (plant)	S	*Triticum aestivum*	crop
Fusarium pseudograminearum	**[85]**	8	AFLP	0.145	-	Gst	0.38[P]	3060	A	So	Hy	n	air	y	P (plant)	S	*Triticum aestivum*	crop
Gibberella circinata	**[86]**	6	microsat	0.204	0.21	Gst	0.042	134	A	So	Hy	n	air	y	P (plant)	S	*Pinus*	tree
Heterobasidion annosum	**[54]**	4	minisa	-	-	ϕ-st	0.68	1930	B	Ag	Ru	n	air	n	P (plant)	S	***Abies*** *Pinus Larix*	tree
Laccaria amethystina	**[87]**	3	DALP	-	-	Fst	0.02	450	B	Ag	Ag	n	air	n	M (ECM)	G	G	tree
Laccaria amethystina	**[88]**	3	RAPD	-	-	Fst	0.0212[P]	0.045	B	Ag	Ag	n	air	n	M (ECM)	G	G	tree
Leptosphaeria maculans	**[89]**	13	microsat	0.1904	0.474	Fst	0.187[P]	2950	A	Do	Pl	n	air, water	y	P (plant)	S	*Brassica napus. oleifera*	crop
Leptosphaeria maculans	**[90]**	3	AFLP	0.17	-	Fst	0.12	14000	A	Do	Pl	y	air	y	P (plant)	S	*Brassica spp*	crop
Leptosphaeria maculans	**[91]**	4	minisat	0.2	-	ϕ-st	0.001	310	A	Do	Pl	n	air	y	P (plant)	S	*Brassica spp*	crop
Lycoperdon pyriforme	**[92]**	7	isozyme	0.2524	0.2874	Gst	0.122	670	B	Ag	Ag	n	air	n	S (wood)	G	G	soil
Marasmius oreades	**[93]**	2	DAF	-	-	Fst	-0.0048	0.230489	B	Ag	Ag	n	air	n	P (grasses)	G	G	grasses
Melampsora larici-epitea	**[94]**	3	AFLP	-	-	ϕ-ct	0.172	500	B	Ur	Ur	y	air	y	P (plant)	S	*Salix Larix*	tree
Melampsora larici-populina	**[95]**	6	AFLP	0.2182	0.248	ϕ-st	0.1468	440	B	Ur	Ur	y	air	y	P (plant)	S	*Populus Larix*	tree
Metarhizium anisopliae var. anisopliae	**[96]**		microsat	0.37	-	Fst	0.1975	9300	A	So	Hy	y	air	y	P (insect)	G	G	insect

species	Reference	#pop	marker	Hs	Ht	Stat.	Pop-st	Study Span (km)	Div.	Class	Order	Ocean?	dispersal	asexual stage	ecology	Specialist	specialized to	host category
Microbotryum lychnidis-dioicae	**[97]**	15	microsat	0.73	-	Fis	0.38	3400	B	Us	Us	n	air	y	P (plant)	S	*Silene latifolia*	flower
Microbotryum silenes-dioicae	**[97]**	15	microsat	0.62	-	Fis	0.34	2250	B	Us	Us	n	air	y	P (plant)	S	*Silene dioica*	flower
Microbotryum violaceum	**[98]**	8	microsat	-	-	Fst	0.419	670	B	Us	Us	y	air	y	P (plant)	S	*Silene latifolia*	flower
Monilinia fructicola	**[99]**	4	microsat	0.3515	-	Fst	0.098[P]	10100	A	Le	He	y	air rain insects	y	P (plant)	S	*Prunus*	crop
Mycena rosea	**[100]**	8	isozyme	0.425	0.457	Gst	0.07	350	B	Ag	Ag	n	air	n	S (soil)	G	*G*	soil
Mycosphaerella fijiensis	**[53]**	10	RFLPs	0.31	-	Fst	0.58	8100	A	Do	Ca	y	air	y	P (plant)	S	*Musa (banana)*	crop
Mycosphaerella graminicola	**[101]**	6	AFLP	0.27	-	Fst	0.04	690	A	Do	Ca	n	air	y	P (plant)	S	*Triticum aestivum*	crop
Ophiocordyceps sinensis	**[102]**	10	ITS, Mat-1-2-1	0.66	0.78	Fst	0.24	1300	A	So	Hy	n	air	y	P (insect)	S	Hepialidae	insect
Ophiostoma clavigerum	**[103]**	7	AFLP	0.0531		phi-st	0.143	1400	A	So	Op	n	insect	y	P (plant)	S	*Pinus Dendroc-tonus*	tree
Phaeomoniella chlamydospora	**[104]**	4	21 AFLP	-	-	Fst	0.029	900	A	Eu	*?*	n	air	y	P (plant)	S	*Vitis*	crop
Phaeosphaeria nodorum	**[105]**	12	microsat mating type locus	0.305167	-	Fst	0.304	1500	A	Do	Pl	n	air water	y	P (plant)	S	*Triticum aestivum*	crop
Phellinus nigrolimitatus	**[106]**	5	RFLPs	0.348333	0.35	Fst	0.042*	830	B	Ag	Hys	n	air	n	S (wood)	G	*conifers*	tree
Phlebia centrifuga	**[107]**	8	microsat	0.26	-	Fst	0.151[P]	1400	B	Ag	Po	n	air	n	S (wood)	S	*Picea abies*	wood
Phytophthora capsici	**[108]**	4	microsat	-	-	Fst	0.344[P]	550	He	Oo	Pe	n	water	y	P (plant)	S	G	crop
Phytophthora capsici	**[109]**	7	AFLP	0.1689	-	ϕ-st	0.34	265	He	Oo	Pe	n	water	y	P (plant)	G	G	crop
Phytophthora	**[110]**	2	isozyme	0.0995	-	Fst	0.71	1100	He	Oo	Pe	n	soil	y	P (plant)	G	G	crop

species	Reference	#pop	marker	Hs	Ht	Stat.	Pop-st	Study Span (km)	Div.	Class	Order	Ocean?	dispersal	asexual stage	ecology	Specialist	specialized to	host category
cinnamomi																		
Phytophthora ramorum	**[111]**	34	microsat	0.4147	-	Φ-st	0.2908	440	He	Oo	Pe	n	air	y	P (plant)	G	G	tree
Pisolithus microcarpus	**[112]**	5	microsat	0.245	-	Fst	0.0606[P]	710	B	Ag	Sc	n	air	n	M (ECM)	S	*Eucalyptu. Acacia*	tree
Pleurotus eryngii	**[113]**	6	allozymes	0.212	0.233	Fst	0.2667	740	B	Ag	Ag	n	air	n	S (can be weak P)	S	*Eryngium campestre (mostly)*	tree
Pleurotus ferulae	**[113]**	13	allozymes	0.165	0.172	Fst	0.1761	750	B	Ag	Ag	n	air	n	P (plant)	S	*Ferula communis*	tree
Puccinia striiformis v. tritici	**[114]**	5	AFLP	0.414	-	Fst	0.004	50	B	Ur	Ur	n	air	y	P (plant)	S	*Triticum aestivum*	crop
Puccinia striiformis* v. *tritici	**[115]**	6	microsat	0.57	-	Fst	0.317	7700	B	Ur	Ur	y	air	y	P (plant)	S	*Triticum aestivum*	crop
Puccinia triticina	**[116]**	6	microsta	0.4137	-	Fst	0.136	9700	B	Ur	Ur	n	air	y	P (plant)	S	*Triticum spp.*	crop
Puccinia triticina	**[117]**	5	microsat	0.323	-	Rst	0[P]	3200	B	Ur	Ur	n	air	y	P (plant)	S	*Triticum spp.*	crop
Pyrenopeziza brassicae	**[118]**	8	AFLP	0.43	0.58	Fst	0.16	1200	A	Le	He	y	splash	y	P (plant)	S	*Brassica sp*	crop
Pyrenophora teres	**[119]**	2	AFLP	-	-	Fst	0.308	400	A	Do	Pl	n	air	y	P (plant)	S	*Hordeum vulgare*	crop
Pyrenophora teres* f. sp. *maculata	**[120]**.	2	microsat	0.39	0.41	θ	0.014	0.5	A	Do	Pl	n	air	y	P (plant)	S	*Hordeum vulgare*	crop
Pyrenophora teres* f. sp. *teres	**[120]**	3	microsatellites	0.37	0.38	θ	0.008	0.5	A	Do	Pl	n	air	y	P (plant)	S	*Hordeum vulgare*	crop
Pythium aphanidermatum	**[121]**	4	AFLP	-	-	Fst	0.118	150	H	Oo	Py	n	water, soil	y	P (plant)	G	G	crop
Rhizoctonia oryzae-sativae	**[122]**	3	microsat	0.56	-	Fst	0.08[P]	40	B	Ag	Ca	n	air, water	y	P (plant)	S	*Oryza sativa*	crop
Rhizoctonia solani	**[123]**	5	RFLPs	-	-	Fst	0.0741	290	B	Ag	Ca	n	air	y	P (plant)	G	G	crop

species	Reference	#pop	marker	Hs	Ht	Stat.	Pop-st	Study Span (km)	Div.	Class	Order	Ocean?	dispersal	asexual stage	ecology	Specialist	specialized to	host category
Rhizopogon occidentalis	**[52]**	4	microsat	0.1933	-	Fst	0.482[P]	40.5	B	Ag	Bo	y	mammal	n	M (ECM)	S	*Pinus*	tree
Rhizopogon vesiculosus	**[124]**	3	microsat	-	-	ϕ-st	0.062[P]	5.5	B	Ag	Bo	n	mammal	n	M (ECM)	S	*Pinus*	tree
Rhizopogon vinicolor	**[124]**	3	microsat	-	-	ϕ-st	-0.002[P]	5.5	B	Ag	Bo	n	mammal	n	M (ECM)	S	*Pinus*	tree
Rhizopogon vulgaris	**[52]**	3	microsat	0.4267	-	Fst	0.249[P]	18.5	Ba	Ag	Bo	y	mammal	n	M (ECM)	S	*Pinus*	tree
Rhynchosporium secalis	**[125]**	3	AFLP	0.66	0.67	Gst	0.05	150	A	So	Ph	n	air	y	P (plant)	S	*Hordeum vulgare*	crop
Russula brevipes	**[126]**	2	microsat	-	-	Fst	0.434	1500	B	Ag	Ru	n	air	n	M (ECM)	G	G	tree
Russula brevipes	**[127]**	3	microsat	0.4573	-	ϕ-st	0.01	1.09	B	Ag	Ru	n	air	n	M (ECM)	G	G	tree
Schizophyllum commune	**[26]**	11	allozymes	0.252	0.287	Gst	0.214	14000	B	Ag	Ag	y	air	n	S (wood)	G	G	wood
Sclerotinia sclerotiorum	**[128]**	6	microsat	0.5288	-	Rst	0.004	0.03	A	Le	He	n	air	n	P (plant)	G	G	crop
Septoria passerinii	**[129]**	9	AFLP	0.32	-	Gst	0.238	600	A	Do	Cas	n	water	y	P (plant)	S	*Hordeum vulgare*	crop
Suillus grevillei	**[130]**	5	microsat	0.3633	-	Fst	0.024	0.7	B	Ag	Bo	n	air	n	M (ECM)	S	*Pinus*	tree
Suillus luteus	**[131]**	9	AFLP	0.326	-	Fst	0.036	25	B	Ag	Bo	n	air	n	M (ECM)	S	*Pinus*	tree
Suillus luteus	**[132]**	9	microsat	0.801	-	Rst	0[P]	25	B	Ag	Bo	n	air	n	M (ECM)	S	*Pinus*	tree
Tetracladium marchalianum	**[133]**	4	microsat	-	-	Fst	0.267[P]	480	A	Do	Pl	n	water	y	S	G	*aquatic*	-
Thelephora ganbajun	**[134]**	9	ITS	0.5782	-	Fst	0.101	600	B	Ag	Th	n	air	n	M (ECM)	S	*Pinus spp esp. P. yunnanen-sis*	tree
Trichaptum abietinum	**[135]**	11	RFLPs	0.36	-	Fst	0.03	1290	B	Ag	Po	n	air	n	S (wood)	G	*conifers*	wood
Tricholoma matsutake	**[136]**	8	SNPs	0.42	-	Fst	0.112[P]	268.5	B	Ag	Ag	n	air	n	M (ECM)	S	*Pinus densiflora*	tree

species	Reference	#pop	marker	Hs	Ht	Stat.	Pop-st	Study Span (km)	Div.	Class	Order	Ocean?	dispersal	asexual stage	ecology	Specialist	specialized to	host category
Tricholoma matsutake	**[137]**	7	microsat	-	-	Fst	0.0132	0.5	B	Ag	A	n	air	n	M (ECM)	S	*Pinus densiflora*	tree
***Tricholoma scalpturatum* group I**	**[138]**	5	microsat	-	-	ϕ-St	0.21	2240	B	Ag	Ag	y	air	n	M (ECM)	G	G	tree
***Tricholoma scalpturatum* group II**	**[138]**	5	microsat	-	-	ϕ-St	0.28	2240	B	Ag	Ag	y	air	n	M (ECM)	G	G	tree
Tuber magnatum	**[139]**	26	microsat	-	-	Fst	0.15	820	A	Pe	Pe	n	air/ mam mal	y	M (ECM)	G	Fagaceae Betulaceae Salicaceae	tree
Tuber melanosporum	**[140]**	13	microsat AFLP, ITS	-	-	Fst	0.177	1260	A	Pe	Pe	n	air mam-mal	y	M (ECM)	G	G	tree
Tuber melanosporum	**[21]**	17	ITS	-	-	Fst	0.2	1020	A	Pe	Pe	n	air mam-mal	y	M (ECM)	G	G	tree
Typhula incarnata	**[141]**	4	RAPD	0.2725	-	Fst	0.1267	1040	B	Ag	Ag	n	air	y	P (plant)	G	G	grasses
Ustilago maydis	**[142]**	2	AFLP	0.2	0.27	Fst	0.25	9430	Ba	Us	Us	y	air	y	P (plant)	S	*Zea mays*	crop
Venturia inaequalis	**[143]**	4	ITS RAPD Btub	0.26	0.28	Gst	0.26	260	As	Do	Pl	n	air water	y	P (plant)	S	*Malus x domestica*	tree
Venturia tremulae	**[144]**	13	RAMS	0.6855	-	Fst	0.023	1140	A	Do	Pl	n	air	y	P (plant)	S	*Populus*	tree

Table 2. Metadata analysis of fungal population genetics studies using highly variable markers does not find significant effect of life history traits on population structure.

Trait	R^2	ANOVA	Study Span p-value	Trait p-value	Span * Trait p-value
Ocean Barrier	0.13992	0.0242*	0.756	0.2107	0.083
Air Dispersal	0.107985	0.0675	0.2729	0.6735	0.4273
Asexual Stage	0.169659	0.0105*	0.0018**	0.0428*	0.4904
Ecology	0.146893	0.0925	0.8962	0.4142	0.7043
ECM v. Pathogen	0.141349	0.035*	0.0045**	0.1958	0.6507

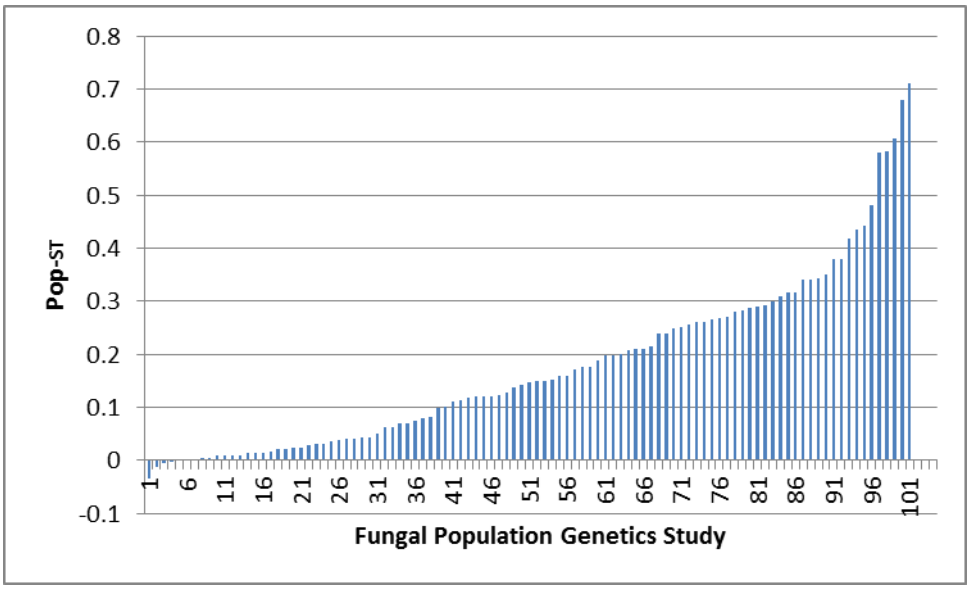

Figure 1. The reported pairwise or global Fst estimator for 102 fungal population studies indicates substantial variation in population structure for the fungi, ranging from no population separation to extremely divergent populations.

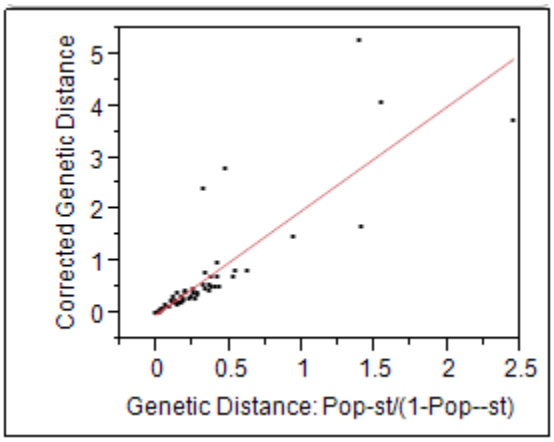

Figure 2. Corrected genetic distance based on Hedrick's G'st versus genetic distance shows that at low genetic distances, the effect of the standardization is minimal, while at higher distances (>0.5) there is extreme variation.

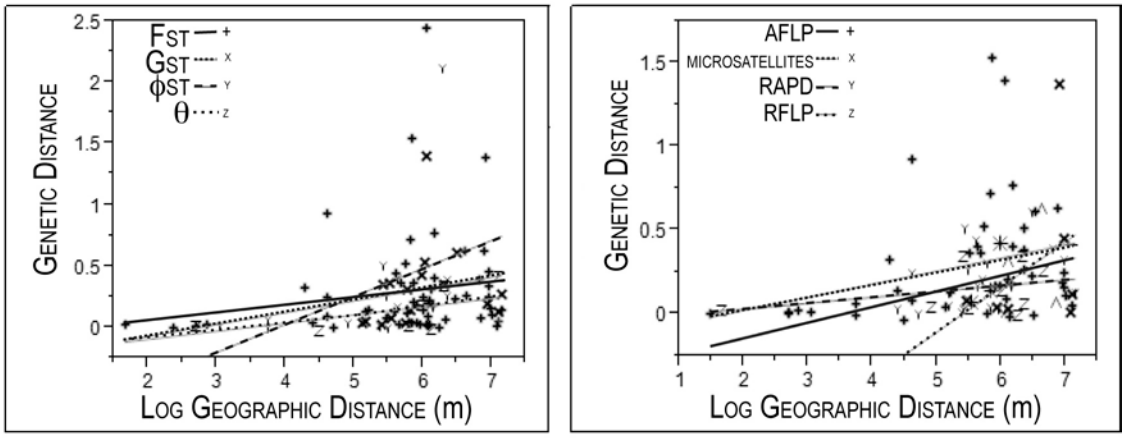

Figure 3. The summary statistic and genetic marker used do not significantly change the regression of genetic distance to geographic distance.

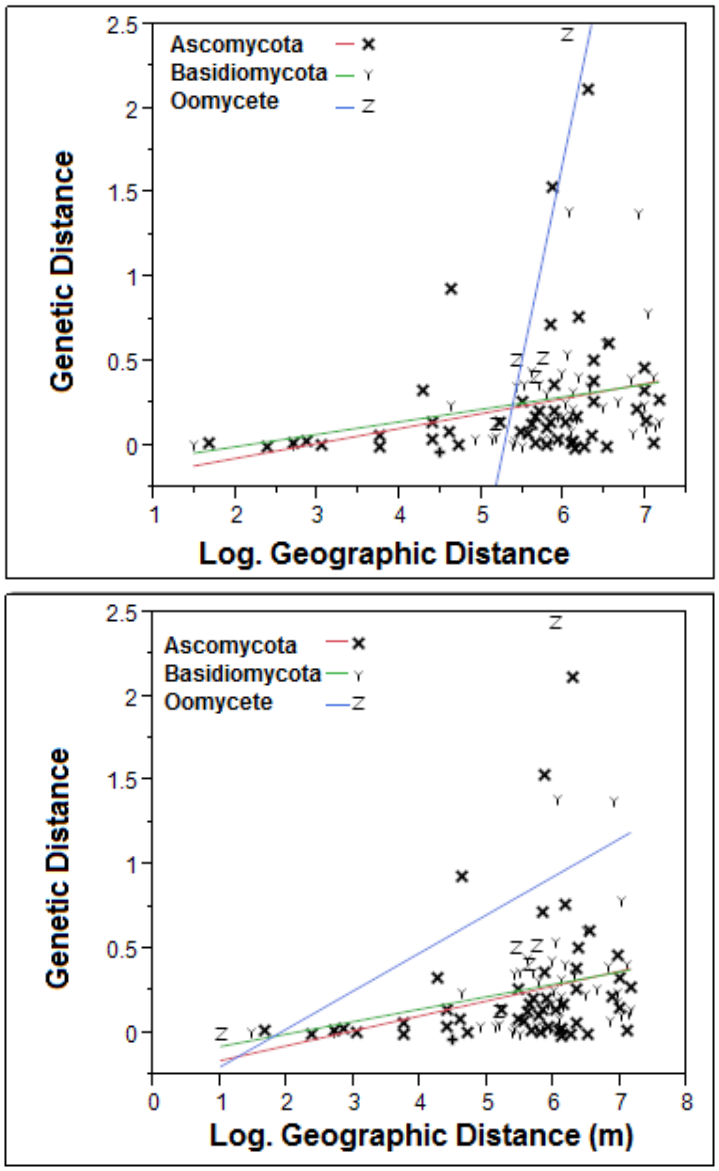

Figure 4. Taxonomic identity appears to affect genetic distance, with the Oomycetes having higher genetic distance over geographic distance than the Ascomycota and Basidiomycota. Top: Unconstrained analysis of all studies. Bottom: Analysis has been constrained by adding an Oomycete at 0 m.

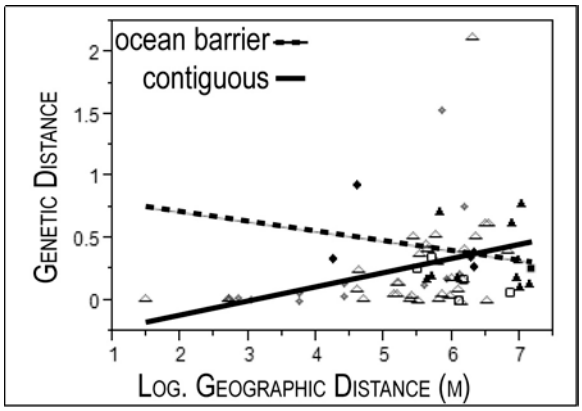

Figure 5. Studies in a contiguous landmass show a positive relationship between geographic and genetic distance, while studies spanning an ocean show a slightly negative relationship. Filled symbols: study spans ocean; open symbols: study in contiguous land mass. Triangles: plant pathogens; diamonds: ectomycorrhizae; squares: saprobes.

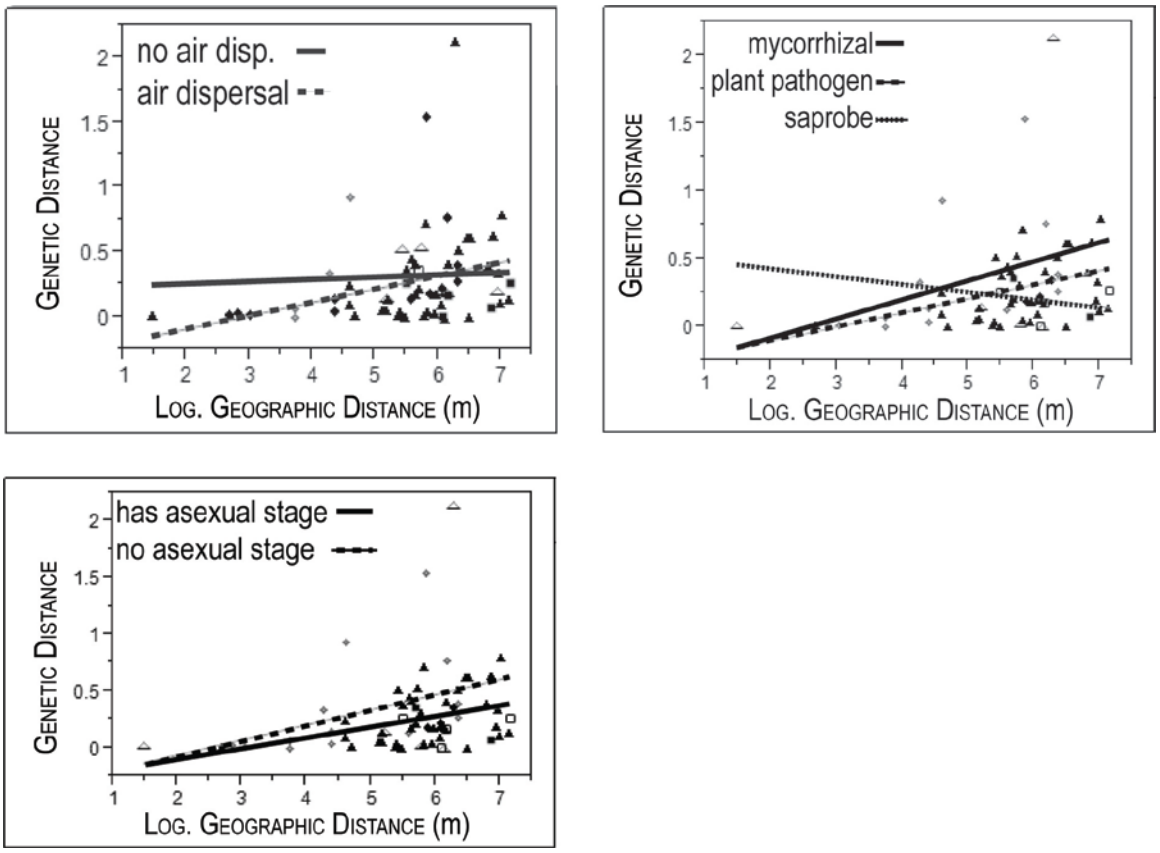

Figure 6. Life history traits show non-significant trends affecting genetic distance. A. Genetic distance increases with geographic distance for air-dispersed fungi, while for fungi lacking air dispersal, genetic distance is high at low distances, with no major increase at greater distances. B. For symbiotic fungi, genetic distance increases with geographic distance, while there is a negative trend for saprobes. C. Fungi lacking a known asexual stage have a slightly steeper increase in genetic distance over geographic distance than fungi known to have an asexual stage in their life cycle.

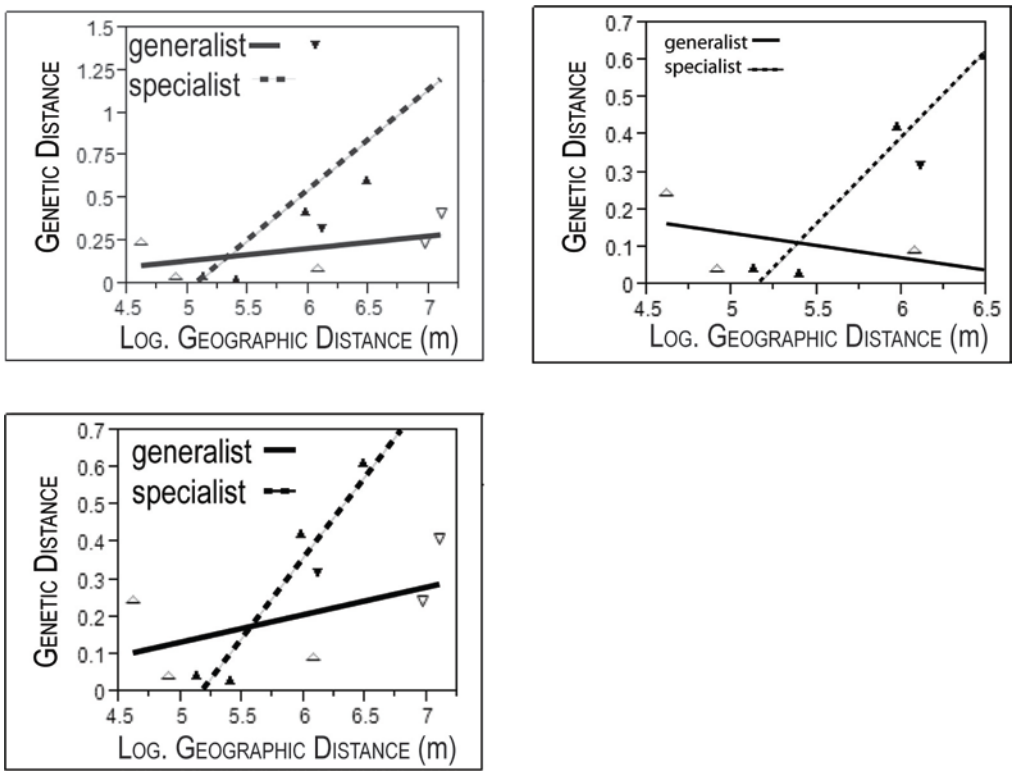

Figure 7. Hypocreales pathogens that are specialized to their hosts show sharper increases in genetic distance with geographic distance than do generalists. All plant and insect pathogens included (n = 11). B. Plant pathogens only (n = 7). C. Exclude outlier (F_{st} > 0.5) (n = 10). Upward-pointing triangles: plant pathogens; down-pointing triangles: insect pathogens; filled: specialist; open: generalist.

Chapter 2. Phylogeography of the stag truffle family Elaphomycetaceae

2.1 Introduction

The Ascomycete stag truffle family Elaphomycetaceae is found worldwide and associates with a variety of ectomycorrhizal hosts, consisting of the truffle genus *Elaphomyces* and a stalked genus *Pseudotulostoma*. *Elaphomyces* has a long taxonomic history, having been described in 1591 as *Tubera cervina* **[145]** and in 1650 as *Cerviboletus* **[146]**. Linnaeus renamed this species *Lycoperdon cervinum **[147]***. The name of the genus derives from folk legends that the fungus would be found wherever the deer had "exercised their desire," though early mycologists pointed out that it had been found in high places were deer were not known to travel **[146]**.

Dodge included two truffle genera in the Elaphomycetaceae: *Elaphomyces* with a cottony core and *Mesophellia*, with a corky or woody core **[148]**. *Mesophellia*, a Basidiomycete, was moved to the Lycoperdales **[149, 150]**. The family name was first proposed as the Mesophelliaceae by Zeller **[151]**, and published officially under that name by Jülich **[152]**. Molecular analysis found that the Mesophelliaceae was polyphyletic, *Mesophellia* was placed in the Hysterangiales as the sister family to the Hysterangiaceae **[153]**.

Elaphomyces remained the sole genus in the Elaphomycetaceae until the discovery of *Pseudotulostoma volvata* in Guyana **[154]** . Another species of this stalked genus, *P. japonica*, was found in Japan. In its early stage, *Pseudotulostoma* resembles a

truffle, and then extends a fibrous, spore-topped stalk **[154]**. The identification of this genus was difficult; the asci can be observed only in the immature, subterranean form, which meant that collectors finding the mature form had frequently thought it to be a basidiomycetous stalked puffball such as *Tulostoma* or *Battaria*.

Dodge had described the Elaphomycetaceae as part of the Plectascales, which included the Trichocomaceae and Onygenaceae **[148]**. Eriksson grouped the families Monascaceae, Trichocomaceae, and Elaphomycetaceae in the Eurotiales with the Onygenales as a separate order **[155]**. Molecular phylogenetics studies using SSU rDNA confirmed Dodge's placement of *Elaphomyces* in the Eurotiales **[156, 157]**, and the relationship between the Eurotiales and Onygenales **[158]**. Eriksson grouped the Onygenalean taxa in the Eurotiales as the suborder Onygenineae, while the Trichocomaceae and Elaphomycetaceae formed the Eurotiineae **[159]**. The Onygenales were once again separated from the Eurotiales by Geiser **[158]**. In the multi-gene analysis by Geiser *et al*., the Elaphomycetaceae were represented by *Pseudotulostoma volvata*, which was most closely related to *Trichocoma paradoxa **[158]***. The Eurotiales have globose asci and multiple fruiting body forms that include highly reduced, stromatic, and cleistothecial forms, of which the *Elaphomyces* are the largest. The Trichocomaceae are named for the shaving-brush fungus *Trichocoma*, a wood rotter that forms a loose, brush-like stroma. Other important members of the Trichocomaceae, *Aspergillus*, *Penicillium*, and *Paecilomyces*, are ubiquitous saprobes and facultative pathogens best

known for their imperfect states that produce phialospores **[160]**. The Monascaceae, containing the genus *Monascus* and *Xerocomus*, has been supported as a monophyletic group in the Eurotiales based on LSU **[161]** and ITS sequences **[162]**. A review of cleistothecial fungi also listed the Thermoascaceae as a member of the Eurotiales **[163]**. The Thermoascaceae was originally erected by Apinis to include *Thermophilus* and *Dactylomyces crustaceus* **[164]**. The placement of the genus *Thermophilus* has alternated between the Onygenaceae **[165, 166]** and the Thermoascaceae **[167]**. Most recently, studies of SSU have shown that *Thermoascus* is a member of the Trichocomaceae, most closely related to *Byssochlamys* and *Talaromycs* species **[168-170]**, but as these studies included only one *Thermoascus* species, it is possible that the Thermoascaceae could in fact be a separate clade.

Dodge enumerated 24 *Elaphomyces* species and suggested multiple sub-generic divisions. He divided the genus into two subgenera: Malacoderma, with a soft, wrinkled, or fleshy cortex and small spores (<15μ), and Scleroderma, with larger spores (15-50 μ) and a hard cortex that could be variously ornamented. He placed 5 species in the Malacoderma, and did not group them further: *Elaphomyces immutabilis*, *E. mutabilis*, *E. papillatus*, and *E. atropurpureus*. At that time, these species had been reported only from Italy. The subgenus Scleroderma contained the majority of the species he described, and was divided into two sections based on cortex ornamentation: Ceratogaster (smooth or nearly smooth) and Ceraunion (warted to spiny). The

Ceratogaster section consisted of *E. leucosporus*, *E. maculatus*, *E. leveillei*, *E. anthracinus*, and *E. septatus* and could be distinguished by their differing sizes, coloration of the cortex, crusts, peridium, spore size, and spore ornamentation. Of these species, only *E. leucosporus*, *E. maculatus* and *E. septatus* had been found solely in Europe. *Elaphomyces anthracinus* was also known in North America, and *E. leveillei* had been reported in Australia. The Ceraunion section was split into two subsections, Phylctospora and Hypogeum, based on the presence or absence of a sterile base, respectively. Phlyctospora contained only three species: *E. cyanosporus*, *E. persooni*, and *E. foetidus*, which were known only from Italy. Hypogeum contained several black or brown spiny and warted species. The following species had been reported from both Europe and North America: *E. variegatus*, *E. muricatus*, *E. decipiens*, *E. reticulatus*, *E. cervinus*, and *E. asperulus*, while others were known either only from Europe (*E. virgatosporus*, *E. morettii*, *E. aculeatus*) or from North America (*E. verrucosus*). In the case of *E. aculeatus*, Dodge had examined possible specimens from California, but as they were immature, he could not readily identify them. The general picture of *Elaphomyces* diversity and geographic range was one dominated by European collections, but with some widespread taxa and some apparently endemic to North America.

As collection of *Elaphomyces* expanded, the understanding of the range of the genus and of several species increased. The current known distribution of the range encompasses the Northern Hemisphere, and in the Southern Hemisphere, South

America and much of Australasia. Both of Dodge's subgenera are represented throughout the genus range, as are Section Ceratogaster and Subsection Hypogeum (Section Ceraunion), while Subsection Phlyctospora has been discovered only in Europe (Table 3). Phlyctospora was defined as having a sterile, rooting base, but other morphological and molecular characters may give rise to a broader definition of the group. New species of *Elaphomyces* have been described from North America, Asia, Australia, and New Zealand, and new, undescribed species have been collected from Costa Rica (R. Halling, pers. comm.) and Guyana (T. Henkel, pers. comm.). An ectomycorrhizal study in Malaysia reported a sequence from the Elaphomycetaceae, but a fruiting body was not found **[171]**.

Assigning the recently described species to traditional morphological clades has been difficult in some cases. Of the Australian species, most were clearly allied with species that had already been described, but others had characters conflicting with easy categorization. The subgenus Malacoderma is defined by small spores (< 15 μ) and a soft cortex that wrinkles on drying. Four *Elaphomyces* species from Australia have small spores but a smooth, carbonaceous cortex (*E. chlorocarpus*, *E. cooloolanus*, *E. pedicellaris*, and *E. rugosisporus*) **[172]**. The section Phlyctospora is defined by larger spores and a smooth cortex. The same combination of smooth cortex and small spores is seen in the North American *E. viridiseptum*. An evident problem with these categories is that a limited number of characters have been used to divide the *Elaphomyces*. It is possible

that the smooth, small-spored species listed above are most closely related to each other and form a separate group, or they could be members of a group that should be more broadly defined.

We addressed three major questions in the Elaphomycetaceae using molecular phylogenetics methods. First, how are *Elaphomyces* and *Pseudotulostoma* related? Initial studies placing *Pseudotulostoma* as the sister species had sampled only a small number of *Elaphomyces* species. We hypothesized that *Pseudotulostoma* would be supported as the sister genus, but also considered that the formation of the fruiting body from a truffle-like form and its lack of a spore-shooting mechanism could indicate an origin from one of the *Elaphomyces* subgenera. The small spore size (7 —9.5) of *P. volvata* and soft surface of the volva could place it in Malacoderma, while the rooting base could indicate a relationship with subsection Phlyctospora. Second, are Dodge's morphological groups supported? Third, what is the phylogeographic history of the Elaphomycetaceae and of the subgenera in *Elaphomyces*? We hypothesized that the primarily animal-dispersed truffles would show a history of dispersal limitation. While the morphological clades were widely distributed, we anticipated that, if supported, they would each show strong geographic structure. On the other hand, *Elaphomyces* and *Pseudotulostoma* have powdery spores that may have potential for passive air dispersal. Though *Elaphomyces* truffles depend on animal excavation, their spores may escape into the wind. If so, the Elaphomycetaceae could have gained its current distribution by long-distance dispersal.

In order to test whether the Elaphomycetaceae have experienced a history of dispersal limitation, we needed to date divergence times at key nodes in their phylogeny.

Dating divergence times is challenging in the fungi due to a general lack of fossils. A 400 million year-old *Paleopyrenomycetes* fossil was used in previous studies to calibrate the timing of major evolutionary developments in the fungi **[173, 174]**. Using this fossil to estimate the age of the Ascomycota/Basidiomycota split, the age of the Pezizomycotina, which contains the Eurotiales, was estimated to be 215 million years old **[173]**. The estimated date of any lineage depends on its calibration, and is particularly problematic when molecular rates calibrated using dates from distantly related clades are applied across a tree. Using multiple calibration points can help test the robustness of an estimated date, and it is preferable to use fossil ages that are relevant to the clade in question.

As we lack fossils of the Elaphomycetaceae that could be used to calibrate their molecular rate of evolution, we searched for phylogenetically and ecologically relevant calibration points to constrain the age of the family. We chose to accept Berbee and Taylor's decision to designate the 400 million year-old *Paleopyrenomycetes* fossil as a date for the Ascomycota, and used their estimated age of the Pezizomycotina (215 mya) to constrain the maximum age of the Elaphomycetaceae. The other two calibrations were chosen for their relevance to the ecology of this group. The *Elaphomyces* truffles are excavated by mammals, and their radiations may have coincided. The mammals

diversified in the Jurassic, with placental mammals and marsiupials diverging at approximately 160 mya **[175]**. The genus *Pseudotulostoma* is not spread by mammals, however, and it is possible that the ancestor of modern *Elaphomyces* were excavated by reptiles or birds; we cannot rely on a calibration based on mammalian evolution alone. The third calibration point is derived from a parasite that attacks multiple *Elaphomyces* species, *Elaphocordyceps*. The divergence date for *Elaphocordyceps* has been estimated to be 70 mya based on a 99—105 million year-old fossil resembling *Ophiocordyceps* discovered on an insect in amber **[176]**. A separate dating study using the SSU nucleotide substitution rate from **[177]** had found the switch from a cicada larva host to *Elaphomyces* to be later, 43±13 mya **[178]**. According to these multiple, ecologically relevant estimated dates, the Elaphomycetaceae likely originated at some point between 160 and 40 mya, with 220 million years as the maximum. While this calibration range is too large to be helpful in understanding the timing of deep nodes in the Elaphomycetaceae, it could help us estimate dates of recent divergences of geographically separate taxa.

2.2. Materials and Methods

2.1.2 Extraction and PCR Conditions

We extracted genomic DNA from spores, glebal tissue, and tissue from the inner peridium, taking approximately 100 to 300 μL of dry tissue. Silica beads and sterile sand were added to the dry tissue and shaken with a BeadBeater to disrupt cells. We then

added 500 μL of 2x CTAB to the samples, placed them in a water bath at 65 C for one hour, and then allowed them to sit at room temperature overnight. We then proceeded with a basic chloroform:IA extraction, using 3 washes of 24:1 chloroform:isoamyl alcohol before precipitating with isopropanol. Pellets of DNA were washed in 80% EtOH 3 times, dried, and re-suspended in 1 M TE buffer.

We used 3 ribosomal genes for the family-scale phylogeny: n-SSU, n-LSU, and mt-SSU. The primers used for PCR were: NS1/NS4 for n-SSU, LROR/LR5 for n-LSU, mt-SSU for MS1/MS2. PCR cocktail for each 25 μL reaction was: 12 μL ddH_2O, 4 μL 1M dNTPs, 2.5 μL 10X PCR Buffer with MgCl, 2.5 μL BSA, 1.25 μL each of the forward and reverse primer, and 0.5 μL of Apex Taq polymerase. We added 1, 0.5 or 0.1 μL of genomic DNA, depending on the concentration. Thermocycler conditions were as follows: 10 minutes at 94°C followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 47°C, and 1 minute 45 seconds at 72°C, and a final extension time of 5 minutes at 72°C. Amplified PCR products were prepared for sequencing using the Exo-AP kits from Qiagen. Sequences were prepared using 3.5 μL ddH_2O, 1 μL sequence buffer, 0.35 μL 10 μM forward primer, and 0.5 μL Big Dye, plus 5 μL of cleaned PCR product.

2.2.2 Specimens used for sequencing

We obtained sequences for the following specimens: **A. Montecchi 2** — *E. muricatus* Spain 10-15-2004 under *Quercus serris* — nSSU, mtSSU, **AHF 146** — *E. verrucosus* Arizona 8-21-1981 — nSSU, **Bingoletto** — *E. persoonii* Ticino Canton,

Switzerland under *Castanea* — mtSSU, **Bolognini** — *E. mutabilis* Gargallo, Spain under conifers — nSSU, mtSSU, **CDC0579** — *E. crabtreeii* Castellano *nom. prov*. Missouri 8-2-07 under *Carya*, *Quercus velutina* — nSSU, nLSU, mtSSU, **H4031** — *E. suejoyaceae* Coll. M. Trappe and N. Malajczuk Davies rd. #2, Queensland, Australia 5-3-1988 under *Eucalyptus* — nSSU, nLSU, **H4091** *E. aurantius* — Coll. N. Malajczuk, M. Castellano, and M. Amaranthus, Mothar Mtn., Queensland, Australia 5-9-1988 under *Lophastemon conferatus*, *Eucalyptus grandis*, *Allocasuarina torulosa*, *Syncarpia* — nSSU, mtSSU, **H4139** *E. cooloolanus* Coll. M. Castellano, Cooloola, Queensland, Australia 5-10-1988 — nSSU, **H5045** *E. aurantius* Coll. M. Castellano, Cradle Mtn., Waldheim, Tasmania 5-13-1991 under *Eucalyptus* and *Nothofagus cunninghamii* — nSSU, **H5539** *E. youngii* Castellano *nom. prov*. Coll. N. Malajczuk and R. Young, Mt. Kaindi, Papua New Guinea 2-11-1992 under *Nothofagus* — nSSU, mtSSU, **HHB 584** *E. sp*. Coll. H. H. Burdsall, Jr., Peninsula Pack Forest, Warren, New York 5-17-1968 under *Pinus resinosa* and *P. strobus* — nLSU, **HR-1009-1** *E. sp*. Coll. M. Smith, Eno State Park, North Carolina 10-6-09 — nLSU, **Kers 6255** *E. aculeatus* Sweden 10-18-1981 — nSSU, nLSU, **KH-PNG-04061** *E. sp. nov*. Coll. K. Hosaka, Papua New Guinea 4-9-2004 under *Castanopsis* — nSSU, mtSSU, **Loeb 4** *E. crescens* Castellano *nom. prov*. North Carolina — nLSU, **Mel 2063443** *E. pedicellaris* TYPE Coll. T. Lebel, Royal Botanic Gardens, Gippsland Plain, Victoria, Australia 8-11-2000 — mtSSU, **Misarisini** *E. cyanosporus* Tirino Canton, Switzerland, September — nSSU, mtSSU, **OSC118789** *E. sp*. Oregon — nLSU, **OSC81094** *E. verruculosus* Castellano *nom*.

prov. Coll. S. L. Stevenson Canaan Heights, Tucker Co., West Virginia 7-20-2001 under *Picea abies* — nLSU, **PDD 56293** *E. aureocrusta* Castellano *nom. prov*. Coll. R. E. Beever Taipo, Kaimanawa, North Island, New Zealand 5-7-1987 — nSSU, mtSSU, **RH 12** *E. hypospinus* Castellano *nom. prov*. Coll. R. Healy, Hickory Grove State Park, Story Co., Iowa, 8-7-1996 under *Prunus serotinus* and *Quercus macrocarpa* — nSSU, mtSSU, **RH 357** *E. variegatus* Coll. R. Healy, Waubonsie State Park, Fremont Co., Iowa under *Ostrya virginiana* — nSSU, **RH 369** *E. variegatus* Coll. R. Healy, Mines of Spain State Park, Dubuque Co., Iowa 6-13-1999 — nSSU, **RH 417** *E. sp*. Coll. R. Healy, Ledges State Park, Boone Co., Iowa, 6-29-1999 — mtSSU, **RH 298, ISC#435953** *E. sp*. coll. R. Healy Hickory Grove Park, Story Co., Iowa, 10-13-1998 — nLSU, **Simpson 1888** *E.mutabilis* Papua New Guinea 12-10-1978 under *Castanopsis acuminatissima* — nSSU, mtSSU, **SLM 692** *E. foetidus* Virginia 10-16-1983 under *Tsuga*, *Pinus strobus* — nSSU, nLSU, **SLM 799** *E. millerii* Castellano *nom. prov*. Virginia 10-14-1984 under *Fagus grandifolia*, *Quercus* — nSSU, mtSSU, **TH7878 (Henkel 7878)** *P. volvata* Coll. T. Henkel, Upper Potoro River, Pakaraima Mtns., Guyana 7-24-2000 under *Dicymbe corymbosa* — nSSU, nLSU, TH8880 *E. sp*. nov. Coll. T. Henkel, Guyana 8-25-2007 under *Dicymbe corymbosa* — nLSU, mtSSU, **TH9039** *P. volvata* Coll. T. Henkel, Region 8 Potaro-Siparuni, Pakaraima Mtns., Upper Potoro River Basin, Guyana 7-12-09 under *Dicymbe corymbosa* — nLSU, **TNS-F-22944** *P. japonica* Coll. F. Daijo, Japan 9-26-1997 under *Quercus crispula* — nSSU, nLSU, **TNS-F-22945** *P. japonica* Coll. F. Daijo, Japan 10-14-2003 under *Quercus crispula* — nSSU, nLSU, **TNS-F-2799** *E. sp*.

Coll. H. Kobaya and H. Neda, Kiyosumi, Chiba, Japan March 1979— nLSU, **Trappe 12300** *E. verrucosus* Castellano *nom. prov.* coll. D. Luoma and M. Castellano, Corvallis, Oregon 12-3-1991 under *Quercus garrayana* — nSSU, mtSSU, **Trappe 12500** *E. cibularii* Castellano *nom. prov.* Coll. D. Mitchell, Hoosier National Forest, Indiana 19-12-1992 under *Quercus alba*, *Q. prunus* — nSSU, nLSU, **Trappe 13300** *E. sp.* nov. Coll. D. Wheeler, Multnomah Co. near Corbett, Oregon 12-4-1993 under *Tsuga* — nSSU, **Trappe 13452** *E.* laetiluteus Coll. M. Castellano and D. Arora, Atherton Tablelands, Queensland, Australia 3-5-1994 — nLSU, **Trappe 13457** *E. queenslandicus* Coll. M. Castellano and D. Arora, Atherton Tablelands, Queensland, Australia 3-6-1994 — nSSU, nLSU, mtSSU, **Trappe 13589** *E. fallax* Castellano *nom. prov.* Coll. M. Castellano, P. Perret, J. Shubzda, G. Semlak, J. Roberts, milepost 383.5 on Blue Ridge Parkway, Buncombe Co., North Carolina 9-4-1994 under *Quercus spp.*— nSSU, nLSU, mtSSU, **Trappe 14919** *E. chlorocarpus* Coll. M. Castellano and J. Trappe, Walpole-Nornalup NP, Perth, Australia 7-22-1993 under *Casuarina* and *Eucalyptus ficifolia* — nSSU, nLSU, **Trappe 15300** *E. rugosisporus* Coll. M. Castellano and J. Trappe, Atherton Tablelands, Queensland, Australia 3-9-1995 — nSSU, nLSU, mtSSU, **Trappe 17510** *E. singaporensis* Coll. M. Castellano, Bukit Timah Nature Reserve, Singapore 9-13-1995 — nSSU, nLSU, mtSSU, **Trappe 17537** *E. kunmingensis* Castellano *nom. prov.* Coll. M. Castellano, Kunming, Yunnan, China under *Keteleeria evelyneaena* 9-21-1995 — nSSU, mtSSU, **Trappe 17811** *E. viridiseptum* Coll. A. J. Cash near Pensacola, Florida 9-30-1970 — nSSU, nLSU, **Trappe**

19323 *E. appalachiensis* Coll. D. Mitchell, Round Knob, Pendleton Co., West Virginia 9-31-1996 under *Pinus virginiana* — nSSU, nLSU, mtSSU, **Trappe 20145** *E. suejoyceae* Coll. E. Cázares, 0.3 km off Omeo Highway (East Side), 2.1 km S of Ash Range Road, East Gippsland, Victoria, Australia 11-11-1996 under *Acacia dealbata*, *Eucalyptus cypellocarpa*, *Eucalyptus muellerana* — mtSSU, **Trappe 20705** *E. sp*. Coll. A. Jumpponen, M. Castellano, Colville National Forest, Pend Orielle Co., Washington 6-25-1997 under *Larix occidentalis* — mtSSU, **Trappe 22547** *E. coralloides* Coll. K. Vernes, Davies Creek, Queensland, Australia 4-19-1996 under *Eucalyptus spp.*— nSSU, nLSU, **Trappe 25762** *E. sp*. Coll. M. Castellano, Deschutes National Forest, Jefferson Co., OR 5-8-2000 — nLSU, mtSSU, **Trappe 25889** *E. sp*. Coll. A. Beverle, Hwy 216 at Forest Service RD. 2110, Waseo Co., Oregon 4-26-2000 under *Abies grandis* — nSSU, mtSSU, **Trappe 26550** *E. sp*. Coll. A. Giacchini, Mt. Hood National Forest, Clackamas Co., Oregon 8-28-2001 under *Pseudotsuga menziesii* — mtSSU, **Trappe 27905** *E. sp*. Coll. M. Castellano, 500 m South of Interamerican Hwy on Road to San Gerardo, Mile post 80 Km, Dota Co., San Jose Province, Costa Rica 11-6-2002 under *Quercus costaricensis*, *Comaro staphylis arbutoides*, *Vaccinium consanguineum* — nSSU, nLSU, mtSSU, **Trappe 27916** *E. hallingii* Castellano *nom. prov*. Coll. M. Castellano, Tapanti National Park, El Guarco Co., Cartago Province, Costa Rica 11-12-2002 under *Quercus* — nSSU, mtSSU, **Trappe 27918** *E. sp*. Coll. M. Castellano, Tapanti National Park, El Guarco Co., Cartago Province, Costa Rica 11-12-2002 under *Quercus* — nSSU, nLSU, **Trappe 28202** *E. scabrellus* Castellano *nom. prov*. Coll.

D. Mitchell, Greenland Gap, Grant Co. West Virginia 3-9-2003 under *Quercus prinus*, *Quercus coccinea* — nLSU, **Trappe 30762** *E. sp*. Coll. M. Dehne, M. Hinds, Crater Lake National Park, Klamath Co., Oregon 6-29-2005 — mtSSU, **Trappe 31432** *E. vernesii* Castellano *nom. prov*. New Brunswick — nSSU, nLSU, mtSSU, **Trappe 31755** *E. brunneohypha* Castellano *nom. prov*. Coll. M. Castellano, Windsor, Alachua Co., Florida 5-3-2006 under *Quercus virginiana* — nSSU, nLSU, mtSSU, **Trappe 32045a** *E. cristosporus* Castellano *nom. prov*. Florida — nSSU, **Trappe 32046** *E. fallax* North Carolina — nSSU, **Trappe 32060** *E. viridiseptum* Florida — nSSU, **Trappe 32069** *E. sp*. Oregon — mtSSU, **Trappe 32257** *E. dunlapii* Castellano *nom. prov*. South Carolina — nSSU, mtSSU, **Trappe 32498** *E. muricatus* Mexico — nSSU, **Trappe 32580** *E. sp*. Mexico — nLSU, **Trappe 4988** *E. anthracinus* Coll. St Remy, Provence, France 1-10-1972 under *Quercus*, *Ilex* — nLSU, **Trappe 6988** *E. bollardii* Castellano *nom. prov*. Coll. R. Beever and J. Trappe, Auckland, Waitakere, North Island, New Zealand 9-29-1982 under *Leptospermum ericoides* — nSSU, nLSU, **Trappe 8648** *E. amicus* Castellano *nom. prov*. Coll. S. Miller Lake Noonan, Alachua, Florida 8-10-1985 under *Quercus virginiana* — nSSU, **Trappe 8650** *E. hypospinus* Castellano *nom. prov*. Coll. S. Miller and M. Castellano, Lake Noonan, Alachua, Florida 8-11-1985 under *Quercus virginiana* — nSSU, **Trappe 8926a** *E. macrosporus* Castellano *nom. prov*. Coll. M. Castellano University of Massachusetts, Hampshire Co., Massachusetts 8-10-1986 under *Tsuga canadensis*, *Betula populifolia* and *Quercus* — mtSSU,

Vidal *E. citrinus* Spain — nLSU, mtSSU, **Zoltan** *E. virgatosporus* Hungary — nLSU, mtSSU.

2.2.3 Tree building methods and dating analyses

Sequences were aligned initially using MUSCLE **[179]** and checked visually in MacClade **[180]** to improve alignment. Ambiguously aligned regions were excluded from phylogenetic analysis. To maximize nucleotide overlap, alignments were pruned of taxa with short sequences. Outgroups included several taxa chosen from the Trichocomaceae and Onygenales: *Hamigera avellanea*, *Monascus purpureus*, *Chrysosporium georgiae*, *Eupenicillium javanicum*, *Penicillium freii*, *Aspergillus sclerotiorum* and *Trichocoma paradoxa*. For the SSU set, there were 38 ingroup taxa, for the LSU set there were 39 ingroup taxa, and for the mtSSU there were 39 ingroup taxa. In the combined analysis that included full overlap of all three genes, there were 11 ingroup taxa.

We used maximum likelihood, Bayesian, and maximum parsimony to generate phylogenies for subsets of our sequence data that included single genes (SSU, LSU, mt-SSU) and all three genes. Maximum parsimony analysis was done using all characters as unweighted in PAUP* **[181]** using the heuristic search algorithm starting with a random tree and MulTrees set to "Off." For the heuristic search, 1000 replications were done. To determine node support, 1000 bootstrap replications were done using a heuristic search with 10 replicates each. Maximum likelihood and Bayesian analysis were run on the Cipres server **[182]**. Maximum likelihood analysis was performed using Garli **[183]**. For

each analysis, we used the model chosen by Modeltest 2 under the Akaike criterion **[184]**, used 2 independent search replicates for maximum likelihood trees, and tested node support using 1000 bootstrap replicates. Bayesian analysis was performed using MrBayes **[185, 186]**. Initial analyses were set at 10 million generations, sampling every 1000 generation. The analysis was stopped when the standard deviation of split frequencies was less than 0.01. The burnin was set to be at least one quarter of the sampled trees, and the convergence on likelihood was checked to ensure that sufficient burnin time was used. In multi-gene analyses, the data were partitioned according to gene identity.

Biogeographic analysis was performed using RASP **[187]** to reconstruct ancestral areas for the LSU dataset. This dataset was used because it had indicated the highest levels of support near the tips of clades of taxa from different continents. We reduced the numbers of outgroup taxa to *Aspergillus sclerotiorum* and used only one representative of each Elaphomycetaceae species in the LSU dataset. This dataset was used as the input for MrBayes using 2,000,000 generations and sampling every 1000 generations. The consensus tree was used as the input for a RASP Bayesian MCMC run using 1,000,000 generations. We coded the geographic area of each taxon based on the continent area: (A=Asian including Malaysia, Japan, and Singapore, B=North/Central American, C=Europe, D=Australia/Tasmania/Papua New Guinea/New Zealand, E=South America). Several *Elaphomyces* species are thought to have Holarctic

distributions, and it is possible that our North American species are also represented more broadly. RASP operates on the basis of known species distributions, and we wanted to consider the possibility that North American taxa could in fact represent Holarctic species. Furthermore, we were interested in how the Australasian taxa might exhibit long-distance dispersal. To this end, we performed a second analysis using different codes that grouped the Northern Hemisphere as one and subdivided Australasia: (A=Holarctic, B=Australia, C=South America, D=New Zealand, E=Singapore). The outgroup species, *Aspergillus sclerotiorum*, was given a global distribution set as ABCDE. The maximum ancestral areas allowed was set to 5.

Dating analysis was conducted in BEAST version 1.6.1 **[188]**. Beauti, part of the BEAST software package, was used to prepare files for analysis. We used the SSU dataset in the dating analysis because it had the higher support at the backbone of the tree and because there have been published estimated rates of evolution for this marker. ModelTest 2.3 **[184]** selected a preferred model of SYM+I+Γ with I=0.8723 and Γ alpha parameter = 0.5623. This model is not available in BEAST, so we used the simpler model of HKY+ Γ+I. Attempts to use the more complex GTR model caused run failure. We conducted two separate analyses, both of which used a relaxed lognormal clock model and the HKY+ Γ+I substitution model. The first used the estimated rate of evolution of the SSU for fungi: The second used a broader prior for the molecular rate and calibrated the root of the tree. The root calibration used fossil-based information relevant to the

ecology of the Elaphomycetaceae. We used a normal distribution with mean of 130 mya and standard deviation of 45 mya, allowing a range of 40-220 mya in the 95% distribution of the prior. The molecular rate prior, based on the estimated rate of fungal SSU evolution (1-1.25% per 100 my) **[177]** was a lognormal distribution with a mean of 1.5E-4 substitutions/million years and standard deviation of 0.5. For each of the two analyses, we performed 2 independent runs in BEAST on the Cipres server of 100 million generations to produce 20,000 trees. Runs were checked for convergence in Tracer and combined in LogCombiner with a burnin of 2000. Nodes with over 50% Bayesian posterior probabilities were annotated with the estimated ranges of divergence times in TreeAnnotator. FigTree was used to examine the final, combined trees.

2.3 Results

We were able to obtain 48 SSU sequences, 37 LSU sequences, and 37 mtSSU sequences for the Elaphomycetaceae. For each of the single-gene datasets, Modeltest 2.2 selected the GTR+I+Γ model using the Akaike criterion. The SSU alignment, after excluding ambiguously aligned regions, had 384 characters, 340 of which were constant and 23 of which were parsimony-informative. The consensus tree combined 3517 equally parsimonious trees with a length of 76 (CI=0.629). The best maximum likelihood tree (lnL=-990.22486) is presented in (Figure 8) with support values from 1000 MP and ML bootstrap repetitions and Bayesian posterior probability. The LSU alignment had 291 included characters, 54 of which were parsimony-informative. The length of the 201

equally parsimonious was 203 (CI=0.478, RI=0.760), and the best maximum likelihood tree (Figure 9) had a likelihood score of (lnL = -1561.750934).

The mtSSU dataset had 421 characters after excluding ambiguous regions, 299 of which were constant, and 72 of which were parsimony-informative. The best tree under equally-weighted parsimony was hit 126 times and had a score of 223 (CI=0.673, RI=0.833). The best maximum likelihood tree (Figure 10) had a likelihood score of (lnL = -1684.717937). The *P. volvata* mtSSU sequence had only 285 bp, making it considerably shorter than the rest of the mtSSU alignment. Including this taxon would include missing data that would risk lowering the resolution and support of the tree, but we wanted to assess the placement of *P. volvata* according to each of the sequenced genes because of its taxonomic importance. We therefore performed two analyses on this dataset. The first, described above, excluded *P. volvata* to maximize the number of homologous nucleotides fully represented in the data matrix. The second analysis (Figure 11) included *P. volvata* and limited the outgroup sampling to *Aspergillus sclerotiorum* to improve the alignment within the Elaphomycetaceae. This second alignment had 38 taxa and 431 included characters, 365 of which were constant and 42 of which were parsimony-informative. ModelTest selected the GTR+I+Γ model under the Akaike criterion. Two tree islands of the shortest length (106 characters) were hit, totaling 392 equally parsimonious trees, and the maximum likelihood score was (lnL = -1174.338953).

The combined 3-gene dataset included only 11 ingroup taxa. We partitioned the data according to gene and used ModelTest 2.2 to determine the appropriate model for each partition: SYM+I for SSU and GTR+I+Γ for LSU and mtSSU. The likelihood score of the best ML tree (Figure 12) was (lnL = -3103.126965).

In each of the ribosomal genes used, the Elaphomycetaceae was indicated as a monophyletic group with varying support. The SSU tree split the Elaphomycetaceae into two major clades, which we term A and B. The A clade was only moderately supported by Bayesian posterior probability and not supported by ML or MP. This clade could be subdivided into the well-supported A1 clade, which included brown, ornamented taxa with large spores and the Australian species *E. suejoyceae*. Clade A2 was not supported, but included black, ornamented species, the brown, small-spored, ornamented species *E. aculeatus*, a smooth, black species *E. hypospinus* nom.prov. The B clade, consisting of most of the Australian species, *Pseudotulostoma*, the species originally belonging to Malacoderma and Phlyctospora, and *E. singaporensis* was strongly supported from Bayesian, ML, and MP analyses.

The LSU analysis also uncovered 2 major clades, but *E. singaporensis*, which had been placed in clade B in the SSU analysis, was included in a small, well-supported clade at the base of clade A. This clade included *E. citrinus*, *E. singaporensis*, *E. crescens nom. prov.* and an undescribed species from Mexico. Clade B again included

Pseudotulostoma, representatives of Phylctospora and Malacoderma, and a clade of brown and black ornamented species.

The mtSSU phylogeny differed in the placement of clades A1 and A2. The first phylogeny, using the full set of outgroups, placed A1 and B together, though with no support. The Phlyctospora species *E. persooni*, *E. cyanosporus*, and *E. fallax* formed a clade that was moderately supported with ML and strongly supported with Bayesian analysis. The analysis with only *Aspergillus sclerotiorum* as outgroup included *P. volvata* with the Phlyctospora species with moderate support. This second analysis could not resolve the placement of several clades within the Elaphomycetaceae. There was strong support for clade A1 with ML, MP, and Bayesian analysis, and strong ML and Bayesian support for clade A2. These formed an unsupported clade with *E. singaporensis*, *E. queenslandicus*, and the new Guyanan species. Other species found in clade B in the SSU analysis were split into two groups, the majority forming clade B, which received moderate ML support and high Bayesian support, and four species forming the aforementioned Phlyctospora/*Pseudotulostoma* group (Clade C).

In each of the individual gene analyses and in the combine analysis, *Pseudotulostoma* was shown to be a terminal group in a clade of *Elaphomyces* rather than being supported as a separate genus. In the SSU topology, *Pseudotulostoma* was found to be most closely related to *E. fallax*, *E. cyanosporus*, *E. queenslandicus*, and an unidentified black, carbonaceous species from Guyana. In the LSU dataset, the topology indicated the

polyphyly of *Pseudotulostoma*, but with no support. *Elaphomyces* species closely related to *Pseudotulostoma*, in addition to those indicated by the SSU tree, were a clade of unidentified species from Japan and eastern North America. The mtSSU tree that included *P. volvata* showed strong support for its relation to *E. persooni*, *E. cyanosporus*, and *E. fallax*, and did not resolve the placement of this clade. The 3-gene topology grouped *P. volvata* with *E. fallax* and *E. queenslandicus* with strong Bayesian support and moderate ML support.

The concatenated dataset of SSU, LSU, and mtSSU included 11 Elaphomycetaceae taxa. The monophyly of the Elaphomyceataceae was supported with moderate ML and Bayesian support. The A clade was supported only moderately by the ML and Bayesian analysis, but both the A1 and A2 clades were strongly supported by all three analysis methods. The B clade, which included *P. volvata*, was strongly supported. *Pseudotulostoma* formed a clade with *E. fallax*, which has a sterile base and *E. queenslandicus*, which does not.

The biogeographic pattern indicated by tree topology indicated broadly-dispersed clades consisting of a mix of Northern and Southern Hemisphere taxa. Clades A1 and B included Australian and Northern Hemisphere taxa for every gene sampled. Clade A2, according to the nSSU sample, included the Australian species *E. coralloides*, but in the nLSU sample, this species was included in the A2 group, and was not sequenced for the mtSSU. In neither case was the placement of *E. coralloides* supported,

however. In clades A1 and A2, the Southern Hemisphere taxa were found at the tips of the tree, while in clade B there was a mix of Australian species with close relatives in Europe and North America, and a large clade of Australian and New Zealand species. South America was represented by Guyanan fungi: *Pseudotulostoma volvata* and a new *Elaphomyces* species. In the mtSSU analyses, the Guyanan *Elaphomyces* species was well supported as sister to *E. singaporensis* from Singapore, but the placement of this clade was not resolved, and this relationship was not found in the LSU analysis.

In the first RASP analysis, which coded North American, Asian, and European taxa separately, the Bayesian constraint analysis on the LSU dataset calculated that the most likely ancestor of clade A was North American, with 86.1% probability (Figure 13). The ancestral distribution of clade B could not be determined. Clade B was divided into two groups, one with a likely New World ancestor with South America at 58.68% probability, and the other consisting of North American and Australian taxa. Taxon sampling was dominated by North American species, which would bias ancestral state reconstruction and increase the chances of finding a North American ancestor.

For the second RASP analysis, we coded all Holarctic species as the same geographic area. This analysis found the *Elaphomyces* ancestor to have a Holarctic distribution with 92.97% probability. Clades A1 and A2 were estimated to have a Holarctic distribution with over 99% probability, and clade B had a 92.97% probability of a Holarctic ancestral distribution.

Node age estimations using the SSU phylogeny in BEAST found similar dates using the estimated SSU rate alone (Figure 15) and using the rate with a large prior (135±45 mya) on the root of the Elaphomycetaceae (Figure 16). The estimated age of clade A ranged from 185 to 40 million years, with an average of 97 million years, while that of clade B ranged from 145 to 30 million years with an average of 73 million years. Clade A1 was estimated as the youngest clade, with an average estimated age of 34 million years (high 70, low 15 my). Clade A2 was estimated to be 62 million years (high 120, low 25 my). In the SSU tree, there were 9 separate nodes of both Southern and Northern Hemisphere taxa. Of these 9 nodes, 6 were estimated to be very recent, with an average age of 3-6 million years and not more than 20 million years. *Pseudotulostoma volvata* from Guyana was, in this analysis, sister to the Japanese species *P. japonica*, and the two species were estimated to have diverged 11 million years ago (high 30, low 1 my). *Elaphomyces coralloides* from Australia was estimated to have diverged from its North American relatives 17 million years ago (high 40, low 2 million years). *Elaphomyces aureocrusta* from New Zealand and *E. cibularii* from Indiana had an average estimated age of 15 million years, but as this node was unsupported, BEAST could not estimate a divergence time range. Finally, an unsupported clade containing 6 species from Australia, Tasmania, and New Zealand was estimated to have an average age of 40 million years. Thus, the connections between the Southern and Northern Hemispheres have been quite recent, ranging from 40 million years at the most distant, to less than 5

million years at the most recent. Combined with the RASP analyses, the estimations in BEAST indicate there were at least 8 instances of long-distance dispersal between the Northern Hemisphere to the Southern Hemisphere in *Elaphomyces*.

2.4 Discussion

2.4.2 Reassessment of Morphological Divisions in Elaphomyces

The subgenus Malacoderma, indicated by small spores and a soft cortex that wrinkles on drying , was indicated as an unsupported clade within Clade B in the LSU analysis, and in the 3-gene analysis but not in the mtSSU or SSU analyses, in which appeared to be polyphyletic. Multiple small-spored, smooth species that do not noticeably wrinkle were shown to be related to Malacoderma species in the SSU analysis: *E. aurantius*, *E. cooloolanus*, and *E. chlorocarpus*. The LSU dataset included *E. chlorocarpus* and the mtSSU dataset included *E. aurantius*; in both analyses, these species were closely related to Malacoderma taxa. In the SSU tree, the smooth, large-spored species *E. singaporensis* was included in the Malacoderma representatives in clade B, but not in the LSU and mtSSU set, where it was found to be in a small clade with clade A as a sister group.

The subgenus Scleroderma, consisting of all harder species of *Elaphomyces*, can be clearly rejected. Subsection Phlyctospora, having a sterile, rooting base, is in clade B, closely related to *Pseudotulostoma*. In the mtSSU analysis, Phlyctospora and *Pseudotulostoma* form a clade, but in the other analyses, this group is not supported. The

3-gene analysis includes the black, warty species *E. queenslandicus* in the clade of Phlyctospora and *P. volvata* with high Bayesian support and moderate ML support. Subsection Ceratogaster, the smooth, large-spored group, is polyphyletic, with members in clades A1, A2, and B. The polyphyly of Ceratogaster is found in each of the single-gene analyses and in the 3-gene analysis. Representatives of subsection Hypogeum are similarly polyphyletic.

2.4.2 Pseudotulostoma nested within *Elaphomyces*

Each of the three markers used in this study placed the stalked genus *Pseudotulostoma* in a terminal clade of the truffle genus *Elaphomyces*. The combined dataset showed high support for the placement of *Pseudotulostoma* in the *Elaphomyces*. The placement of *Pseudotulostoma* in a terminal clade of *Elaphomyces* indicates that this epigeous fungus evolved from a truffle ancestor. Truffles have evolved independently multiple times in several different fungal families, and in most cases, the systematics is not sufficiently supported to clearly delineate relationships with epigeous relatives. However, the unsupported topologies indicate that while most truffles have evolved from epigeous lineages, there may have been reversals to an epigeous state. In the Basidiomycota, there is a strongly-supported instance of a stalked fungus arising from a truffle ancestor: the Phallales stinkhorns **[153]**. This order of fungi is a terminal clade in the gomphoid-phalloid and is most closely related to the truffle families Claustulaceae

and Trappeaceae. Ancestral character state reconstruction found strong support for a truffle-to-stalked transition for the Phallales.

In the Ascomycota, truffles also occur in the Discinaceae (*Gyromitra*), Helvellaceae (*Balsamia* and *Barssia*), Morchellaceae (*Fischerula*, *Imaia*, *Kalapuya*,and *Leucangium*), Pezizaceae (*Amylascus*, *Calongea*, *Cazia*, *Eremiomyces*, *Hydnootryposis*, *Kalaharituber*, *Mattirolomyces*, *Pachyphloeus*, *Peziza ellipsospora*, *Peziza infossa*, *Peziza whitei*, *Ruhlandiella*, *Terfezia*, and *Tirmania*), Pyronemataceae (*Genabea*, *Genea*, *Geopora cooperi*, *Gilkeya*, and *Otidea subterraneus*), and Tuberaceae (*Choiromyces*, *Dingleya*, *Labyrinthylomyces*, *Reddellomyces*, and *Tuber*). The Tuberaceae consist entirely of truffle genera, and the truffle genera in the Morchellaceae form one truffle lineage. In the other families, truffles have evolved multiple, independent times. This is particularly pronounced in the Pezizaceae, which has 16 independent truffle lineages described. The Elaphomycetaceae represent the first family in the Ascomycota to show a well-supported topology for an epigeous clade derived from a hypogeous lineage. The transition from the stalked to truffle state had been thought to be irreversible because of the loss of active spore propulsion mechanisms associated with this state. Both the Phallales and *Pseudotulostoma* lack active spore shooting; the Phallales rely on fly dispersal, and *Pseudotulostoma* produces an airborne stroma filled with spores that may be passively wind-dispersed or dispersed by rain. Other possible cases of epigeous taxa derived from truffles are not as clear, and morphology indicates a more likely scenario

of multiple epigeous-to-truffle transitions. Another epigeous taxon that may have arisen from a truffle lineage is the cup fungus *Humaria* in the Pyronemataceae **[189]**. *Humaria* was found to be sister to the truffle genus *Genea*, with the truffles *Gilkeya* and *Genabea* closely related to this clade. The authors suggested that *Humaria* could have evolved from a truffle ancestor, but because the deeper nodes were not well-supported, the results were not conclusive. While the topology was not supported and is therefore not conclusive, this could represent either: 1) a transition from epigeous to truffle state, followed by diversification of the clade and a reversal to the epigeous state for *Humaria* or 2) independent transitions for each truffle genus, with three epigeous-to-hypogeous transitions. While the three truffle genera in question have lost spore-shooting and ellipsoid spores, the apothecial *Humaria* has operculate asci. We therefore think it unlikely that *Humaria* regained the epigeous state along with spore-shooting asci.

In the Basidiomycota, there are other instances of a complex picture of truffles and epigeous relatives. For instance, in the Boletaceae there are multiple origins of truffle genera, including *Chamonixia*, which was found to be polyphyletic in a group of *Leccinum* species **[190]**. While the tree topology could indicate either multiple origins of truffles or multiple reversions to the stalked form, it is most likely, based on the hypothesis that the loss of ballistospory is irreversible, that *Chamonixia* arose multiple times. Further research in the close relationships of truffles and their epigeous relatives that includes broad taxon sampling in each lineage and multiple genetic markers is

needed to estimate the frequency of the transitions between epigeous and hypogeous forms and the morphological changes accompanying these transitions.

2.4.3 Biogeographic Pattern of Frequent Long-Distance Dispersal

The Elaphomycetaceae display a biogeographic pattern of broadly dispersed lineages and recent endemism. Southern Hemisphere taxa were in many cases nested within clades found in the Northern Hemisphere. Dating analyses using the SSU phylogeny estimated that connections between Northern and Southern Hemisphere lineages occurred as recently as 2 million years ago, indicating that frequent long-distance dispersal events have shaped the biogeographic history of this subterranean fungus.

Both BEAST analysis suggested an origin for the Elaphomycetaceae in the early Cretaceous (~135 million years), though with a large 95% confidence interval spanning 243-60 mya. Clades A and B were estimated to have a late Cretaceous diversification at 97 my and 73 mya, respectively. During the late Cretaceous, North America was separated from Gondwana, but exchange of some taxa could have been possible through island-hopping **[191]**. The breakup of Gondwana was complete by the end of the late Cretaceous period. The Cretaceous origin of the Elaphomycetaceae suggests it may have at first been widespread due to vicariance. The radiation of clades B and A2 (73 and 62 my) are in accordance with the radiation of mammals, particularly the rodents, which have been estimated to have diversified at approximately 70-77 mya **[192]**. Clades A1

and A2 diversified at 34 and 62 million years. At this point, the continents were fully separated; the distributions of these clades cannot be explained through continental vicariance.

In the case of the Elaphomycetaceae, ancestral character state reconstruction was either ambiguous or indicated a Holarctic ancestor for the family. In both analyses, North America was indicated as an ancestral area for multiple clades, but this may have been caused by a preponderance of North American species being included. The majority of described Australian species were included in the analysis, but we were unable to obtain sequences for many of the known Asian species. Additionally, many areas of ecological interest are under-sampled for subterranean fungi, particularly in the tropics. Future collections from Southeast Asia should aid in attempting to understand the connection between Australian and Northern Hemisphere taxa. Many of the Australian species with close relatives in the Northern Hemisphere may be found to have links in Southeast Asia. A survey of ectomycorrhizal fungi in Malaysia uncovered an *Elaphomyces* species that was most closely related to the Australian species *E. laetiluteus* (Fig 2-1).

When combined with the dating analysis showing that most of the clades spanning both Northern and Southern Hemispheres are only a few million years old, the indication of a Holarctic ancestor suggests that recent geological events have had

profound effects on the phylogeographic history of this group. In Guyana, *Pseudotulostoma volvata* was found to be an average estimate of 11 million years old (high 30, low 2 my). The Great American Interchange between North and South America occurred when the Panama isthmus formed during the Pliocene approximately 3.1 million years ago, allowing the incursion of numerous North American mammals into South America **[193]**. The interchange between the two continents occurred more recently than the average estimated date of *P. volvata*'s divergence from *P. japonica*, but is within the 95% confidence interval for the *Pseudotulostoma* clade. *Pseudotulostoma* is unknown from North America, but it is possible that North America held either an extinct ancestor or an extant, but undiscovered species. *Pseudotulostoma* was only recently discovered and identified as an ascomycete **[154, 194]**, and more species throughout the world may be uncovered from other locations.

The majority of independently derived *Elaphomyces* from Papua New Guinea and Australia were separated from Northern Hemisphere taxa by only 3-40 million years. The most recently derived taxa could have migrated from Southeast Asia into Australia and Papua New Guinea after the Australian plate collided with Asia 15 million years ago **[195]**. This migration, unlike the Great American Interchange, could not have been vectored by mammals; Wallace's Line marks a historic biogeographic division between Southeast Asia and Australian species for numerous animal species, including birds **[196]**. While Wallace's Line is a significant feature in animal biogeography, plants have

clearly been able to migrate between the islands in the south Pacific **[197]**. In our sampling, the Northern Hemisphere species related to the Australasian taxa are from North America; however, we think it likely that a thorough survey of tropical Asian and Australasian species would find closer relations, indicating migrational tracks. *Elaphomyces* lineages thus are more similar to plants than to animals in their ability to traverse Wallace's Line, a further indication that *Elaphomyces* dispersal does not depend entirely on the animals that excavate and consume it. As for the older clade, its placement in clade B is not well supported. The clade could have had a longer term presence in Australia if it in fact branched early from clade B rather than being derived.

2.4.4 Re-examining biogeographic hypotheses for truffles

The indication of frequent long-distance dispersal contradicted our initial hypothesis that the truffle family Elaphomycetaceae would show signs of dispersal limitation due to its reliance on animal dispersal. Rather, it shows a pattern similar to that found in many air-dispersed fungi (Chapter 1). It is possible that, in addition to mammalian dispersal, *Elaphomyces* may also be air dispersed; its powdery, hydrophilic spores could become airborne after being maturation. Long-distance dispersal has been shown to have occurred in other truffles, so it is possible that air-borne dispersal is common in powdery-spored truffles.

In the Hysterangiales, another powdery-spored truffle, while much of the phylogeny was concordant with Gondwanan vicariance events, a biogeographic history

based strictly on vicariance could be rejected **[19]**. Ancestral character state reconstruction of species area in the Hysterangiales indicated that the order evolved in the Southern Hemisphere, then spread at least twice to the Northern Hemisphere. Generally, the phylogeny of this highly diverse order indicated strong geographic structure; three of four major clades were restricted to the Northern Hemisphere, while the fourth contained a mix of Northern and Southern Hemisphere species. At least six long-distance dispersal events were indicated between New Zealand and Australia, indicating that even these apparently mammal-dispersed fungi can experience transoceanic dispersal.

The truffle genus *Tuber* is widespread through the Northern Hemisphere. Bonito *et al* conducted a broad sampling of *Tuber* ITS sequences from Asia, Europe, and North America found that each species was local to one continent, save for those species thought to have been introduced to other countries not in their native range (e.g. New Zealand) **[198]**. Clades of this diverse group were distributed throughout the Northern Hemisphere, which is unsurprising given recent connections between continents in the Pleistocene. An intensive survey of Japanese species by Kinoshita *et al*. uncovered multiple species that were highly distinct from European, North American and continental Asian taxa, as well as species very closely related (ITS similarity > 98%) to species from China and Taiwan **[199]**. While the biogeographic history of *Tuber* in Japan could not be conclusively determined, the authors suggested that the mix of species

highly endemic to Japan along with broadspread Asian taxa could have been produced by two factors 1) the effect of geographic isolation on Japanese species and 2) the higher migratory ability for select taxa. The higher migratory ability of the broadly distributed species was further indicated by their broad distribution within Japan. While *Tuber* is known in the literature only from the Northern Hemisphere, there have been reports of the *T. puberulum* group from Argentina (G. Bonito, pers. comm.). Overall, *Tuber* exhibits a geographic pattern marked by the dispersal limitation we hypothesized would influence truffles.

Studies in truffle biogeography are challenging primarily because the subterranean fungi are difficult to find. We relied heavily on herbarium materials, which in many cases did not yield sequences for any of the genes surveyed. Developing markers is also a challenge, as the obligate symbionts may be inculturable. However, the importance of truffles in the forest ecosystems as mycorrhizal symbionts and as a food source for many animals, and the varied methods of dispersal make them an attractive target for phylogeography and biodiversity. The Elaphomycetaceae, a group with powdery spores, may be able disperse by air, permitting more frequent long-distance dispersal. This additional dispersal mode could explain the biogeographic history of this family, which is marked by long-distance dispersal events as well as geographic isolation. Other truffles with sticky spores may either be truly restricted or may have developed alternate dispersal strategies. For instance, the genus *Tuber*, spread

apparently only by mammals in its sexual stage, also produces an asexual stroma that may permit either splash or air-dispersal (Healy, pers. comm.). More ecological research in hypogeous fungi may discover a suite of dispersal modes employed by different clades, yielding a spectrum of dispersal abilities. Phylogeographic studies in other hypogeous lineages may find the highly structured pattern of endemism marked by continental vicariance we had hypothesized for the Elaphomycetaceae. Future studies in the phylogeography of truffle species will be necessary to continue revising hypotheses on the impact of ecological constraints on fungal dispersal and evolution.

Table 3. Distributions of morphological clades indicate global distribution for every clade but subsection Phlyctospora. Abbreviations: Location (As = Asia, Au = Australia, E = Europe, NA = North America, T = Tasmania); Cortex Texture (O = Ornamented, S = Smooth, W = Wrinkled); Morphological Clade (* we assigned species to morphological clade; 1 = Subgenus Malacoderma (Vitt.) Dodge, 2 = Section Ceratogaster (Corda) Dodge, 3 = Hypogeum (Persoon) Dodge, 4 = Subsection Phlyctospora (Zobel) Dodge)

Elaphomyces species **(Author)**	**Location**	**Cortex Texture (W,S,O)**	**Sporocarp Color**	**Sterile Base**	**Carbonaceus Peridium**	**Spore Size (μm)**	**Morph. clade**
appalachiensis **(Linder)**	NA	W	purplish	N	N	6.6 -- 9	1*
atropurpureus **(Vittadini)**	E	W	purplish	N	N	<10	1
carbonaceus **(Corner et Hawking)**	As	O	black	N	Y	15 -- 16.5	1*
chlorocarpus **(Castellano, Trappe & Vernes)**	Au	S	black	N	Y	10 -- 12	1|2?
citrinus **(Vittadini)**	E	W	brown	N	N	8 -- 10	1
cooloolanus **(Castellano, Trappe & Vernes)**	Au	S*	black	N	N	11 -- 13	1?
immutabilis **(Spegazzini)**	E	W	gray-blue, black on drying	N	N	7 -- 15	1
mutabilis **(Vittadini)**	E	W	black	N	N	< 13	1
papillatus **(Vittadini)**	E	W	brown	N	N	10 -- 15	1
pedicellaris **(Castellano, Trappe & Vernes)**	Au	S/W	black	N	Y	11 -- 14	1?
rugosisporus **(Castellano, Trappe & Vernes)**	Au, T	W	black	N	Y	10 -- 12	1*
striatosporus **(Kers)**	E	W	brown-black	N	N	10 -- 18	1
viridiseptum **(Trappe)**	E	S	black	N	N	5 -- 8	1?

Elaphomyces species (Author)	Location	Cortex Texture (W,S,O)	Sporocarp Color	Sterile Base	Carbonaceus Peridium	Spore Size (µm)	Morph. clade
***anthracinus* (Vittadini)**	E, NA, As	O	black	N	Y	17 -- 21	2
***asahimontanus* (Kobayasi)**	As	S	black	N	Y	13.5 -- 22	2*
***aurantius* (Castellano, Trappe & Vernes)**	Au, T	S	black	N	Y	14 -- 16	2*
***laetiluteus* (Castellano, Trappe & Vernes)**	Au	S	black	N	Y	22 -- 28	2*
***leucosporus* (Vittadini)**	E	O	brown-black	N	N	17 -- 20	2
***leveillei* (Tulasne)**	E, Au	O	black	N	N	22.5	2
***maculatus* (Vittadini)**	E	S	brown-black	N	N	35 -- 50	2
***nopporensis* (Imai)**	As	S	brown-black	N	Y	15 -- 17.5	2*
***nothofagi* (Castellano, Trappe & Vernes)**	Au	S	black	N	Y	43 -- 55	2*
***reticulosporus* (Zhang)**	As	S	black	N	Y	17 -- 22	2*
***septatus* (Vittadini)**	E	O	brown-black	N	Y	28 -- 32	2
***singaporensis* (Corner et Hawking)**	As	S	black	N	Y	30 -- 36	2*
***subviscidus* (Trappe)**	NA	S	yellow-brown	N	N	14 -- 23	2*
***suejoyceae* (Castellano, Trappe & Vernes)**	Au	S	black	N	Y	24 -- 30	2*
***trappei* (Galán & Moreno)**	E	S	brown	N	N	15.5 -- 18.5	2*

Elaphomyces species (Author)	Location	Cortex Texture (W,S,O)	Sporocarp Color	Sterile Base	Carbonaceus Peridium	Spore Size (µm)	Morph. clade
***aculeatus* (Vittadini)**	E, NA	O	yellow-brown	N	Y	14 -- 17	3
***asperulus* (Vittadini)**	E, NA	O	yellow-brown	N	N	25 -- 34	3
***austrogranulatus* (Castellano, Trappe & Vernes)**	Au, T	O	yellow-brown	N	N	24 -- 28	3*
***cervinus* (granulatus) (L. ex S.F. Gray)**	E, NA, As	O	yellow-brown	N	N	21 -- 25	3
***coralloides* (Castellano, Trappe & Vernes)**	Au	O	black	N	Y	18 -- 23	3*
***decipiens* (Vittadini)**	E, NA	O	yellow-brown	N	N	22 -- 28	3
***echinatus* (Vittadini)**	E	O	black	N	Y	20	3
***fragilisporus* (Imai)**	As	O	yellow-brown	N	Y	30 -- 40	3*
***guangdongensis* (Zhang)**	As	O	black	N	Y	17 -- 22	3*
***japonicus* (Lloyd)**	As	O	yellow-brown	N	N	12 -- 30	3*
***miyabeanus* (Imai)**	As	O	brown-black	N	Y	15 -- 22	3*
***morettii* (Vittadini)**	E	O	brown	N	N	17 -- 42 *	3
***muricatus* (Fries)**	E, NA, As	O	yellow-brown	N	N	19 -- 25	3
***neoasperulus* (Kobayasi)**	As	O	red-brown	N	N	17 -- 27	3*
***nikkoensis* (Imai)**	As	O	black	N	Y	23 -- 30	3*

Elaphomyces species (Author)	Location	Cortex Texture (W,S,O)	Sporocarp Color	Sterile Base	Carbonaceus Peridium	Spore Size (μm)	Morph. clade
***porcatosporus* (Zhang)**	As	O	brown	N	N	16 -- 25	3*
***queenslandicus* (Castellano, Trappe & Vernes)**	Au	O	black	N	Y	23 -- 26	3*
***reticulatus* (Vittadini)**	NA, As	O	yellow-brown	N	N	14 -- 20	3
***shimizuensis* (Kobayasi)**	As	O	yellow-brown	N	N	15 -- 27	3*
***symeae* (Castellano, Trappe & Vernes)**	Au	O	black	N	Y	12 -- 14	3*
***titibuensis* (Kobayasi)**	As	O	black	N	N	25 -- 30	3*
***tuberculatus* (A-Sheng)**	As	O	yellow-brown	N	N	19 -- 40	3*
***variegatus* (Vittadini)**	E, NA	O	yellow-brown	N	N	17 -- 24	3
***verrucosus* (Dodge)**	NA	O	purple-brown	N	N	22 -- 25	3
***virgatosporus* (Hollós)**	E	O	brown	N	Y	16 -- 22	3
***cyanosporus* (Tulasne)**	E	O	brown	Y	Y	23 -- 25	4
***foetidus* (Vittadini)**	E	O	brown	Y	N	24 -- 30	4
***persooni* (Vittadini)**	E	O	black	Y	N	21 -- 28	4

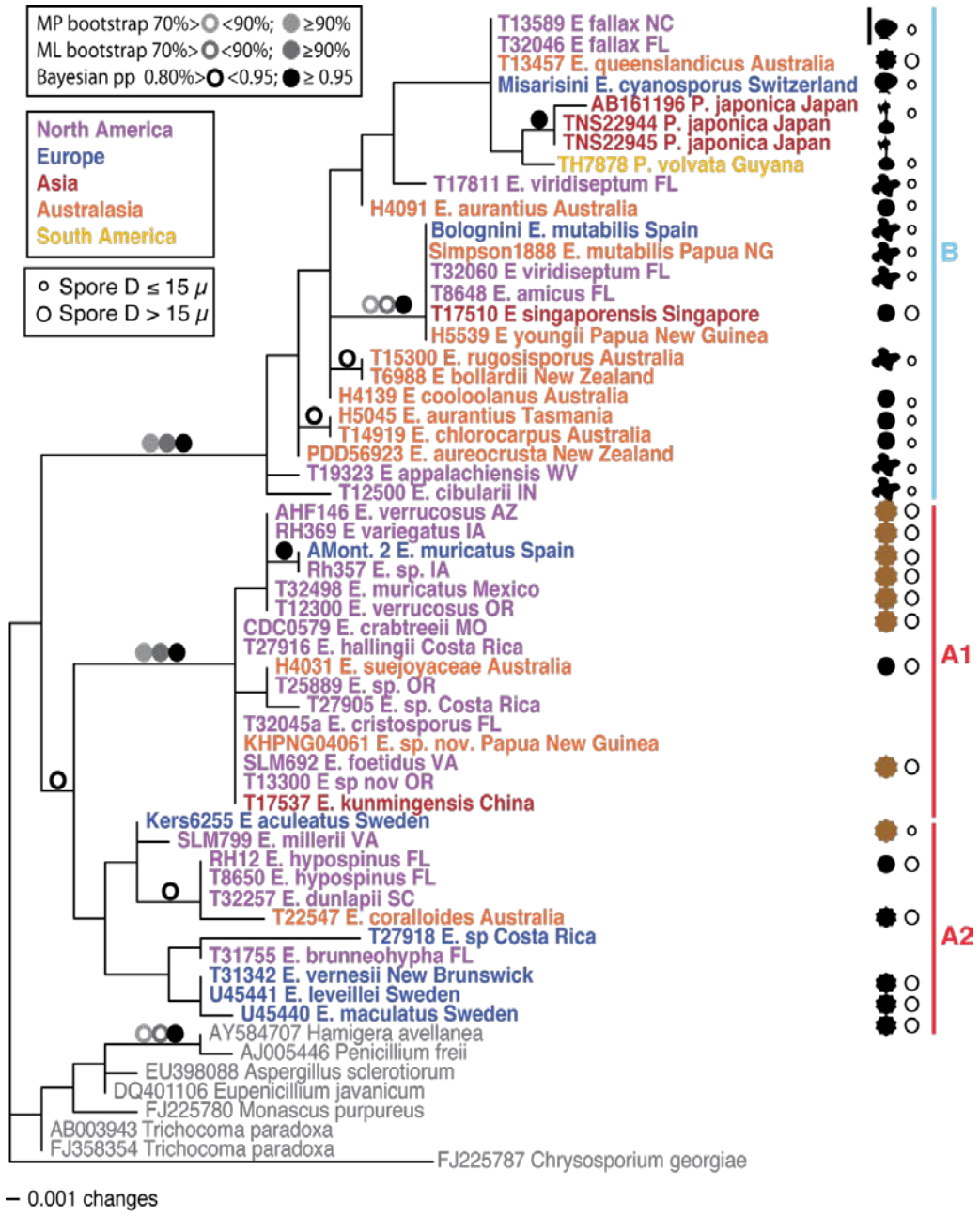

Figure 8. Best Maximum Likelihood tree of Elaphomycetaceae using SSU. Symbols indicate sporocarp shape (wrinkled, smooth, ornamented) and color (black/brown)

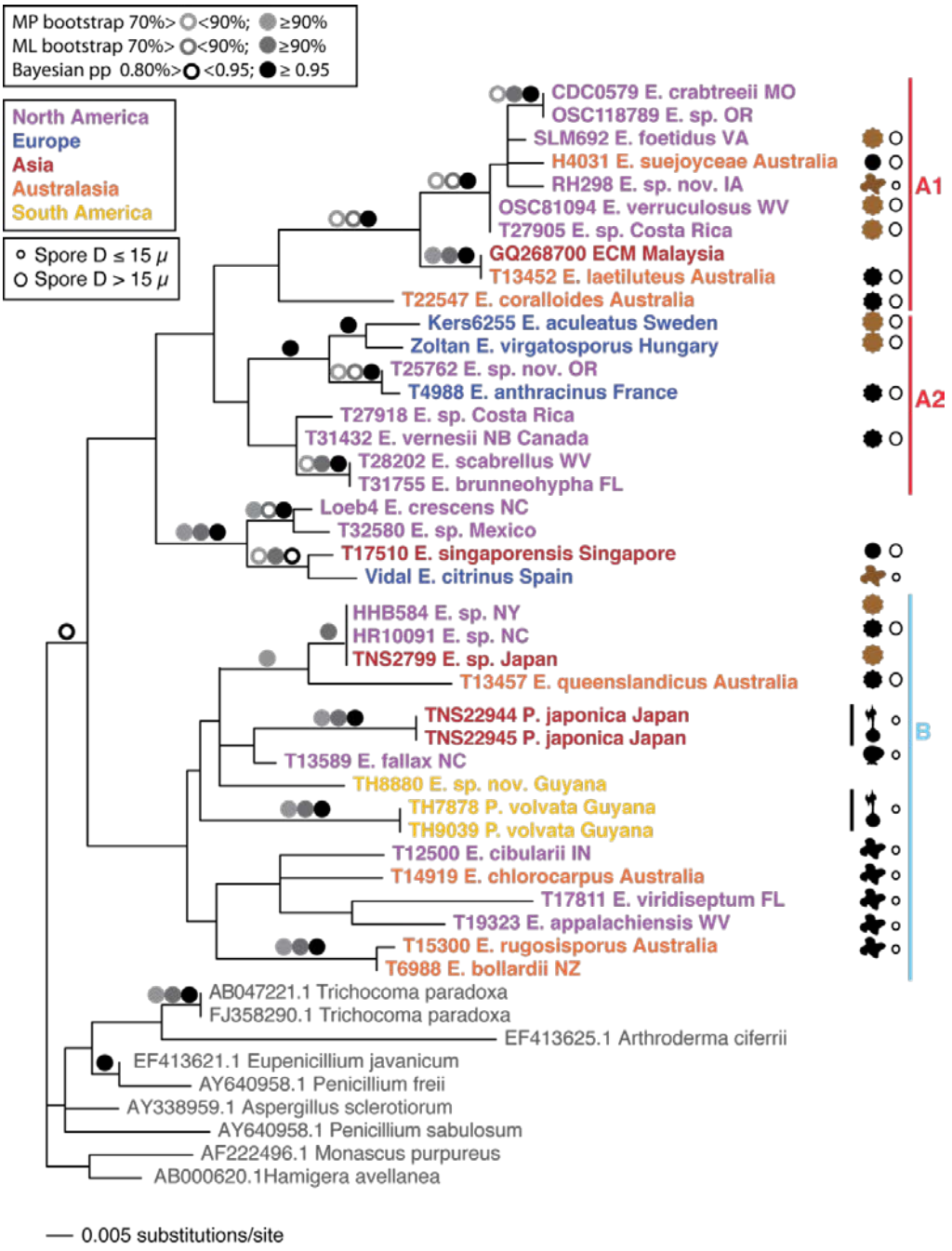

Figure 9. Best Maximum Likelihood Phylogeny for Elaphomycetacaeae using LSU.

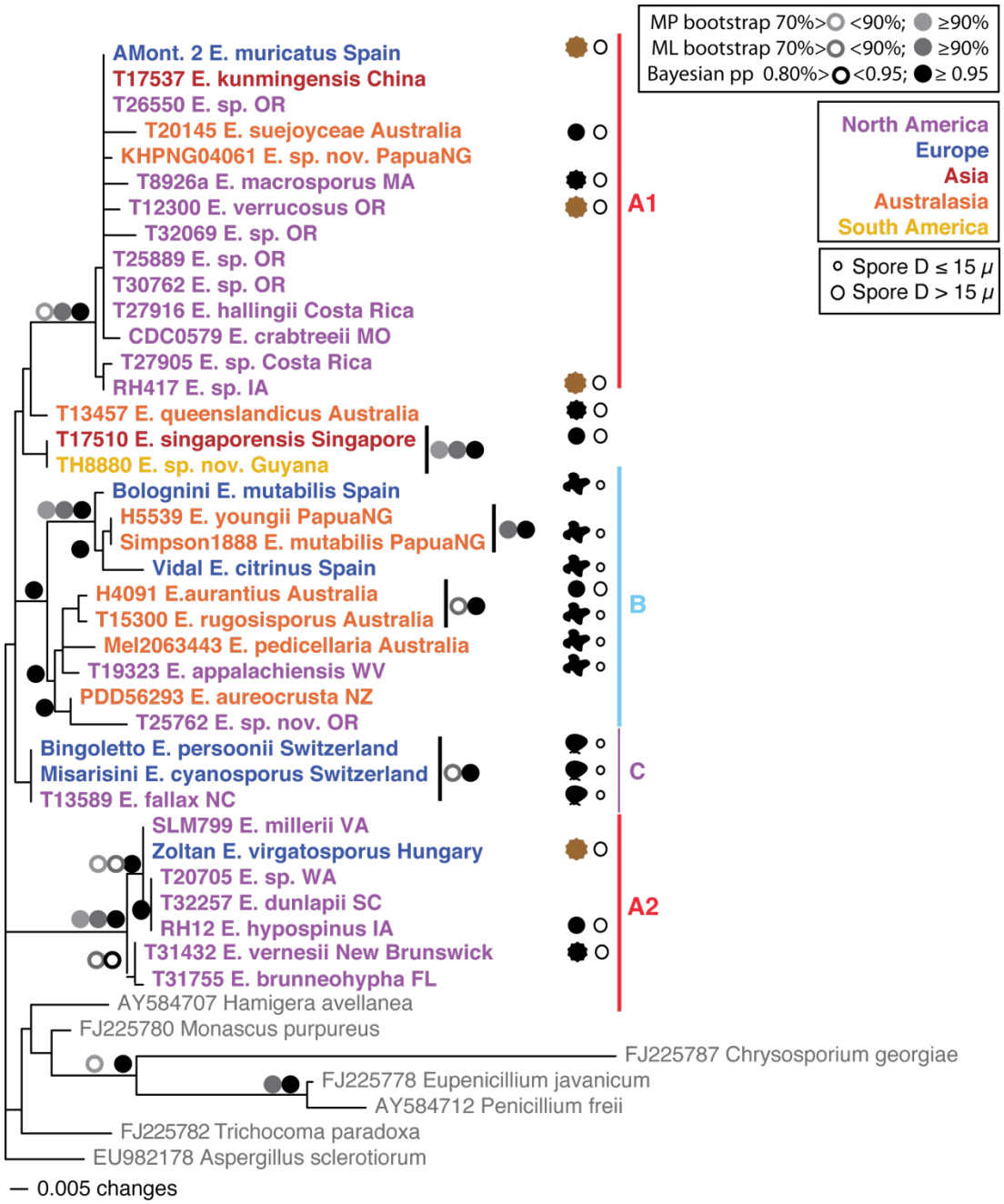

Figure 10. Best Maximum Likelihood Phylogeny for Elaphomycetaceae using mtSSU. *Pseudotulostoma* is excluded.

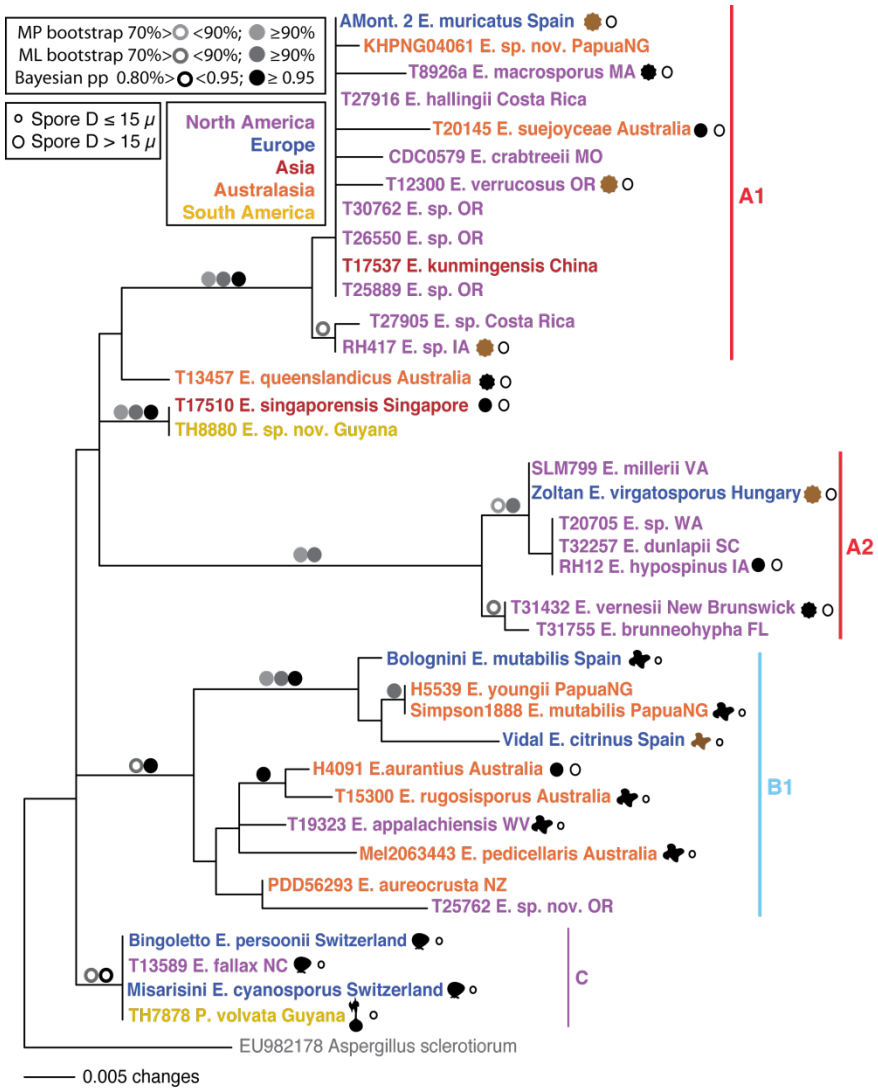

Figure 11. Elaphomycetaceae mtSSU phylogeny including *Pseudotulostoma volvata*.

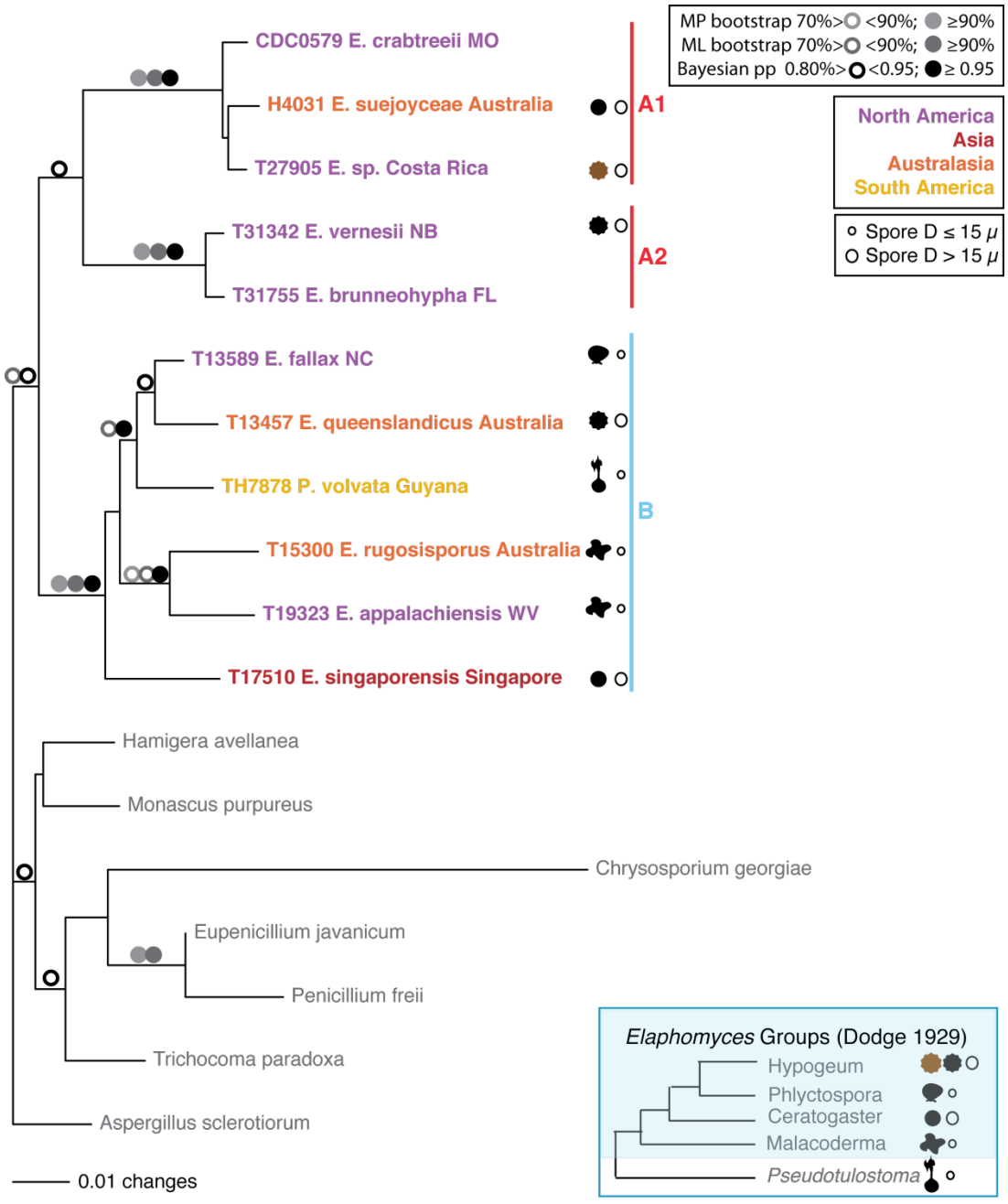

Figure 12. 3-Gene Elaphomycetaceae Phylogeny. Inset: Dodge's Morphological Classification.

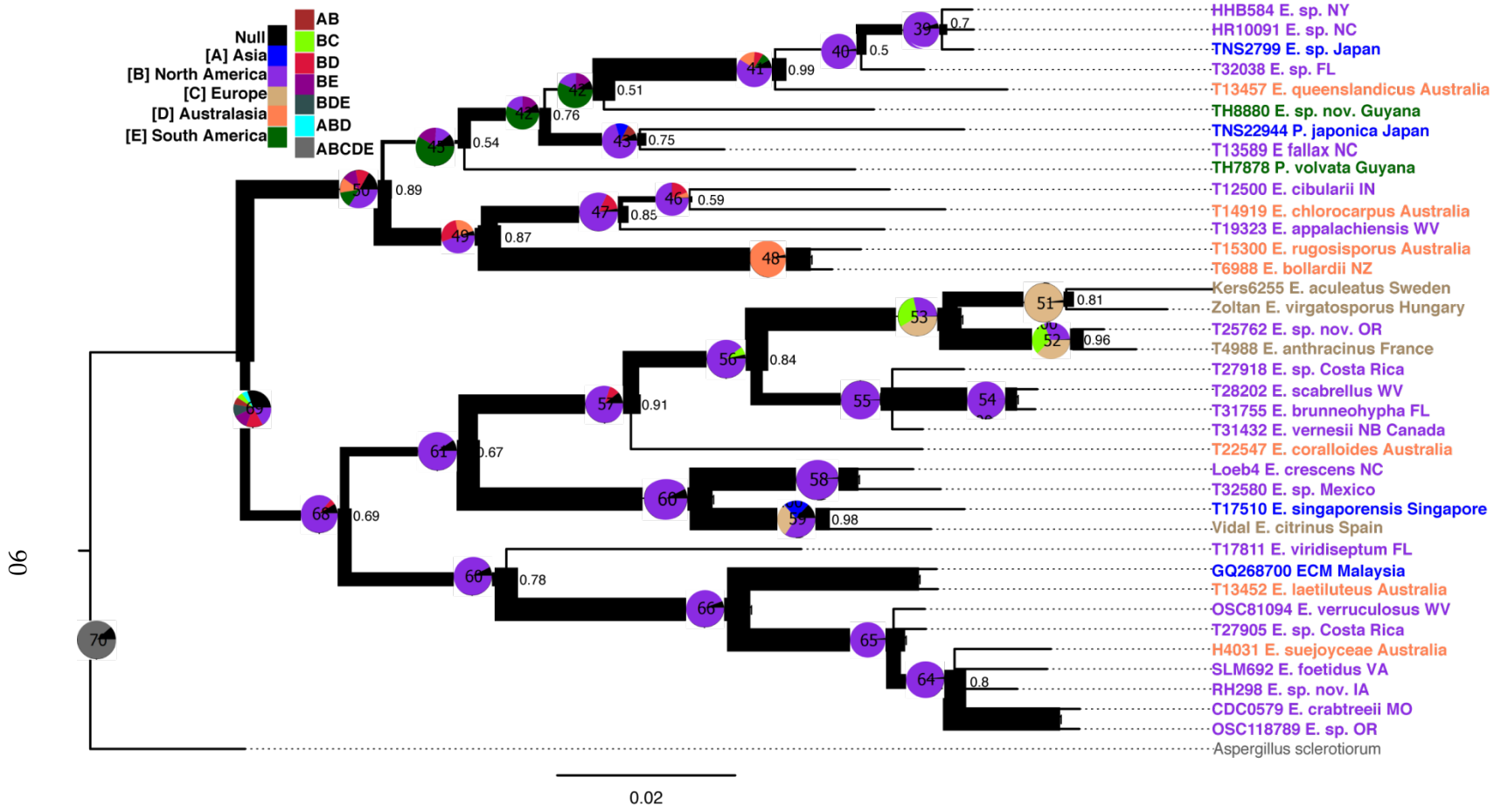

Figure 13. Bayesian LSU tree with attached RASP Bayesian ancestral distributions estimated with Holarctic divided. Bayesian posterior probability indicated next to nodes and by branch width.

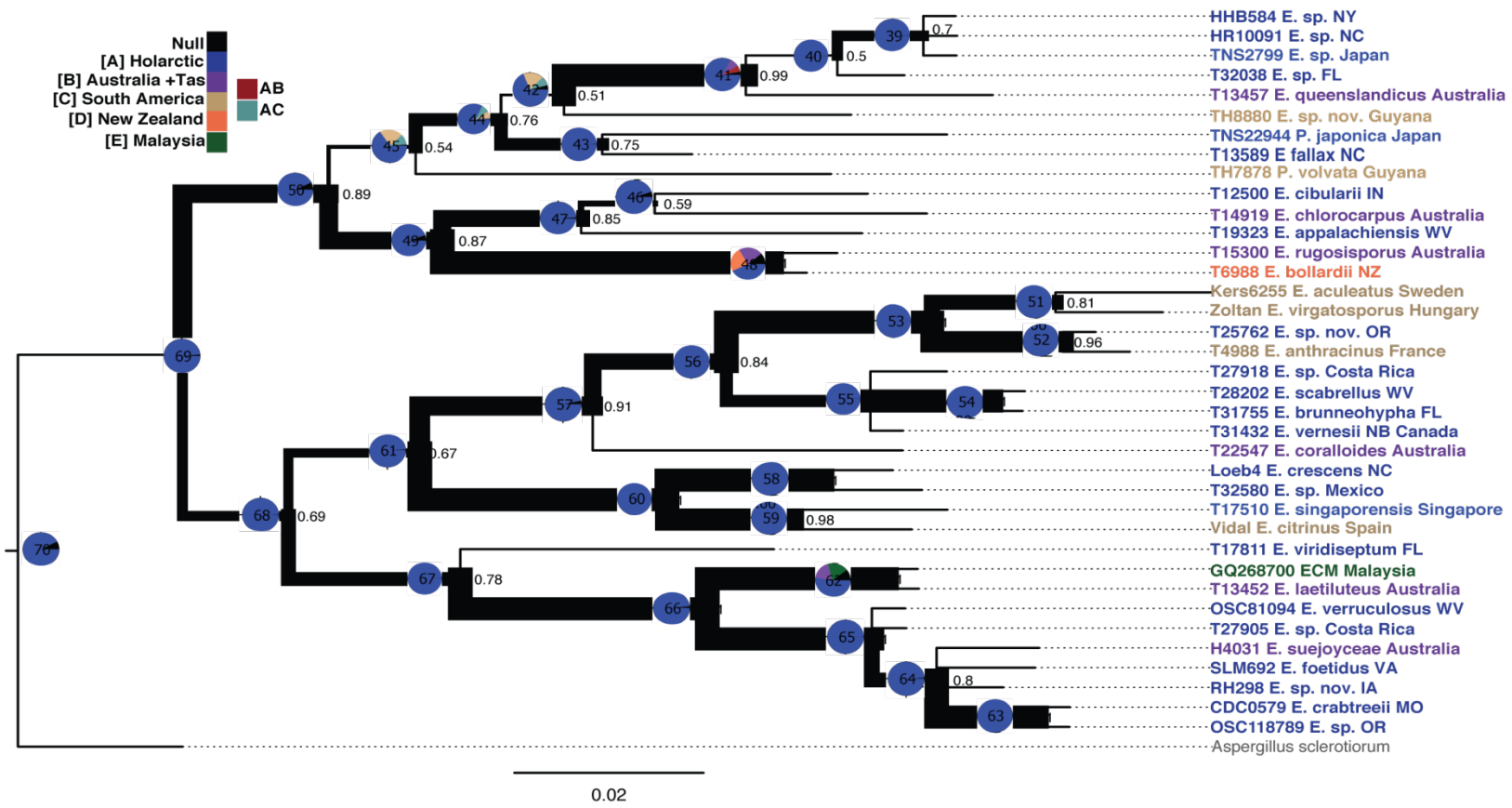

Figure 14. Bayesian LSU tree with attached RASP Bayesian ancestral distributions estimated with Holarctic grouped and Australasia divided. Bayesian posterior probability indicated next to nodes and by branch width.

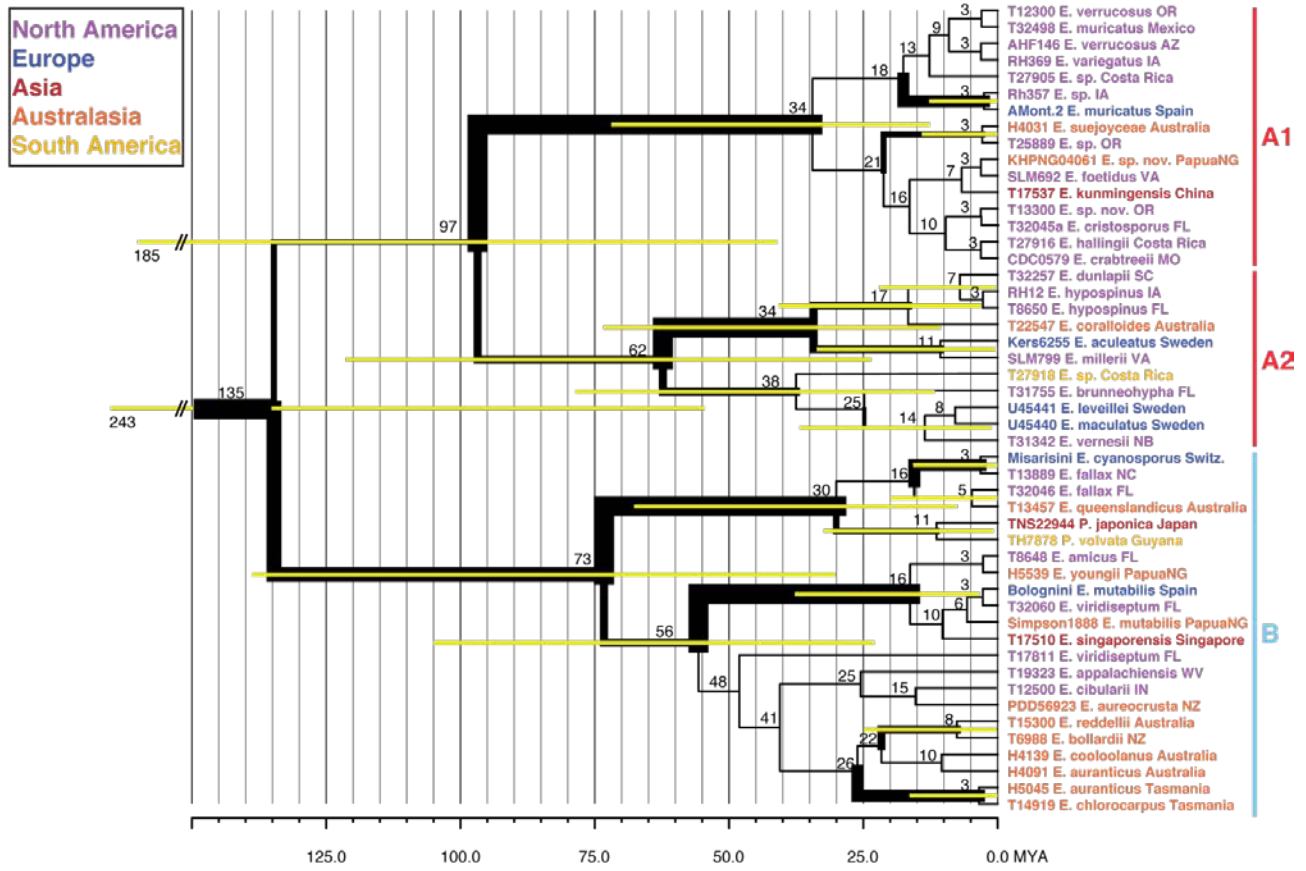

Figure 15. Estimated ages of Elaphomycetaceae divergence using SSU topology (constraint: rate prior).

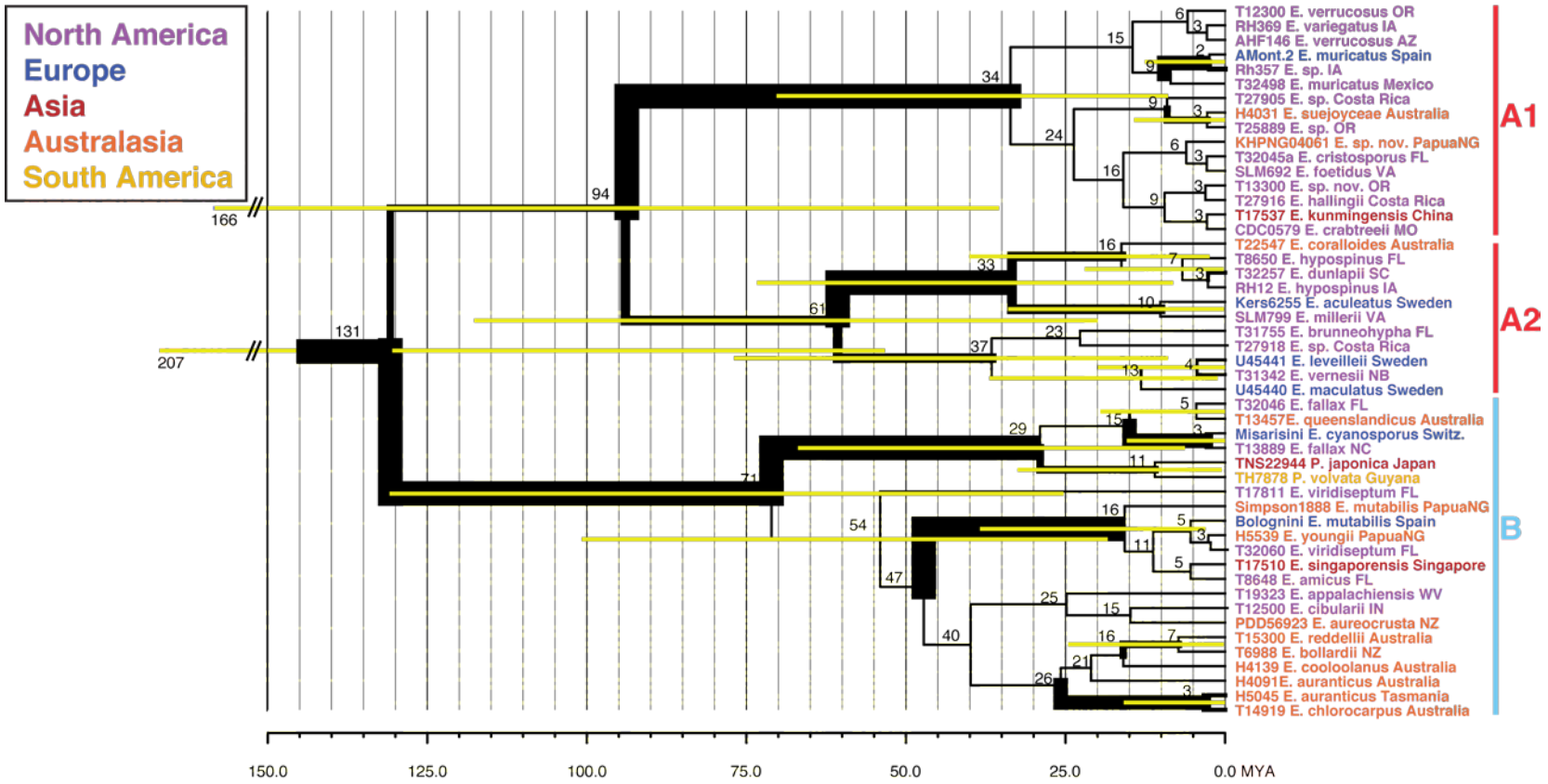

Figure 16. Estimated divergence times of Elaphomycetaceae based on SSU using (constraints: rate prior + Elaphomycetaceae age prior of 135±45 mya)

Chapter 3. *Elaphocordyceps* diversity and variable effects on fungal host sporulation

3.1 Introduction

Elaphocordyceps, which include 22 described species, infects a wide range of *Elaphomyces* truffles and was found to have evolved from cicada parasites, an instance of a host-kingdom switch **[178]**. The genus *Elaphocordyceps* was erected by Sung *et*. *al* to include all of the mycoparasites as well as some closely-related insect pathogens **[200]**. Early literature on this suite of mycoparasites indicated a mix of specialists and generalists infecting a morphologically diverse set of hosts. The reported species of *Elaphocordyceps* are from the Northern Hemisphere on a limited set of *Elaphomyces* species. Parasitized *Elaphomyces* are occasionally collected before the parasite has fully developed. We tested a large sample of *Elaphomyces* species, including recently discovered Southern Hemisphere taxa, for the presence of *Elaphocordyceps* in order to describe phylogeographic patterns in *Elaphocordyceps*. We hypothesized, based on our findings of frequent long-distance dispersal events in *Elaphomyces* (Chapter 2), that *Elaphocordyceps* would, like its host, also show evidence of high dispersibilty. We had found that some Australasian *Elaphomyces* were closely related to Northern Hemisphere taxa, with a strong indication that these were relatively recent migrants into the Southern Hemisphere (10-3 mya). We hypothesized that some Australian and South

American *Elaphomyces* might be infected with *Elaphocordyceps*, which might show a similar pattern of recent invasion from the Northern Hemisphere.

The morphological classification system indicated that hosts of *Elaphocordyceps* were phylogenetically diverse, and we reassessed the host diversity using a recent molecular *Elaphomyces* phylogeny (Chapter 2). Imai described several Japanese *Elaphocordyceps* and *Elaphomyces* species, noting that in Japan, *C. capitata* parasitizes *E. fragilisporus* , and C. *intermedia* on *E. subvariegatus* ***[201]***. Mains reported seven species of *Cordyceps* as parasites on *Elaphomyces*: *C. capitata* on *E. granulatus*, *C. valliformis* on *E. cervinus* (= *granulatus*), *C. canadensis* (= *longisegmentis*) and *C. ophioglossoides* on *E. granulatus*, *E. muricatus*, and *E. variegatus*, *C. fracta* on *E. appalachiensis* and unidentified species, *C. japonica* on *E. japonica*, and *C. tenuispora* on unidentified species **[202]**. Kobayashi enumerated more Japanese host-parasite relationships: *C. jezoensis* on *E. nopporensis*, *E. anthracinus*, and *E. miyabeanus*, *C. canadensis* (= *C. longisegmentis*) on *E. reticulatus*, *C. intermedia* on *E. variegatus*, and *C. ophioglossoides* on *E. titibuensis*, *E. reticulatus*, *E. shimizuensis*, and *E. neoasperulus* in addition to *E. muricatus* and *E. granulatus* ***[203]***. The *Elaphomyces* hosts are morphologically diverse: *E. granulatus*, *E. muricatus*, *E. variegatus*, *E. japonica*, *E. subvariegatus*, *E. reticulatus*, *E. shimizuensis*, *E. neoasperulus*, *E. japonicus* and *E. fragilisporus* are brown and warted, *E. titibuensis* is black and warted, *E. anthracinus* is black and smooth, and *E. appalachiensis* is black with a

bright mycelial crust and small spores. Our molecular phylogenies placed most brown, warted species, including *E. muricatus* and *E. variegatus*, in one major clade, and *E. appalachiensis* in another; known *Elaphocordyceps* fungal hosts are thus known from both major clades of *Elaphomyces* (Chapter 2, Figures 8-12).

Mature *Elaphomyces* sporocarps are filled with powdery spores, but we noticed that in multiple parasitized specimens, there was little sporulation evident. Young specimens will of course show little sporulation, whether or not they are parasitized, so we would expect to see a certain number of *Elaphocordyceps* truffles showing no spores. If *Elaphocordyceps* slows host development or stops it altogether quickly after the start of infection, the degree of maturation would indicate only the age at which the host had been parasitized. On the other hand, if *Elaphomyces* development continues in the initial stages of parasitism, the range of developmental states could also indicate varying degrees of host resistance.

The variation in sporocarp development of parasitized *Elaphomyces* may indicate how the parasite impacts host fitness. The prevention of host reproduction could impose a cost on the parasite if its virulence decreases the population of available hosts. At the same time, the host would be under selective pressure to increase reproduction. Several *Elaphocordyceps* species are known to be generalists on *Elaphomyces*, while others may be specialists. Parasites may show preference for certain hosts, rather than strict

specialization, and this preference may vary geographically. The observed variation in sporulation could be due to some combination of host resistance, parasite virulence, and seasonality. The most straightforward explanation of a sterile sporocarp is that it was parasitized when immature, while a parasitized specimen full of spores may be thought to have encountered its host at a later age. Under this model, *Elaphomyces* would be under selective pressure to develop and sporulate before parasitism, while *Elaphocordyceps* would face similar pressure for early activity. Should the majority of *Elaphocordyceps* become active more early, some *Elaphomyces* could have an advantage if they fruited later than the majority of the population. The tension between selective pressure on the host species to have a fruiting time unanticipated by its parasite and the pressure on the parasite to optimize the numbers of hosts it could infect could lead to the diversification of fruiting seasons for both host and parasite. This diversification could potentially take place within species or lead to speciation. As *Elaphomyces* depends on its ectomycorrhizal hosts for nutrients, it cannot develop while the trees are inactive, setting a limit to how early it could feasibly grow. We therefore hypothesize that 1) the degree of sterilization of *Elaphomyces* would correspond to season, and 2) the degree of sterilization may also vary according to the host, indicating either a variation in host virulence or seasonal activity. Hosts could be weaker parasites or might initially parasitize only the external tissues, acting slowly enough that *Elaphomyces* could

continue developing spores. In the second case, *Elaphocordyceps* species might vary in seasonal activity. It is also possible that *Elaphocordyceps* spores are capable of parasitizing *Elaphomyces* whenever they form, allowing multiple infection cycles in a single growing season. In this case, we would expect the percentage of *Elaphomyces* collected with *Elaphocordyceps* to not vary according to season, but to be constant. In any of these assessments, we must caution that the collection date may differ from the actual date the fungus fruited; these specimens may persist in the environment and be collected well after they have sporulated. We hypothesized that different species of *Elaphocordyceps* might have different seasons of activity, thus infecting hosts of different ages.

3.2 Materials and Methods

3.2.1 *Elaphocordyceps* prevalence and diversity in *Elaphomyces*

We had extracted DNA from 427 *Elaphomyces* specimens for a phylogenetics study (extraction and PCR protocols are in Chapter 2). We screened these samples for *Elaphocordyceps* DNA using a forward primer for ITS developed to exclude *Elaphomyces* and include all sequenced species of *Elaphocordyceps*: 5′-CAA CGG ATC TCT TGG CTC TGG-3′ (PCR conditions described in Chapter 2). As the updated phylogeny of *Elaphomyces* (Chapter 2, Figures 8-12) had indicated that many of the morphological categories were not monophyletic, we assessed the diversity of *Elaphocordyceps* hosts. We attempted to obtain sequences for both host and parasite from each specimen, but

generally were unable to do so. For this reason, we were not able to compare the host and parasite phylogenies using sequences from the same specimen. In most cases, we were also unable to identify the host species using morphology, as the observable characters, such as peridium structure and color, may be altered, and the host may not have produced spores. Therefore, we compared the general phylogenetic diversity of known *Elaphomyces* hosts at a broad scale, testing whether *Elaphocordyceps* hosts would be found in all major *Elaphomyces* lineages. We also assessed the phylogenetic diversity of *Elaphocordyceps* recovered from parasitized truffles. Using MUSCLE **[179]** for initial alignments corrected visually in MacClade **[180]**, we aligned our sequences with a broad sampling of *Elaphocordyceps* ITS sequences from Genbank and performed maximum parsimony and Bayesian analysis to determine species identity and relationships. The Cipres server **[182]** was used for Bayesian and maximum likelihood analyses. Maximum likelihood analysis in Garli **[183]** used a starting tree determined by stepwise addition and two independent runs, sampling every 100 generations. Support values were determined with 100 ML bootstrap repetitions in Garli **[183]** and 1000 unweighted parsimony bootstrap repetitions in PAUP* **[181]**. Bayesian support was determined from 10 million generations in MrBayes **[185, 186]** using 2 runs and 4 chains, sampling every 1000 generations for a total of 10000 trees. The consensus tree was calculated after

discarding the burnin of 2500 trees. The best ML tree was used to present the topology of *Elaphocordyceps*, with the support values from MP and Bayes added (Figure 17).

3.2.2 Assessment of Seasonal and Parasite Effect on Host Development

While processing multiple *Elaphomyces* specimens for extraction, we noticed a range in glebal development. Our observations of parasitized specimens gave the impression that these specimens were less developed, showing no sporulation at all in the most extreme cases. In order to quantify these observations for analysis, we designed a visual rubric for scoring the glebal development of *Elaphomyces* based on the coloration and texture of the gleba (Figure 18). We designated 4 categories of glebal development: **(1)** gleba dark, spores powdery, and are released from the sporocarp if tapped **(2)** gleba dark, but spores are not released if tapped, and there may be a mix of white or pink mycelium with dark areas of fertile tissue **(3)** the sporocarp is not filled with spores, and is either just turning pink to brown, or may be white mycelium with pink areas showing sporulation (4) the sporocarp is mostly white and cottony mycelium, indicative of little to no sporulation. To show the effect of *Elaphocordyceps* on *Elaphomyces* spores, we photographed spores at 1000x magnification from a parasitized specimen (LGD 7640 from Queretaro, Mexico, collected by L. Guzmán-Dávalos 10-12-1998) using a Canon PowerShot SX110 IS (Figure 19). Though this specimen did not include an

Elaphocordyceps sporocarp, parasite hyphae can be seen attached to *Elaphomyces* spores. One of these spores is collapsed.

Using the rubric, we examined 175 parasitized and non-parasitized specimens from the New York Botanical Garden (NYBG), the National Fungus Collection (BPI), and the Universidad de Guadalajara (IBUG) (Table 4). For each collection, we assigned a glebal score to each truffle in the collection and recorded the numbers of host and parasite sporocarps. Of the collections we examined, 69 contained more than one host sporocarp, and 34 had sporocarps showing different levels of glebal development. We used the average glebal score for each collection in statistical analyses. In order to assess the effect of climate, we coded the collections according to the Trewartha climate classification scheme **[204]**, a modification of Köppen's climate classification **[205]**.

We used JMP 9 for several statistical tests examining the roles of season, climate, and host-parasite interactions on *Elaphomyces* development and *Elaphocordyceps* distribution **[45]**. We tested whether *Elaphocordyceps* species identity varied according to either season, defined by month of collection, or Trewartha climate code with X^2 tests. We tested host specificity with X^2 analysis to find whether host species varied significantly according to parasite species. To understand factors that may correlate with *Elaphomyces* development, we used ANOVA and 2-tailed t-tests. We first tested whether *Elaphomyces* specimens with and without visible *Elaphocordyceps* sporocarps differed in

glebal development, based on both the distribution of each individual score (1 to 4, from most to least developed) and the average score for each collection. We tested for possible effect of collection month, climate, and the combined effect of month and climate on glebal development. *Elaphomyces* species may vary in development rate; species slower in development would be more likely to be collected when immature. To test whether different species of *Elaphocordyceps* may have different effects on host development, we performed three analyses. The first tested the effect of species identity using data for the three most common parasite species in our sample: *E. ophioglossoides*, *E. capitata*, and *E. longisegmentis*. The second and third analyses examined host-parasite interactions in the individual *E. ophioglossoides* and *E. capitata* datasets, testing for the influence of host species on glebal development. The majority of specimens in the collections had unidentified hosts, but we were able to examine the effect of *Elaphomyces* species on development for 35 specimens.

3.3 Results

We obtained 32 *Elaphocordyceps* ITS sequences from parasitized *Elaphomyces* truffles. Modeltest 2.2 selected the HKY+Γ model under the Akaike criterion; this model was used for maximum likelihood and Bayesian analysis. The best maximum likelihood tree from two independent Garli runs was used to present the topology of the *Elaphocordyceps* (Figure 17). The parasites were phylogenetically diverse, and all

recovered sequences were from hosts from *Elaphomyces* clade A1. These specimens were morphologically placed in the traditional subsection Hypogeum, having a brown, warted cortex and large spores. While the ITS region was not able to resolve the *Elaphocordyceps* tree, it was evident that *Elaphocordyceps* species complexes have wide geographic ranges, as does its host. Sequences from Genbank included *Elaphocordyceps* insect pathogens, which are polyphyletic in this genus.

The *Elaphocordyceps* specimens we examined for the ecological analysis were dominated by *E. ophioglossoides*, *E. capitata*, and *E. longisegmentis*. We assessed the possible effect of seasonality on the presence of these three species (Figure 20), and did not find a significant effect (n = 153, $X^2 = 2.588$, p-value = 0.2741). While the three common species had been collected throughout the year, the late summer was the most common collecting time, particularly for *E. ophioglossoides*. They were found from the temperate to the tropical zones, and in the highlands in Mexico. Parasite species spanned a broad range of climates (Table 5), and the effect of climate on the three most common species was not significant (n = 158, $X^2 = 48.90315$, p-value = 0.0741). In this collection, *E. granulatus* and *E. variegatus* were infected by all three common *Elaphocordyceps* species, while *E. asperulus* was infected by *E. longisporus* and *E. ophioglossoides*, and *E. muricatus* was infected only by *E. ophioglossoides*. The host effect,

while showing a strong trend toward specialization (Table 6), was not significant (n = 37, X^2 = 18.15797, p-value = 0.0524).

The glebal development score averaged for each individual collection was examined for possible seasonality, climate, and host-parasite effects. The effects of seasonality and climate (Figure 21) were found to be non-significant, both when assessed individually and when combined (n = 161, Month: ANOVA F-Ratio = 0.17, p-value = 0.6807l; Climate: n = 167, ANOVA F-Ratio = 1.0715, p-value = 0.3842; Combined: n = 163, effect test F-Ratio = 0.9807, p-value = 0.4476). We evaluated whether specimens with and without *Elaphocordyceps* fruiting bodies differ in maturity score, and found a strongly significant difference (n = 173, ANOVA = 15.9927, p<0.0001*). The identity of the parasite and host were assessed individually, as sampling of identified *Elaphomyces* host species was not sufficient to test the combined effect (Figure 22). The effect of parasite species was non-significant (n = 155, ANOVA = 2.01, p-value = 0.115), but the effect of host species was weakly significant (n = 35, ANOVA = 3.169, p-value = 0.0276*). The latter analysis excluded unidentified species. For those specimens parasitized by *Elaphocordyceps ophioglossoides* and *E. capitata*, the average development score varied significantly according to host species (*ophioglossoides:* n = 21, ANOVA = 3.2006, p=value = 0.0498*; *capitata*: n = 9, ANOVA = 12.4483, p-value = 0.0073**). For specimens parasitized by *E. ophioglossoides*, *E. asperulus* specimens were significantly more

immature than *E. muricatus* and *E. variegatus* specimens (t-Ratio = 2.58, p-value = 0.0194*), while for *E. capitata*, *E. granulatus* specimens showed significantly higher development than *E. varigatus* (t-statistic 4.79, p-value = 0.003*).

3.4 Discussion

The geographic and host diversity of *Elaphocordyceps* is not surprising given the generalist nature of this parasite. *Elaphocordyceps* sequences were in some instances recovered from hosts that were apparently uninfected: RH688 from Iowa, SM10053 from Guyana, and LGD7640 from Mexico. The ITS sequence of RH688 was identical to Genbank sequences identified as *E. ophioglossoides* and *E. capitata* and to specimens that we identified as *E. ophioglossoides*. LDG764 was closely related to Genbank sequence AJ786568, *E. longisegmentis*, from Norway. *Elaphocordyceps jezoensoides*, known previously only from Japan, was shown to have close representatives in North America and Europe. There is a clear indication that, like its host, the biogeographic history of *Elaphocordyceps* is marked by frequent long-distance dispersal. While our study uncovered sequences from only one clade of *Elaphomyces*, the literature indicates that it can attack hosts from other major clades. Because of the rarity of these species, we were unable to use them for sequencing, but based on the morphological descriptions, it is clear that there are at least some species in *Elaphomyces* clade B parasitized by *Elaphocordyceps*. The survey also indicated the presence of *Elaphocordyceps* in Guyana and

Australia, showing that these parasites span the full range of their broadly-distributed hosts. The close relationships with extant insect pathogens further indicate the ability of *Elaphocordyceps* to switch hosts; Sung *et al* had also found multiple insect parasites in the *Elaphocordyceps* and suggested these represent multiple reversals to entomopathogenesis **[176, 200]**. To better understand the evolution of *Elaphocordyceps* morphology and host associations, a better-resolved phylogeny is needed.

We had hypothesized that Southern Hemisphere *Elaphocordyceps* would show a similar pattern to its host, which showed evidence of long-distance dispersal from the Northern to Southern Hemisphere. However, we were unable to assess this hypothesis adequately, given that we uncovered only two sequences of Southern Hemisphere *Elaphocordyceps*, one from Guyana and the other from Australia, and that the placement of these taxa was not supported. The unsupported topology indicated that the Guyanan species was closely related to *E. japonica* from Japan and *E. gracilis* from Norway; if confirmed with further analysis, this species could be an example of long-distance dispersal between continents. Future studies in *Elaphocordyceps* should expand taxon sampling from the Southern Hemisphere.

The seasonality and climate assessment showed that the three common species of *Elaphocordyceps* in our sample, *E. ophioglossoides*, *E. capitata*, and *E. longisegmentis* have a broad distribution through the temperate to tropical zones and have similar fruiting

seasons. *Elaphocordyceps ophioglossoides* and *E. capitata* are also found in the subarctic zone. The three common species can be found nearly year-round, and are most commonly found in late summer through fall (August through October). While sampling from the tropics was too sparse to fully assess seasonality, the range of fruiting season was similar to that in the temperate zone. We hypothesize that expansion of tropical sampling would find that fruiting peaks in different seasons than in the temperate zone. Our ITS phylogeny of *Elaphocordyceps* indicated that *E. capitata* and *E. ophioglossoides* are polyphyletic. Our ecological analyses are based on morphological identifications and herbarium records, and it is crucial to be clear that these collections could in fact contain multiple cryptic or misidentified species. However, given that our phylogenetic analysis supported the existence of widespread species in the Northern Hemisphere, the cosmopolitan nature of at least some *Elaphocordyceps* species is probable.

When we first noted the variation in sporocarp maturity evident in the collection, we were curious as to whether the sporulation could represent some level of host resistance, or whether this effect was due to a possible seasonal or climate effect. We tested whether host immaturity would correlate with season, climate, or the species identity of host and/or parasite. The effect of season and climate were non-significant. We found that specimens lacking the parasite, at least according to morphological

observation, had significantly more development. Furthermore, the identity of the host species was shown to correlate significantly with the maturity score, both in the overall analysis and when analysis was limited to hosts of *E. capitata* and *E. ophioglossoides*. The apparent variation in development according to host species gives initial support to the hypothesis that host-parasite interactions affect host development.

However, there are several potential problems with this assessment. First, it could be that the limited sampling of identified host species yielded a false positive, and that, with expanded sampling, the apparent effect of host identity on development would vanish. Second, identifying *Elaphomyces* based on the current morphological taxonomy is problematic, particularly for parasitized specimens. Spore size and ornamentation are important characters for identifying *Elaphomyces* species, and several parasitized specimens show either immature spores or no spores. Third, the rubric we employed in evaluating sporocarp maturity divides a continuous process of sporulation into four discrete categories, which will miss subtle gradations of maturation. We would therefore hesitate to conclude that host-parasite interactions in the *Elaphomyces-Elaphocordyceps* system are indeed driving an apparent difference in maturation state. However, based on this initial foray into the developmental effects of *Elaphocordyceps* on its host, we present three models of possible host-parasite interactions.

It is clear that the most commonly discovered species of *Elaphocordyceps* are generalists, but their interactions with the individual host species are little understood. Based on our initial findings, we hypothesize that different host-parasite combinations arrest the host in different stages of maturity. Should this hypothesis be supported by future research, there are three major models that could explain this variability. First, the variability could be explained by host resistance: parasitized fungi have some capacity to suppress latent infections while sporulating. The hypothesis of host resistance would be supported if evidence of spore viability could be found in parasitized specimens. Under this model, the resulting variation in reproduction would provide the material for natural selection. Second, the variability could be explained by parasite preference: the parasite sterilizes all hosts regardless of their maturation stage at infection, but is better adapted to infect certain species earlier or later in their development cycle, perhaps because of chemical cues or physical properties. Third, the variability could be explained by different rates of development in different *Elaphomyces* species: host species with longer maturation times would have a higher probability of being infected at an early stage of development. Under this model, *Elaphocordyceps* could either fully or partially sterilize its host. If the latter, *Elaphomyces* with faster maturation rates would have higher fitness. The most critical question for testing the above models is the viability of parasitized *Elaphomyces* spores. *Elaphocordyceps* hyphae attach to *Elaphomyces* spores and

can cause the collapse of immature spores (Figure 20), but we do not know whether mature spores are destroyed or if all spores are destroyed. Tests of spore viability and germination may prove difficult in *Elaphomyces*. Germination tests in *Elaphomyces granulatus* found that pre-digested spores in *Spermophilus saturates* feces germinated after 1 year in storage, while the non-digested spores did not **[206]**. The appropriate control tests for normal germination may be difficult to determine. Future research should examine the physical effects of *Elaphocordyceps* on the spores of multiple species and developmental stages of *Elaphomyces* in order to test for possible host resistance.

Table 4. *Elaphocordyceps* specimens examined. (sp = unidentified species, x=absent from collection)

Herb.	#	Parasite	Host	M	D	Y	Locale		collector
BPI	634755	*agariformu capitata*	*sp*	2	5	1945	Gainesville	FL	WA Murrill
BPI	634538	*capitata*	*sp*	2	x	1940	Maitland	FL	CL Shear
BPI	634537	*capitata*	*sp*	11	7	1966	Patuxent River near Belair	MD	KH McKnight
BPI	802573	*capitata*	*sp*	12	21	1993	Pinar de Vigo	Spain	CM Martinez
BPI	634533	*capitata*	*granulatus*	10	19	1910	Calvados	France	R Maire
BPI	634535	*capitata*	*sp*	2	10	1941	Apopka	FL	VK Charles
BPI	634751	*capitata*	*granulatus*	11	x	1904	Sharon	MA	WR Dudley
BPI	869550	*capitata*	*sp*	10	4	1936	Burning Wells, Elk Co.	PA	LO Overholts, WA Campbell
BPI	869549	*capitata*	*sp*	8	26	1931	Montreal	Quebec	HAL Jackson
BPI	634752	*capitata*	*variegatus*	11	12	1934	Dedham	MA	GD Darker
BPI	634546	*capitata*	*sp*	8	25	1938	Duchesnay	Quebec	CL Shear
BPI	634754	*capitata*	*sp*	10	7	1901	South Billerica	MA	JB Rorer
BPI	878359	*longisegmentis*	*sp*	10	13	2006	Lipovska kotlina, 9 km N of Hybe village	Slovakia	V Kautman
BPI	634631	*ophioglossoides*	*cervinus*	8	x	1915	Chocorua	NH	WG Farlow
BPI	634634	*ophioglossoides*	*sp*	8	x	1916	Chocorua	NH	WG Farlow
BPI	634622	*ophioglossoides*	*sp*	x	x	x	x	Belgium	N Taymans
BPI	634638	*ophioglossoides*	*variegatus*	8	24	1938	Duchesnay	Quebec	JA Stevenson
BPI	634632	*ophioglossoides*	*sp*	8	18	1939	Indian Gap Trail, Great Smoky Mtns Natl Park	TN	JA Stevenson & RW Davidson
BPI	634637	*ophioglossoides*	*cervinus*	9	2	1938	Pentwater	MI	CB Stifler
BPI	634625	*ophioglossoides*	*sp*	8	24	1938	Duchesnay	Quebec	JW Groves
BPI	634853	*ophioglossoides*	*muricatus*	1	x	1882	x	x	x
BPI	634858	*ophioglossoides*	*sp*	7	21	1917	Ithaca	NY	JH Faull
BPI	634849	*ophioglossoides*	*sp*	8	12	1903	Ithaca	NY	EJ Durand
BPI	634851	*ophioglossoides*	*sp*	8	21	1935	Ithaca	NY	JA Stevenson
BPI	634856	*ophioglossoides*		9	19	1961	Diever	Netherland	GRA Maas
BPI	634859	*ophioglossoides*	*sp*	x	x	1900	Algonquin Park	Ontario	JH Faull
BPI	634855	*ophioglossoides*	*sp*	8	19	1914	Pegnaket	NH	GE Morris

Herb.	#	Parasite	Host	M	D	Y	Locale		collector
BPI	634867	*ophioglossoides*	*cervinus*	9	19	1899	Rovensko	Czecho-slovakia	F Bubak
BPI	634865	*ophioglossoides*	*cervinus*	10	16	1949	Oberbayern, Traunstein	Germany	K Starcs
BPI	634864	*ophioglossoides*	*cervinus*	9	x	1910	x	x	F Bubak
BPI	634863	*ophioglossoides*	*asperulus*	8	x	1897	x	x	G Bresadola
BPI	634866	*ophioglossoides*	*cervinus*	x	x	x	Petropolis (=St Petersburg)	USSR	A Jaczewski
BPI	634852	*ophioglossoides*	*cervinus*	10	17	1897	Sjaell, Hornbaek Plantage	Denmark	E Rostrups Svampeamling
BPI	634860	*ophioglossoides*	*sp*	10	x	1888	West Haven	CT	x
BPI	634861	*ophioglossoides*	*sp*	9	x	1907	Chocorua	NH	x
BPI	634854	*ophioglossoides*	*sp*	7	x	1949	x	x	x
BPI	634850	*ophioglossoides*	*sp*	7	24	1949	Shanandoah National Park, Pinnacles Picnic Area	WV	JA Stevenson
BPI	634857	*ophioglossoides*		Summer	x	1907	Algonquin Park	Ontario	JH Faull
BPI	634626	*ophioglossoides*	*sp*	8	30	1939	Pocono Lake Preserve	PA	CB Stifler
BPI	721708	*x*	*pyriformis*	10	2	1899		Hungary	L Hollos
BPI	721709	*x*	*rubescens*	9	18	1900		Hungary	L Hollos
BPI	684657	*x*	*singaporensis*	10	30	1934	Botanic Gardens Jungle	Singapore	Corner
BPI	684522	*x*	*carbonaceus*	3	x	1940	Bukit Timah	Singapore	Corner
BPI	684654	*x*	*piriformis*	9	27	1899	Kecskemet	Hungary	L Hollos
BPI	684658	*x*	*uliginosus*	3	x	1896	Oberfranken, Langheim, near Lichtenfels	Germany	F Rohnfelder
BPI	722021	*x*	*virgatisporus?*	x	x	x	x	Italy	Mattirolo
BPI	684515	*x*	*aculeatus*	7	x	1900	Kecskemet	Hungary	L Hollos
BPI	684655	*x*	*piriformis*	6	x	1921	Trento	Italy	G Bresadola
BPI	684516	*x*	*aculeatus*	7	x	1900	Kecskemet	Hungary	L Hollos
BPI	634862	*ophioglossoides*	*sp*	9	16	1962	Rind Plantage, near Brande, Jylland	Denmark	C George
Guerora collecton	Guerora 38	*x*	*sp*	9	23	2007	Mojonera, Hidalgo	Mexico	G Guerora
NYBG	x	*agariciformes*	*sp*	1	10	1946	Gainesville	FL	WA Murrill
NYBG	x	*agariciformes*	*sp*	1	10	1945	Gainesville	FL	WA Murrill
NYBG	x	*canadensis*	*sp*	10	-	1898		MA	H Webster

Herb.	#	Parasite	Host	M	D	Y	Locale		collector
NYBG	x	*canadensis*	*sp*	10	29	1899		MA	Pierce
NYBG	x	*canadensis*	*sp*	10	30	1904		MA	SS Crosby
NYBG	x	*canadensis*	*sp*	11	9	1901		MA	GEM
NYBG	MEBB 6386	*canadensis*	*sp*	10	7	1976		MA	HE + ME Bigelow
NYBG	x	*canadensis*	*sp*	12	23	1965		MA	H Hinds
NYBG	MEBB 4132	*canadensis*	*sp*	9	4	1963		NH	HE + ME Bigelow
NYBG	00424283	*capitata*	*sp*	1	31	1969		LA	AL Welden
NYBG	x	*capitata*	*granulatus*	8	x	1975		MA	F Helwig
NYBG	x	*capitata*	*granulatus*	10	x	1910	Calvados	France	M Maine?
NYBG	REH 4978	*capitata*	*sp*	11	5	1986	Santa Rosa de Osos	Colombia	RE Halling
NYBG	01115358	*capitata*	*sp*	7	9	2001	9 32'2" N, 83 48'27"W	Costa Rica	RE Halling
NYBG	00034698	*capitata*	*sp*	6	22	1990	Belmira	Colombia	AE Franco-Molano
NYBG	00424282	*capitata*	*sp*	1	31	1969		LA	AL Welden
NYBG	1268	*capitata*	*sp*	Winter		1886		FL	WW Calkins
NYBG	Herb U lass 3524	*capitata*	*sp*	1	x	1945	x	FL	WA Murrill
NYBG	x	*capitata*	*sp*	10	x	1950		OH	HE Bigelow
NYBG	MEBB 6263	*capitata*	*sp*	9	21	1975		MA	Springfield Museum Foray
NYBG	x	*capitata*	*sp*	10	29	1983	Cranberry Lake Nature Reserve	NY	S Stein
NYBG	x	*capitata*	*sp*	10	14	1984	Mianus Gorge Nature Reserve	NY	S Stein, CT Rogerson
NYBG	x	*capitata*	*sp*	10	29	1899	Weston	MA	Q Pond
NYBG	x	*capitata*	*sp*	Summer	x	1981	Lloyd Cornell Reservation State Park	NY	S Ristich
NYBG	x	*capitata*	*sp*	10	x	1961	Harriman State Park	NY	S Stein
NYBG	x	*capitata*	*sp*	10	15	1969	Mianus Gorge Nature Reserve	NY	CT Rogerson
NYBG	x	*capitata*	*sp*	10	29	1983	Cranberry Lake Nature Reserve	NY	S Stein
NYBG	x	*capitata*	*sp*	10	14	1984	Mianus Gorge Nature Reserve	NY	S Stein

Herb.	#	Parasite	Host	M	D	Y	Locale		collector
NYBG	x	*capitata*	*variegatus*	10	x	1891	Zehlendorf, Berlin	Germany	T Sydow
NYBG	3587* Ellis #?	*capitata*	*variegatus*	10	x	1891	Zehlendorf, Berlin	Germany	T Sydow
NYBG	00424285	*capitata*	*sp*	1	16	1988		LA	CL Ovebro
NYBG	00424285	*capitata*	*sp*	1	16	1988		LA	CL Ovebro
NYBG	MEBB 2665	*capitata*	*sp*	11	7	1959		MA	HE + ME Bigelow
NYBG	MEBB 3777	*capitata*	*sp*	10	21	1962		MA	HE + ME Bigelow
NYBG	00218411	*capitata*	*x*	4	22	1954		KS	CL Kramer
NYBG	x	*longisegmentis*	*granulatus*	9	26	1989		NC	WR Buck
NYBG	REH 6069	*longisegmentis*	*sp*	11	8	1998	Llanos de Cuiva	Costa Rica	RE Halling
NYBG	00795928	*longisegmentis*	*sp*	6	30	1998	9 33'3" N, 83 40'56" W	Costa Rica	RE Halling
NYBG	00795927	*longisegmentis*	*sp*	12	14	1998	9 42'52" N, 83 58'28" W	Costa Rica	RE Halling
NYBG	no #	*ophioglossoides*	*cervinus*	8	x	1936	x	PA?	CB Stifler
NYBG	Sydow 1280	*ophioglossoides*	*granulatus*	10	x	1881	Zehlendorf, Berlin	Germany	Sydow
NYBG	no #	*ophioglossoides*	*granulatus*	9	24	1891	West Haven	CT	x
NYBG	Allescher 156	*ophioglossoides*	*granulatus*	8	x	1891	Munich	Germany	Allescher
NYBG	x	*ophioglossoides*	*muricatus var. variegatus*	9	24	1971	Lake Sherman	NY	S Smith, CT Rogerson H Haines GJ Samuels, S Faro
NYBG	no #	*ophioglossoides*	*muricatus*	10	x	1952	Slatesville	NY	DP Rogers
NYBG	x	*ophioglossoides*	*muricatus*	8	x	1976	Greenwood	ME	F Helwig
NYBG	Bommer 2387	*ophioglossoides*	*muricatus* : *variegatus*	8	x	1982	Brussels	Belgium	E Bommer M Rousseau
NYBG	00796186	*ophioglossoides*	*sp*	6	24	2003		Costa Rica	RE Halling
NYBG	Baroni 3501	*ophioglossoides*	*sp*	8	16	1978	Highlands Biological Station	NC	T Baroni
NYBG	x	*ophioglossoides*	*sp*	2	12	x	Magnolia Springs	FL	Miss. SB Foray
NYBG	x	*ophioglossoides*	*sp*	8	11	1968	Cades Cove, Great Smoky Mtns. Natl. Park	NC	CT Rogerson
NYBG	x	*ophioglossoides*	*sp*	8	11	1968	Cades Cove, Great Smoky Mtns. Natl. Park	NC	CT Rogerson
NYBG	61-50	*ophioglossoides*	*sp*	8	6	1961	Highlands Biological Station	NC	CT Rogerson
NYBG	Rogerson 1905	*ophioglossoides*	*sp*	9	5	1947	Highlands	NC	RH Peterson CT Rogerson

Herb.	#	Parasite	Host	M	D	Y	Locale		collector
NYBG	x	*ophioglossoides*	*sp*	8	8	1961	Swain County, Great Smoky Mtns. Natl. Park	NC	RH Peterson + CT Rogerson
NYBG	x	*ophioglossoides*	*sp*	8	17	1964	Highlands Biological Station	NC	RH Peterson + CT Rogerson
NYBG	x	*ophioglossoides*	*sp*	8	15	1961	Upper Horsepasture Gorge	NC	RH Peterson + CT Rogerson
NYBG	Anchel 766	*ophioglossoides*	*sp*	8	3	1967	Cherry Valley Gorge	NC	CT Rogerson
NYBG	MEBB 5206	*ophioglossoides*	*sp*	7	30	1968	Conway	MA	HE+ME Bigelow
NYBG	MEBB 5035	*ophioglossoides*	*sp*	8	25	1967	Mt Toby	MA	HE+ME Bigelow
NYBG	MEBB 4810	*ophioglossoides*	*sp*	10	19	1965	Conway State Forest	MA	HE+ME Bigelow
NYBG	MEBB 3118	*ophioglossoides*	*sp*	8	30	1961	Conway State Forest	MA	HE+ME Bigelow
NYBG	Halling 4843	*ophioglossoides*	*sp*	9	5	1986	Indian Lake	NY	HE+ME Bigelow
NYBG	MEBB 6007	*ophioglossoides*	*sp*	7	18	1972	Emerson Hollow	MA	HE+ME Bigelow
NYBG	MEBB 3030	*ophioglossoides*	*sp*	7	23	1961	N Amherst	MA	H Hinds
NYBG	MEBB 3044	*ophioglossoides*	*sp*	7	26	1961	Pittsfield State Forest	MA	HE+ME Bigelow
NYBG	N0027-5	*ophioglossoides*	*sp*	8	30	2001	Roosevelt	NJ	RE Tulloss
NYBG	MEBB 2626	*ophioglossoides*	*sp*	8	30	1959	Mt Toby	MA	HE+ME Bigelow
NYBG	x	*ophioglossoides*	*sp*	9	x	1900	Holbrook	MA	Grinnell
NYBG	x	*ophioglossoides*	*sp*	10	x	1898	Arlington	MA	H Webster
NYBG	MEBB 4980	*ophioglossoides*	*sp*	7	18	1967	Conway	MA	HE+ME Bigelow+J Grow
NYBG	x	*ophioglossoides*	*sp*	9	23	1995	Roundout Reservoir	NY	S Sheine
NYBG	MEBB 2842	*ophioglossoides*	*sp*	9	21	1960	Conway	MA	HE+ME Bigelow
NYBG	x	*ophioglossoides*	*sp*	9	8	1967	2 mi E of Lewis	NY	CT Rogerson + SJ Smith
NYBG	x	*ophioglossoides*	*sp*	9	28	1969	Pack Demonstration Forest of NY, State College of NY	NY	CT Rogerson
NYBG	x	*ophioglossoides*	*sp*	9	6	1975	Canoe Hill, NYBG	NY	CT Rogerson
NYBG	x	*ophioglossoides*	*sp*	8	18	1949	Harvard Forest near Petersham	MA	CT Rogerson

Herb.	#	Parasite	Host	M	D	Y	Locale		collector
NYBG	Halling 3490	*ophioglossoides*	*sp*	8	1	1982	Lincoln, 1 mi W of Walden Pond	MA	RE Halling
NYBG	x	*ophioglossoides*	*sp*	8	7	1982	Alan Seeger State National Area	PA	CT Rogerson
NYBG	Rogerson 2188	*ophioglossoides*	*sp*	8	23	1947	Lloyd-Cornell Reserve	NY	CT Rogerson
NYBG	Rogerson 1698	*ophioglossoides*	*sp*	8	x	1947	Ithaca	NY	V Cutter
NYBG	x	*ophioglossoides*	*sp*	9	19	1970	Camp Arnott of Cornell	NY	CT Rogerson
NYBG	x	*ophioglossoides*	*sp*	9	24	1956	Pound Ridge	NY	DP Rogers
NYBG	KPD 69	*ophioglossoides*	*sp*	9	11	1965	Bloomingdale	NY	
NYBG	MEBB 5840	*ophioglossoides*	*sp*	8	25	1971	Carrabassett	ME	HE+ME Bigelow
NYBG	MEBB 4546	*ophioglossoides*	*sp*	8	18	1964	Bolton	VT	HE+ME Bigelow
NYBG	MEBB 4526	*ophioglossoides*	*sp*	8	14	1964	Mt Mansfield	VT	HE+ME Bigelow
NYBG	MEBB 3737	*ophioglossoides*	*sp*	9	1	1962	Baxter State Park	ME	HE+ME Bigelow
NYBG	MEBB 4725	*ophioglossoides*	*sp*	9	26	1964	Stowe	VT	HE+ME Bigelow
NYBG	MEBB 3736	*ophioglossoides*	*sp*	9	1	1962	Baxter State Park	ME	HE+ME Bigelow
NYBG	MEBB 3761	*ophioglossoides*	*sp*	9	6	1962	Baxter State Park	ME	HE+ME Bigelow
NYBG	MEBB 3704	*ophioglossoides*	*sp*	8	27	1962	Upper Togue Park	ME	HE+ME Bigelow
NYBG	MEBB 3527	*ophioglossoides*	*sp*	8	2	1962	Baxter State Park	ME	HE+ME Bigelow
NYBG	MEBB 4661	*ophioglossoides*	*sp*	9	14	1964	Lake Mansfield	VT	HE+ME Bigelow
NYBG	MEBB 1254	*ophioglossoides*	*sp*	8	8	1953	Miller's Bog, Burt Lake	MI	AH Smith
NYBG	MEBB 3720	*ophioglossoides*	*sp*	8	30	1962	Baxter State Park	ME	HE+ME Bigelow
NYBG	MEBB 4367	*ophioglossoides*	*sp*	7	22	1964	Stowe	VT	HE+ME Bigelow
NYBG	MEBB 3716	*ophioglossoides*	*sp*	8	29	1962	Norcross	ME	HE+ME Bigelow
NYBG	MEBB 4575	*ophioglossoides*	*sp*	8	27	1964	Lake Mansfield	VT	HE+ME Bigelow
NYBG	MEBB 3719	*ophioglossoides*	*sp*	8	30	1962	Baxter State Park	ME	HE+ME Bigelow
NYBG	MeBB 3487	*ophioglossoides*	*sp*	7	27	1962	Millinocket	ME	HE+ME Bigelow
NYBG	MEBB 3735	*ophioglossoides*	*sp*	9	1	1962	Baxter State Park	ME	HE+ME Bigelow
NYBG	MEBB 3639	*ophioglossoides*	*sp*	8	16	1962	Baxter State Park	ME	HE+ME Bigelow
NYBG	MEBB 1457	*ophioglossoides*	*sp*	8	20	1953	Grapevine Pt, UMBS	MI	ME Bigelow

Herb.	#	Parasite	Host	M	D	Y	Locale		collector
NYBG	x	*ophioglossoides*	*sp*	8	11	1971	Spectacle Lake near Mission Hill Cemetery	MI	CT Rogerson
NYBG	x	*ophioglossoides*	*sp*	10	16	1983	Catlin Woods, SW of Litchfield	CT	S + J Sheine
NYBG	x	*ophioglossoides*	*sp*	8	26	1972	Cloquet Forest Research Center	MN	CT Rogerson
NYBG	x	*ophioglossoides*	*sp*	8	5	x	Alstead	NH	Noyes
NYBG	no #	*ophioglossoides*	*sp*	10	x	1896	Shelburne	NH	WG Sturgis
NYBG	no #	*ophioglossoides*	*sp*	9	8	1905	Duck Point Camp near Milo	ME	WA Murrill
NYBG	x	*ophioglossoides*	*sp*	8	29	1981	Bennington	VT	NE Foray Group
NYBG	Lowen 1145	*ophioglossoides*	*sp*	9	21	1996	Natchaung State Forest	CT	R Lowen
NYBG	Halling 3441	*ophioglossoides*	*sp*	9	19	1981	Camp Jewell near Colebrook	CT	RE Halling
NYBG	CTR 81-53	*ophioglossoides*	*sp*	9	19	1981	Camp Jewell near Colebrook	CT	RE Halling
NYBG	00307838	*ophioglossoides*	*sp*	8	15	1931	Bear Island, Lake Temagami	Ontario	SM Pady
NYBG	no #	*ophioglossoides*	*sp*	9	3	1959	Duchesnay	Quebec	CT Rogerson
NYBG	Guzman 1736	*ophioglossoides*	*sp*	10	17	1958	Nevado del Toluca	Mexico	G Guzmán
NYBG	Plowright 596	*ophioglossoides*	*variegatus*	5	x	1875	Lyme	England	CB Plowright
NYBG	MEBB 6234	*ophioglossoides*	*sp*	10	1	1974	Conway	MA	ME Bigelow
NYBG	Lowen 959	*ophioglossoides*	*sp*	9	12	199x	Warrensburg	NY	R Lowen with R Korf & students
NYBG	x	*ophioglossoides*	*sp*	9	25	1983	Colebrook	CT	COMA Foray
NYBG	64-56	*parasitica = ophioglossoides*	*sp*	8	6	1964	Topinabee	MI	CT Rogerson
NYBG	00307839	*parasitica = ophioglossoides*	*sp*	8	12	1931	Lake Temagami	Ontario	SM Pady
NYBG	00307837	*parasitica = ophioglossoides*	*sp*	8	14	1930	Lake Temagami	Ontario	SM Pady
NYBG	no #	*parasitica = ophioglossoides*	*sp*	8	15	9131	Bear Island, Lake Temagami	Ontario	RF Cain
NYBG	01115362	*sp*	*sp*	7	9	2001	9 32'2" N, 83 48'27"W	Costa Rica	RE Halling
NYBG	00815472	*sp*	*sp*	6	2	2004	9 36'13" N, 84 47'26" W	Costa Rica	RE Halling

Herb.	#	Parasite	Host	M	D	Y	Locale		collector
NYBG	00796173	*sp*	*variegatus*	6	25	2003	9 41'56" N, 83 56'31" W	Costa Rica	RE Halling
NYBG	01115365	*sp*	*sp*	7	1	2001	9 32'2" N, 83 48'27"W	Costa Rica	RE Halling
Univ. de Guadalajara	LGD 4039	*capitata*	*sp*	9	11	1987	San Sebastian, Jalisco	Mexico	L Guzmán-Dávalos
Univ.de Guadalajara	LGD 5875	*capitata*	*sp*	8	28	1994	Tapalpa, Jalisco	Mexico	L Guzmán-Dávalos
Uni. de Guadalajara	IBUG 507	*ophioglossoides*	*sp*	8	24	1974	La Cebado, Jalisco	Mexico	J Manzi
Univ. de Guadalajara	LGD 7640	*x*	*sp*	10	12	1998	Puerto del Pino, Queretaro	Mexico	L Guzmán-Dávalos
Univ. de Guadalajara	LGD 5876	*x*	*sp*	8	28	1994	Tapalpa, Jalisco	Mexico	L Guzmán-Dávalos

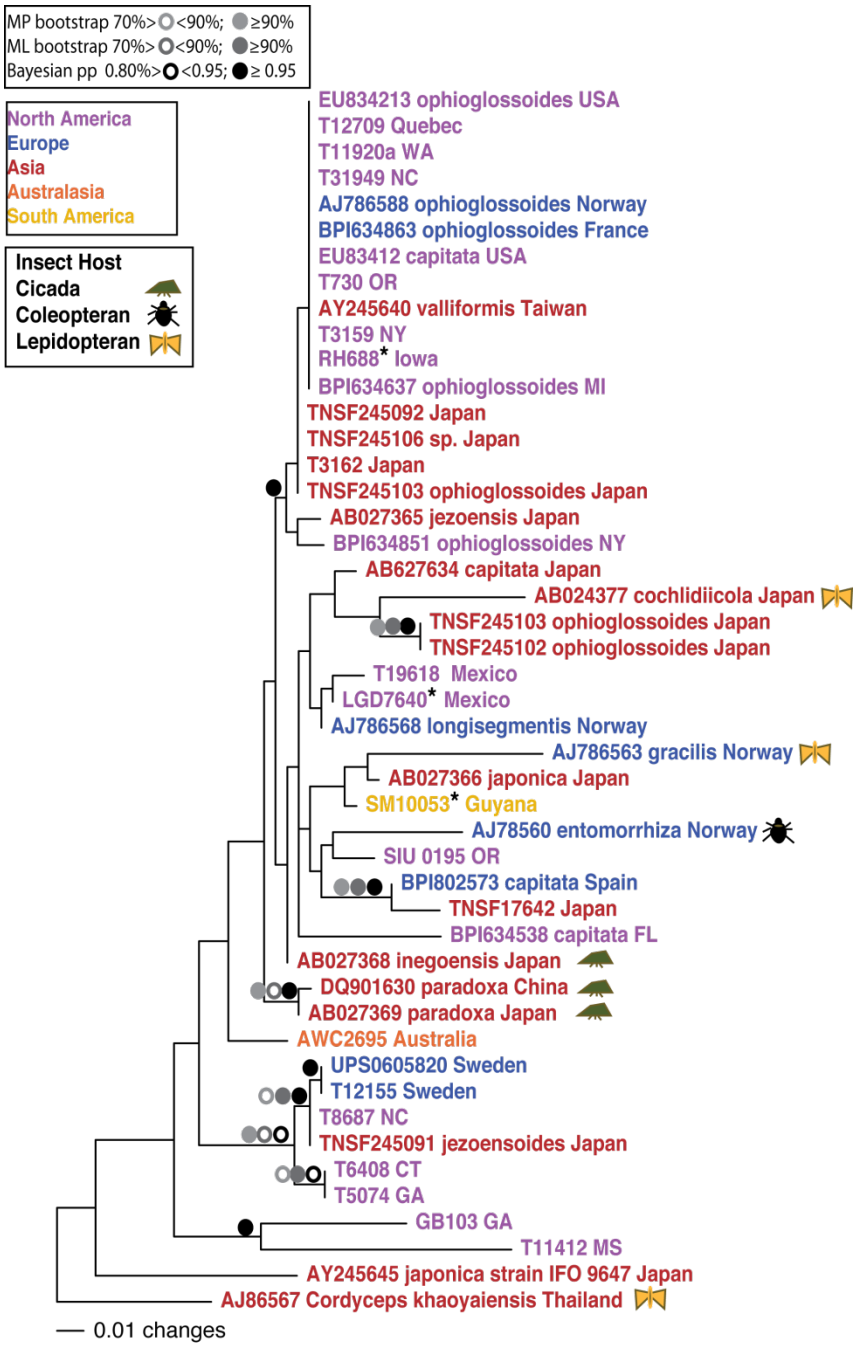

Figure 17. Best Maximum Likelihood phylogeny of *Elaphocordyceps* (LnL=-1321.29832). Parasites were found on *Elaphomyces* unless otherwise indicated. Asterisks indicate specimens known to lack *Elaphocordyceps* fruiting bodies.

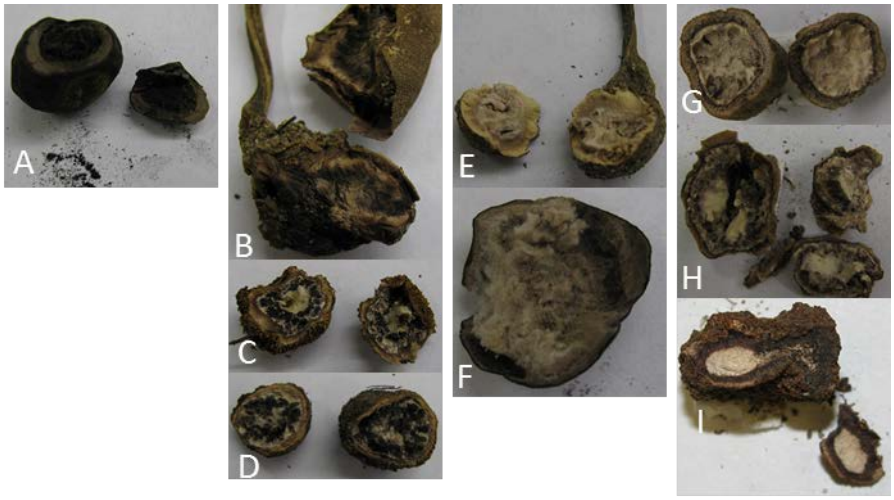

Figure 18. Glebal development of *Elaphomyces*, showing a range of maturation states. A: (Code = 1) filled with powdery spores. B-D: (Code = 2) Shows dark sporulation through most of the specimen. At the most mature, it is filled with dark, but not loose or powdery, spores. E-F: (Code = 3) Most of the specimen is turning pink or pale brown. G-I: (Code=4) Most or all of the specimen tissue is white and shows little to no areas of spore generation.

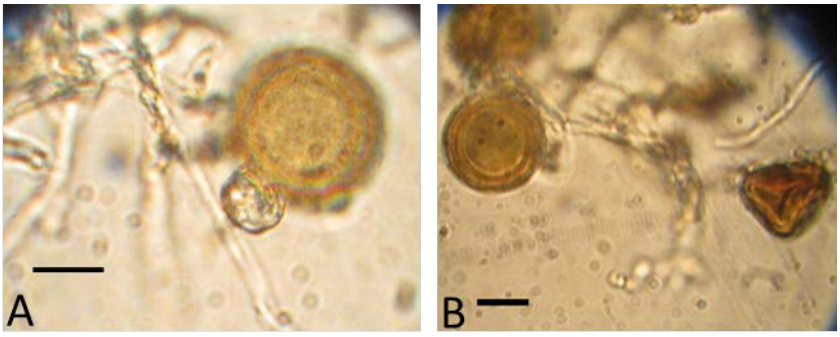

Figure 19. *Elaphocordyceps* hyphae found attached to immature *Elaphomyces* spores from unidentified specimen. A: Appressoria visible on immature spore. B: Hyphae contacting spores, one of which is collapsed.

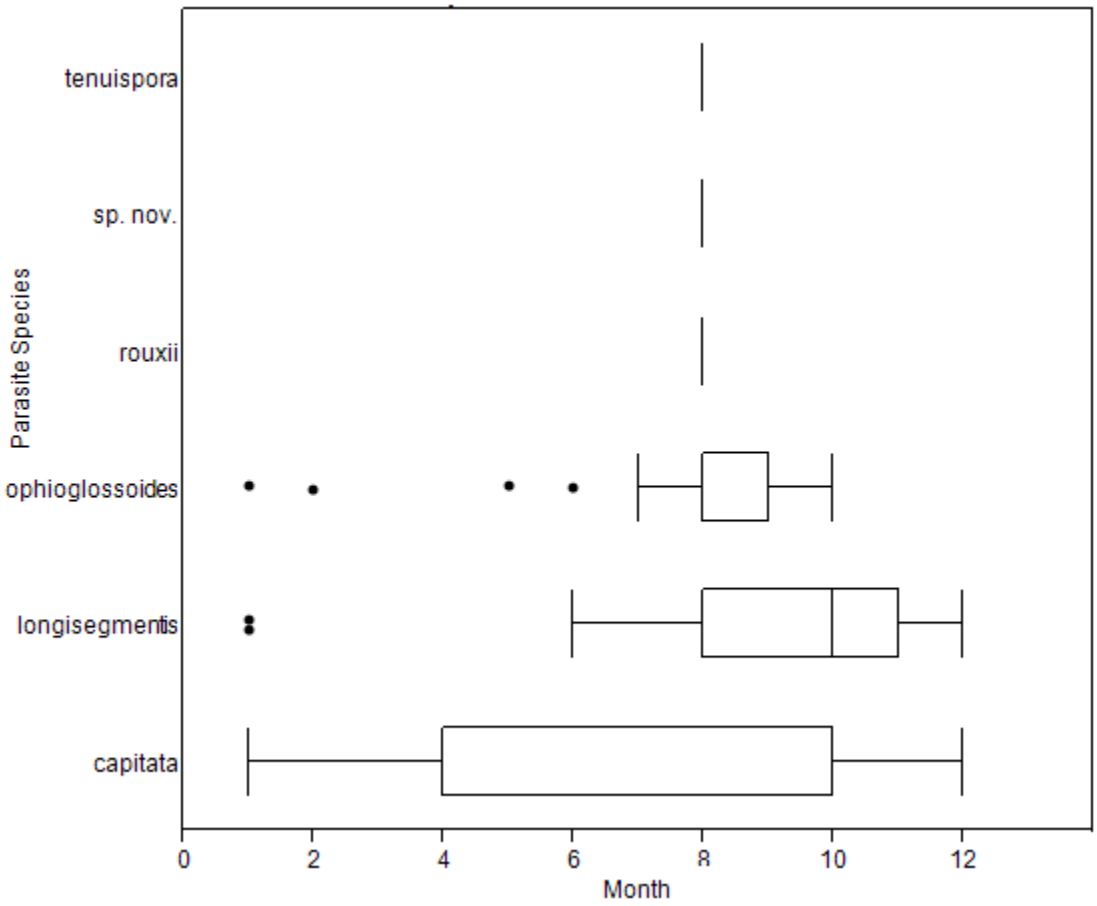

Figure 20. ***Elaphocordyceps*** **collection seasonality. The top 3 species are represented by only one collection, and fall in the typical range of** ***E. ophioglossoides*****,** ***E. longisegmentis*****, and** ***E. capitata*****.**

Table 5. Climate distribution of examined *Elaphocordyceps* collections. Trewartha climate codes: (Ar = Tropical Wet, Aw = Tropical Dry, Cf = Subtropical humid, Dca = Temperate Continental (south), Dcb = Temperate Continental (north), Do = Temperate Oceanic, E = Sub-arctic, H = Highlands)

	Ar	Ar/Aw	Aw	Cf	Dca	Dcb	Do	E	H
capitata	**1**	0	**2**	**9**	**19**	**2**	**3**	**1**	0
longisegmentis	0	**1**	**1**	**2**	**3**	**6**	**3**	0	**1**
ophioglossoides	0	**1**	0	**10**	**37**	**41**	**8**	**9**	**2**
rouxii	0	0	0	0	0	0	0	**1**	0
tenuispora	0	0	0	0	0	0	0	**1**	0
sp.	0	0	3	0	0	**1**	0	0	0

Table 6. Host associations of *Elaphocordyceps* collection. Rows: parasite, Columns: host.

	asperulus	*granulatus*	*muricatus*	*virgatosporus*	*sp.*	**absent**
capitata	0	**6**	0	**1**	27	1
longisegmentis	**1**	**2**	0	0	12	0
ophioglossoides	**5**	**8**	**8**	0	87	3
rouxii	0	**1**	0	0	0	0
tenuispora	0	0	0	0	1	0

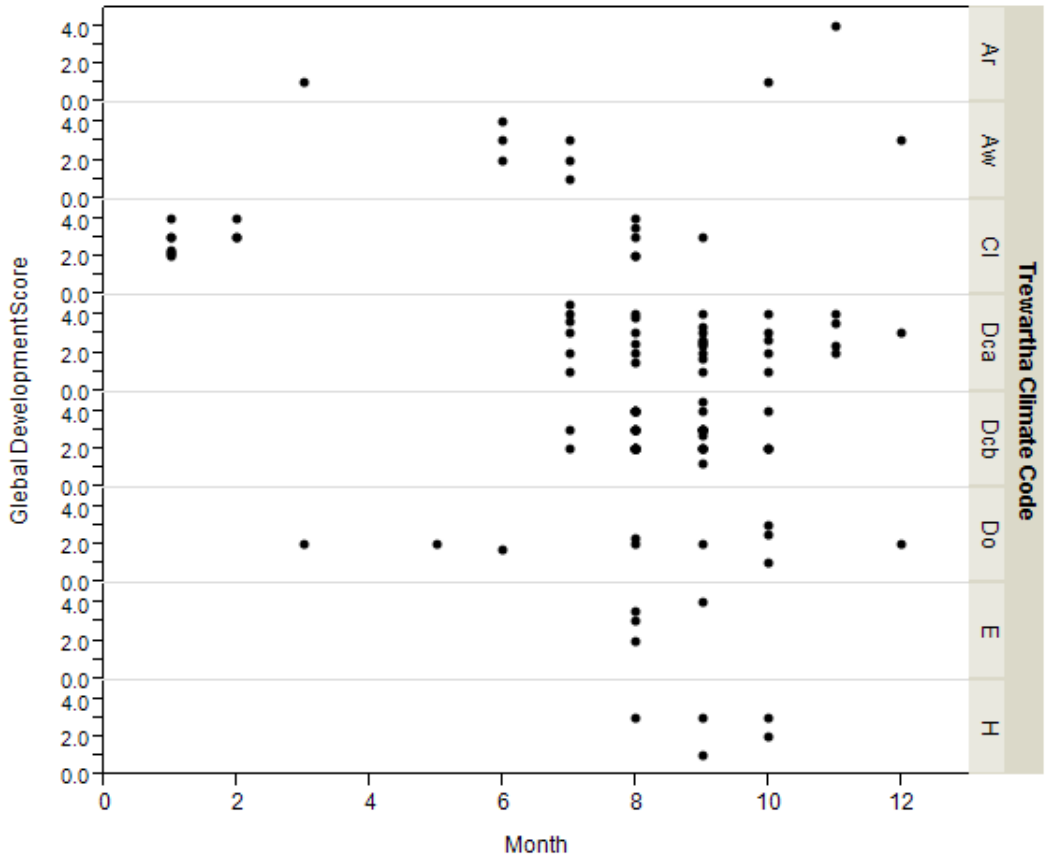

Figure 21. Glebal development (1= fully developed, 4 = least developed) by month and climate zone indicates year-round development.

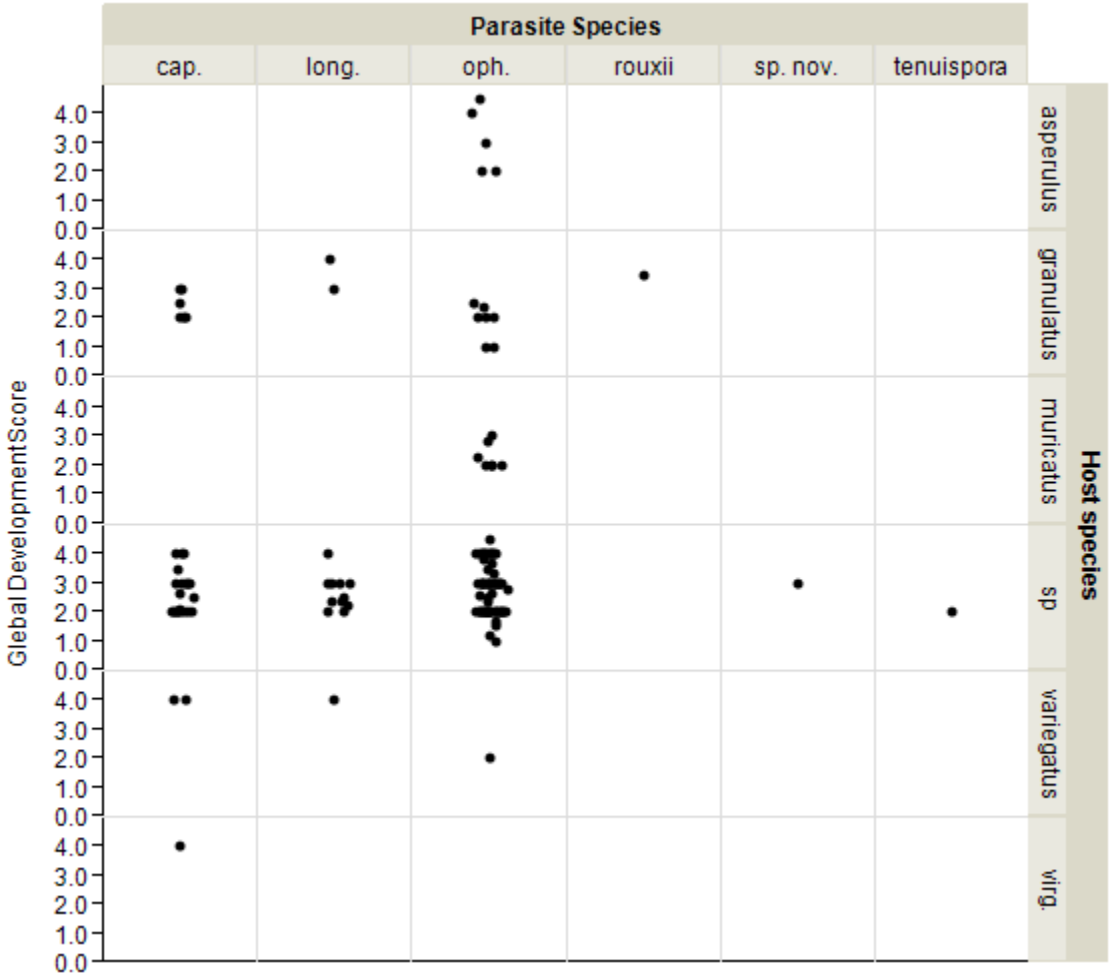

Figure 22. Glebal development (1 = fully developed, 4 = least developed) by host and parasite species significantly varies according to host, but not parasite, species.

Chapter 4. Testing powdery-spored truffles for air-dispersal potential: do hypogeous fungi employ a dual dispersal strategy?

4.1 Introduction

Truffles, fungi that form subterranean fruiting bodies, are generally thought to be dispersal-limited compared to air-dispersed because of their dependence on mycophagous animal vectors. The apparent limitations on their dispersal may not be particularly severe, however, for three main reasons. First, animal based-dispersal may be a highly efficient strategy over a small scale, and may also allow long-distance dispersal. Small mammals may be highly effective dispersers over their foraging ranges of several hundred meters **[207]** . Animal vectors could traverse hostile areas to connect patches of appropriate habitat. Ectomycorrhizal truffles might further benefit from animals defecating near the roots of ectomycorrhizal host trees while they forage **[208]**. Truffles eaten by animals with large foraging ranges or migration paths may also experience long-distance dispersal. For example, *Phaeangium lefebvrei* is excavated and eaten by migratory birds **[209]**. Second, the truffle may have an asexual stage with an alternate mode of dispersal. In the Pezizales, there have been recent reports of diverse truffle genera that form anamorphs on the soil surface and may be dispersed through air or splash dispersal (R. Healy, pers. comm.) Finally, there are truffles that, though dependent on animals for the initial excavation, might additionally have the capacity for

air dispersal. When the truffle *Geopora cooperi* was broken open, it was found to forcibly discharge its spores from operculate asci **[210]**. *Elaphomyces* truffles have prototunicate (spherical) asci that break down before the truffle matures, and thus lack a mechanism for spore-shooting, but form powdery spores that could be passively air-dispersed. Molecular phylogenetics indicates that multiple long-dispsersal events have occurred in *Elaphomyces* (Chapter 2). Here, we investigate the dispersal avenues for *Elaphomyces*. We review the diversity of its mycophagists and investigate 1) how spores from *E. morettii* compare with *Calvatia cyathiformis* in short-range air dispersal and 2) whether large *Elaphomyces* spores could theoretically remain in the turbulent air column long enough for long-distance dispersal. The spore deposition pattern for *Elaphomyces* and *Calvatia* was not significantly different overall, and calculations based on previously published spore terminal velocities indicated that *Elaphomyces* spores are within suggested size limits for long-distance dispersal via air. The darkly pigmented spores may survive UV radiation in the atmosphere, but it is possible that *Elaphomyces* spores cannot germinate without first being partly digested by a mycophagist.

4.1.1 Mycophagy of *Elaphomyces*

Fungi eaten by multiple mycophagists occupying different habitats could be better dispersed in a patchy environment **[211]**. The truffle *Elaphomyces* is found associated with diverse tree hosts on every continent but Africa and Antarctica and is

consumed by multiple species of small mammals and some large mammals. As these animals have different foraging patterns and territory sizes, it may therefore be dispersed over a wide range to multiple areas.

In North America, *Elaphomyces granulatus* is a major component of the subterranean community in several geographic locations and has been suggested as the most common truffle **[212]**. In Arizona, it was found in the spring and fall in Ponderosa pine forests **[213]** and abundantly in the spring in Oregon Douglas-fir forests **[214]**. *Elaphomyces muricatus*, found at a lower frequency than *E. granulatus*, was also a common truffle in Oregon Douglas-fir forests, representing over 5% of the total biomass of truffles collected. This abundance of fruiting bodies in multiple seasons may explain why the globose, ornamented, pigmented spores of *Elaphomyces* have been identified frequently in studies of the northern flying squirrel *Glaucomys sabrinus*, which has a diet largely based on fungi. It was found in *G. sabrinus* scat in the California Sierra Nevadas in the spring **[211]**. In old-growth *Picea sitchensis* and *Pseudotsuga heterophylla* forests on Prince of Wales Island, Alaska, *Elaphomyces* spores were the most common fungal spore in *G. sabrinus* feces in the spring and were in the top five most common fungal spores in the fall **[215]**. *Elaphomyces* was the truffle most consumed in the spring and fall by the endangered West Virginia northern flying squirrel, *G. sabrinus fusca* in a mixed forest in the Monangahela National Forest **[216]**. In a northern Albertan mixed forest, it was also

found in *G. sabrinus* feces in the winter and summer **[217]**, and was found at relatively low numbers in *G. sabrinus* scat in the summer in the South Dakota Black Hills in mixed deciduous/conifer forests **[218]**. The year-round importance of this truffle as a mycophagist extends its season of dispersal.

Elaphomyces spores have been found in the scat of several other small mammals in the Northern and Southern Hemispheres. *Elaphomyces* spores were found in the feces of red squirrel in Alberta, *Tamiasciurus hudsonicus* ***[217]***, of pika (*Ochotona princeps*), yellow-pine chipmunks (*Eutamias amoenus*), and hoary marmots (*Marmota caligata*) in the Cascade Mountains (Cázares & Trappe, 1994), of the lodgepole chipmunk *Neotamias speciosus* in the California Sierra Nevadas **[211]**, and of the woodland jumping mouse *Napaeozapus insignis* in mixed mesophytic and Eastern hemlock forests in the southern Appalachians **[219]**. In Lithuania, *Elaphomyces* was the dominant spore type found in the scat of four different small mammals: mice (*Apodemus spp*.), bank voles (*Myodes glareolus*), common shrews (*Sorex araneus*), and pygmy shrews (*Sorex minutus*) **[220]**. *Elaphomyces* is also consumed by marsupials. Unidentified *Elaphomyces* spores were found in the scat of the Tasmanian rat-kangaroo *Bettongia gaimardi* **[221]**, and 11 species of *Elaphomyces* were found in northern bettong scat (*Bettongia tropica*) in north-eastern Australia **[222]**. In a study in eucalyptus-dominated sclerophyll forests, it was found in scat of three different marsupials: the northern bettong (*B. tropica*), the rufous bettong

(*Aepypyrmnus rufescens*), and the northern brown bandicoot (*Isoodon macrourus*) **[223]**. Two distinct *Elaphomyces* species were found in stomach samples from the lesser forest wallaby (*Dorcopsulus vanheurni*) in a *Nothofagus* forest on New Guinea **[224]**.

Large mammals are also known to eat fungi, and *Elaphomyces* is one of the top hypogeous fungi consumed. In fact, the common name of *Elaphomyces*, the stag truffle, refers to the folklore reports of its popularity with deer **[148]**. *Elaphomyces* was the most common truffle genus and the fourth most common fungal genus found in the feces of roe deer (*Capreolus capreolus*) in Slovenia **[225]**. In the Palatinate Forest in Germany, the stomach contents of wild boars (*Sus scrofa*) contained remains of *Elaphomyces* to an extent that the authors estimated the boars had eaten up to 300 g of truffles **[226]**.

Truffles in general are considered to be low-quality food sources for rodents, but marsupials may be able to better digest them. A study on the effects of diet in the marsupial *Bettongia gaimardi* found that body condition and pouch young growth tended to be higher on a fungal diet rather than a fruit-heavy diet **[227]**. The long-nosed potoroo (*Potorous tridacytlus*) was able to digest 72% of nitrogen in the truffles *Mesophellia glauca* and *Rhizopogon luteolus*, possibly because of its fermenting foregut **[228]**. *Elaphomyces* specifically has been indicated as a poor food source for some of its mycophagists both through observation that some rodents consume it in low quantities compared to other fungi despite its relative abundance and through direct feeding tests. While it was

clearly a part of the diets of the northern flying squirrel and lodgepole chipmunk, it was consumed in low quantities compared to its abundance relative to other truffles **[211]**. The same pattern was seen in the Tasmanian rat-kangaroo *Bettongia gaimardi* **[221]**. When golden-mantled ground squirrels (*Spermophilus saturatus*) were fed *Elaphomyces granulatus* for one week, they lost body mass **[206]**. The truffles had similar nitrogen and energy content to pine nuts and Douglas fir seeds, but only 50-56% digestibility. The authors proposed that *Elaphomyces* may serve as a low-quality food source when high-quality foods are in low supply, and that small amounts of high-quality foods could supplement a truffle diet. In addition to its low digestibility, *Elaphomyces* has also been found to be a source of radioactive cesium; in a German forest contaminated by fallout from Chernobyl, *Elaphomyces* fruiting bodies had high levels of radioactive cesium and were found in the stomachs of boars whose meat was most contaminated **[226]**. High mercury levels in roe deer were found to coincide with diversity of fungal spores in the deer scat and with the months fungi were abundant **[225]**. Fungi are known to sequester pollutants, and it is likely that *Elaphomyces* in other polluted locations serves as a source of contamination for its mycophagists. Thus, *Elaphomyces* seems both low in available nutrients and, at times, a dangerous food source for many of its animal vectors, but by its prevalence in the ecosystem, it is able to attract a wide range of mycophagists.

Animal-dispersed fungi make spores that must remain viable after passage through the mycophagist gut, but the effect of digestion on the spores is not well understood. Passage through the gut may 1) have no effect on spore germination being simply a dispersal mechanism 2) could speed germination by altering spore physical structure or providing chemical cues or 3) could be required for the spore to germinate. Tests on the germination ability of spores in animal scat give contradictory evidence as to the role of the gut. *Glomus* spores were able to germinate from the feces of the Oregon vole (*Microtus oregoni*), while those of *Hymenogaster* in the feces of a chickaree (*Tamiasciurus douglasi*) did not **[229]**. The authors noted one of the primary difficulties with using germination tests to understand spore viability: the ectomycorrhizal truffles can be notoriously difficult to germinate, and may require special cues from the plant host. Seedlings of *Eucalyptus calophylla* and *Gastrolobium bilobum* inoculated with fecal pellets from the rat kangaroo *Bettongia penicillata* formed ectomycorrhizae, while seedlings inoculated with fresh *Mesophyllia* spores did not **[230]**. The same effect was seen using feces from the long-nosed potoroo (*Potorous tridactylus*) and fresh *Mesophyllia pachythrix* spores on *Eucalyptus sieberi* and *E. globoidea* **[231]**. In both studies, fecal pellets contained multiple fungal spores, with *Mesophyllia* as the most common spore type, so it is possible that the mycorrhizae were from another fungus.

Elaphomyces appears to have increased germination rates after passing through an animal. *Elaphomyces granulatus* germinated at much higher rates in golden-mantled ground squirrel (*Speromphilus saturatus*) feces if they had passed through the digestive tract; 70% of the consumed spores ruptured or germinated after 1 year of storage, while no change was seen in spores taken from fruiting bodies **[206]**. This indicates that *Elaphomyces* spores require passage through the gut in order to germinate, but it is also possible that some other mechanical or chemical cue could prepare the spore for germination. Fungal spores exposed in the spore bank are subject to a variety of mechanical stresses, temperature changes, and chemical cues from their plant hosts that are absent in lab trials comparing spores from feces and spores from fruiting bodies.

4.1.2 Possibility of Air-Dispersed Truffles

It is clear that *Elaphomyces* truffles are excavated and consumed by a diverse set of mycophagists in habitats around the world, but they may also have the capacity for passive air dispersal. There are two types of air-dispersed fungi: actively-dispersed fungi that have some spore-shooting mechanism that propels spores into the air column, and passively-dispersed fungi that have dry, powdery spores that are picked up by the wind. The passively-dispersed fungi include puffballs (e.g. *Lycoperdon*, *Geastrum*, *Calvatia*, *Scleroderma*), uredospores from rust fungi, and downy mildews (e.g. *Phytophthora infestans*), and numerous micro-fungi (*Penicillium*, *Aspergillus*, *Alternaria*)

[232]. Scientists observing the powdery spores of *Elaphomyces* have speculated that *Elaphomyces* spores could be passively dispersed through air (Trappe, Castellano pers. comm.).

Mycophagists may carry fungi into trees to eat them, which could improve the chances of truffles being air-dispersed. One of the advantages of spore-shooting is that it helps spores escape the layer of calm air near the ground and move into the turbulent air that could help them disperse. Actively dispersed fungi must overcome the drag on the spores to help them escape. There are four typical ways fungi have adapted to conquer drag: 1) propel smaller spores at higher speeds, 2) make larger spores to lower drag, 3) shoot individual spores simultaneously and 4) puff packets of spores **[233]**. The size of the boundary layer depends on the location, habitat, and weather; on a forest floor on a still day, the boundary layer could be up to 30 cm, while it would be lower on a plant leaf in the wind. It would therefore be easier for *Elaphomyces* spores to enter the turbulent air if they were taken into trees and eaten than if they were consumed on the forest floor. A red squirrel (*Tamiasciurus hudsonicus*) cache containing 52 *Elaphomyces granulatus* truffles was found in an abandon robin's (*Turdus migratorius*) nest in New Brunswick approximately 2 meters above the ground **[234]**. Early reports of fungus-cacheing by the North American red squirrel (*Tamiasciurus hudsonicus*) indicated that this squirrel commonly stores mushrooms in holes and branches of trees **[235]**, and

Ingold recorded an incident of a grey squirrel dropping a partially eaten *Elaphomyces* truffle from a tree **[236]**. These reports are important because they indicate that truffles could more easily enter the turbulent air than if they were only eaten on the ground. Squirrels fed *Elaphomyces granulatus* avoided the spore mass; 90% of what they consumed was peridium tissue **[206]**. If the spores are routinely rejected by mycophagists, they would be left exposed to the elements and could behave similarly to fungi that depend exclusively on passive air dispersal.

Elaphomyces has not been reported from air spore sampling, it may have been present but unidentified due to problems with its identification. Studies on fungal spores in air use either culture-based or spore morphologies to identify fungal genera. Like many other ectomycorrhizal fungi, *Elaphomyces* is difficult to culture in the absence of its host, and would thus not be discovered with culture-based spore traps. Additionally, many studies do not identify all ascospores to genus, but identify the most common fungal genera, typically *Cladosporium* and *Alternaria*, grouping the rest of the rare ascospores together **[237-240]**.

Our aim was to test the capacity of *Elaphomyces* for passive air dispersal by quantifying spore deposition when spores were released into the air. This would test how spores behaved under ideal circumstances. We used the giant puffball *Calvatia cyathiformis*, a passively air-dispersed saprobe, as a control. *Calvatia* is dispersed when

the exoperidium dehisces, exposing the spore mass. It has been found to persist in the air in high numbers. In Spain, a spore trap placed at 15 m found that *Calvatia* spores were the seventh most common basidiomycete spore type, made up 1% of the basidiospore sample, and were found 319 days over a 2 year sampling period **[241]**. *Calvatia*, clearly well-adapted for air dispersal, is an ideal control both because its globose, ornamented spores are shaped similarly to *Elaphomyces*, spores and because it produces huge quantities of spores that would allow repeated tests.

Fungal spore size could potentially limit their dispersal range if the spores settle out of the air quickly. Models of spore long-distance dispersal found that the distance a particle could travel increased decreased with particle terminal velocity **[242]**. The terminal velocity, or rate at which a particle settles in a fluid, is derived from Stokes' Law:

$$v = 2r^2g(\rho_s * \rho_a) / 9\mu$$

where r is the spore radius, g is the acceleration due to gravity (981 cm/s^2), ϱ_s is the sphere density, ϱ_a is the air density, and μ is the viscosity of the fluid. Thus, terminal velocity increases with the square of the radius, indicating that a spore twice as large as another will settle out of still air four times faster. This is important in understanding spore dispersal because if spores are lifted by air currents into the mixed layer of the atmosphere, they can travel hundreds to thousands of kilometers in a very

short time **[242]**. Models of particles in the atmosphere found that over 50% of spores with terminal velocities of 0.04 m/s^2 and lower released from a height of 9.8 m would still be airborne after 1000 meters **[242]**. Terminal velocities of air-dispersed fungi and myxomycetes in one empirical study ranged from .00035 to .00385 m/s^2 for spores ranging from 3.89 to 12.64 μm in diameter **[243]**. *Elaphomyces* spores range from 8 to over 40 μm and would thus have much higher terminal velocities. We used an exponential equation to calculate the terminal velocities for 5 sizes of fungal spore within the range of *Elaphomyces* using published terminal velocities for fungi and myxomycete spores.

4.2 Materials and Methods

4.2.1 Short Distance Dispersal Tests

The capacity for *Elaphomyces morettii* spores to disperse over short distances was tested indoors using a series of spore traps. The highest point of the ceiling in the room was 2.36 m. All spores were taken from two collections: spores of *E. morettii* specimen SB-2-08-2 (Collector ID: "*E. granulatus*" Coll. S. Björnefeldt Feb 2008 in Stadsskogen, Uppsala, Sweden) and of *Calvatia cyathiformis* (Duke Herbarium #HN1072, Coll. R. Vilgalys 15 Sep. 1988, Durham, NC). The *Elaphomyces* collection contained several truffles that were individually too small for the entire experiment, so we combined spores from 2 truffles with diameters of 2 cm and 3 cm for the dispersal tests. The *Calvatia* specimen contained two large puffballs that still had a fairly fibrous gleba. To

separate the spores from the fibers as much as possible, small amounts of glebal tissue from one fruiting body (H = 11 cm, D = 9 cm) were agitated repeatedly. We measured the spore diameters of 10 spores from each specimen at 1000x magnification. To make spore traps, we cut transparency film to 2.7 x 7.6 cm strips and coated the central 4 cm^2 with petroleum jelly. The spore traps were placed horizontally 0 m, 0.1 m, 0.2 m, 0.3 m, 0.5 m, 1 m, 2 m, 3 m, 4 m, 5 m, 6 m, 8 m, and 10 m distance from the release point. All spore traps were 95 cm from the floor. For each trial, 1 mL of spores was briefly homogenized in a plastic bag. A corner was cut from the bag, and it was held open in front of a fan, allowing air to blow through and disperse the spores at a height of 23.5 cm above the spore traps. This height was chosen to maximize the initial air speed. The fan was run for 5 minutes and then switched off. The spores were allowed to settle for 10 minutes before the spore traps were covered with transparency film covers. The air speed at each spore trap location was measured with a La Crosse EA3010U handheld anemometer at 0 cm and 23.5 cm height. Measurements were taken every 5 seconds over a 25 second period at each collection point. Depending on spore density, spores were either counted for a 1 cm^2 area or estimated by counting spores in ten 1 mm^2. Spore dispersal trials were repeated for 10 trials for *Calvatia* and 11 trials for *Elaphomyces*.

Statistical analyses were conducted in JMP 9.0. The spore count data were log-transformed and the linear regressions of spore number over distance for *Calvatia* and

Elaphomyces were compared using a combined effect test for distance and species. The difference in variance at each sample point was tested using a 2-sided F test. The means of *Elaphomyces* and *Calvatia* spores at each sample point were compared using the Wilcoxon X^2 test. The cumulative percentage of spores deposited at each spore trap was calculated for each individual trial.

4.2.2 Calculating Terminal Velocity

We recorded the published spore sizes of 54 *Elaphomyces* species to determine the range of spore diameters (Table 7). We estimated the terminal velocity of 7 possible large spore diameters: 20, 30, 40, 45, 50, 55, and 60 μm. We calculated terminal velocity of each based on the spore diameter and terminal velocity for each of the eight species studied in **[243]**. Tesmer and Schnittler had studied the passively air-dispersed puffball *Lycoperdon perlatum* and seven myxomycetes species. We used the following formula for the terminal velocity of the suggested *Elaphomyces* spores:

$$V_{TE} = (D_E/D_S)^2 / V_{TS}$$

where D_E is the diameters of the *Elaphomyces* spore and D_S and V_{TS} are the observed diameter and terminal velocity of the species previously studied. For each of the spore sizes, we calculated the mean terminal velocity and standard deviation, and compared these to the terminal velocities used in dispersal models from **[242]**.

4.3 Results

We measured air speed and spore deposition over 10 m (Figure 23). Air speed declined from an average of 8.18 km/h at the release point to 0 km/h at the 5 m distance trap. At this distance, the breeze generated by the fan could be felt, but was not detectable by the anemometer. Air speed at the level of the spore traps was highest 1 m from the release point, averaging 2 km/h and declining to 0 km/h at 5 m from the release point.

A high number of spores for both species were deposited at 0-10 cm from the release point, and then declined. There was an increase in spore deposition for both species at 200 cm (average log spores = 2.316), which was the point after the air speed at the spore trap level had risen, and after a significant drop in air speed at the release height. *Elaphomyces* deposition then declined linearly, but *Calvatia* deposition dropped sharply at 300 cm (avg. log spores = 1.57) and then declined linearly. The mean number of *Elaphomyces* spores collected was generally higher and less variable than the number of *Calvatia* spores, though the overall effect test for distance*species did not significantly differ (F-ratio = 0.702, p-value = 0.4029). There was higher variance in *Calvatia* spore count at each collection point after 20 cm (Table 8). There were significantly more *Elaphomyces* spores deposited at 300 cm and 500 cm (300 cm: Wilcoxon $X^2 = 7.165$, p-value = 0.0074, 500 cm: Wilcoxon $X^2 = 5.7322$, p-value = 0.0167). At all other distances, the

mean number of spores for *Elaphomyces* and *Calvatia* was not significantly different. At the farthest distance measured (10 m), *Elaphomyces* spores were collected each trial in low numbers (25-50 spores), while *Calvatia* showed a more variable range (3-357 spores). *Elaphomyces* was found on every spore trap in every trial, whereas *Calvatia* had 4 spore traps over the 10 trials for which no spores were seen. On average, the majority of captured spores for both species were deposited within 1 m of the release point, and 80% of the spores were deposited before 2 m (Figures 24-25).

The range of *Elaphomyces* spore diameters for the publications we reviewed was 8—55 μm (Figure 26). The average range was 17.91±7.46 to 22.7±9.37 μm, and the majority of the spores (43 of 54 species, 79.6%) were between 10 and 30 μm in diameter. *Elaphomyces morettii* had a mean diameter of 30.1±6.97μm, and *Calvatia cyathiformis* spores had a mean diameter of 5.9±0.876 μm (mean ± standard deviation). Terminal velocities for large spore sizes calculated from the diameter and observed terminal velocity of smaller spores ranged as follows: (20 μm, 0.0098 ± 0.0013 m/s^2; 30 μm, 0.0221 ± 0.0028 m/s^2; 40 μm, 0.0392 ± 0.0078 m/s^2; 45 μm, 0.04964 ± 0.0063 m/s^2; 50 μm, 0.0613 ± 0.005 m/s^2; 55 μm, 0.0742 ± 0.009 m/s^2; 60 μm, 0.0882 ± 0.011 m/s^2). The calculated terminal velocity for the smaller spores is within the range of terminal velocities allowing easy long-distance dispersal (<0.04 m/s^2) **[242]**.

4.4 Discussion

Over the short distance studied, 10 m, *Elaphomyces morettii* and *Calvatia cyathiformis* spores were deposited at rates that were not significantly different. For both species, the amount of deposited spores was highly variable at the spore traps nearest the release point, where most of the spores were trapped. We observed that large, visible clumps of spores fell in the 0-10 cm area, landing on or around the spore traps at random. *Calvatia cyathiformis* also had small pieces of glebal tissue containing spores that landed on some spore traps, greatly increasing the numbers of spores counted at some of the 0-10 cm traps. In counting the spores, we noticed a tendency of *Calvatia* spores to clump in groups of sometimes hundreds of spores, whereas *Elaphomyces* spores were more evenly dispersed. This clumping effect is the major cause of the higher variability of the *Calvatia* spore count, and is likely a product of our experimental method, which extracted spores from *Calvatia* tissue. In the field, the mature puffball loses all structure as the exoperidium dehisces, becoming a dwindling mass of spores that are blown away. It is not generally collected in the fully mature state because of difficulty drying and storing it, and the specimen we used was full of mature spores but still had an exoperidium that was beginning to crack. Using fully matured spores in our tests may have led to more evenly dispersed spores, and therefore lower variance. Fewer spores would have been deposited on the spore traps because more could have remained in the

air; however, it is also possible that the more even mixture of spores would have led to higher deposition at the more distant spore traps. *Elaphomyces*, like *Calvatia*, settled in the highest numbers near the release point, but it was also collected in moderately high numbers at 10 m distant, indicating that there was still a high spore density in the air. The cumulative percentages of deposited spores for both species show that the majority of spores under a low wind are deposited within 1 m of the release point. This limitation of dispersal was also found in outdoor tests of wind dispersal; multiple, independent spore dispersal tests suggested that most spores are deposited locally, within about 60 m of the parent **[232]**.

Much of the research on spore dispersal has investigated active spore shooting mechanisms, and research on actively dispersed spores has application for understanding how all spores are transported in air. Actively dispersing fungi produce high velocities to help spores escape the still air near the spore-generating surface, but because of the high drag on small particles, lose their initial velocity once they have actually reached the turbulent air layer. For example, *Gibberella* spores were calculated to lose 99.997% of its initial velocity of 125 km/h and *Sordaria* spores lost 99.96% of their initial velocity before they escaped the still air **[244]**. The smaller the spores, the higher the initial launch speed must be for the spore to reach the turbulent air **[245]**, but in the air column, actively dispersed spores behave similarly to any other particle in the air,

and their dispersal will be determined by how long they can remain in the air stream and whether they survive the journey.

The shape and size of a spore could potentially affect how far it might ultimately travel. *Elaphomyces* produces ornamented spores with warts, spikes, or ridges. One could hypothesize that these ornamentations could increase drag, but models of spore dispersal indicate that fungal spores and their ornamentations are too small for these differences in texture to change their aerodynamics **[246]**. We calculated spore terminal velocities for 7 sizes of large fungal spores, and found that for the first three sizes (20, 30, and 40 μm), the terminal velocities were below 0.04 m/s^2, which under Kuparinen's model allowed most spores to disperse over 1000 m. These first three spore sizes are within the range of known *Elaphomyces* spores. The *E. morettii* specimen used in this study had an average spore diameter of 30 μm, and under this model, is predicted to have high dispersal ability. The spores of *E. morettii* are larger than the average *Elaphomyces sp*. spore, and we would expect most *Elaphomyces* species have similar or better passive air dispersal abilities. *Elaphomyces nothofagus* from Australia has the largest spores known for this genus, ranging from 43-55 μm in diameter, and would thus have a predicted terminal velocity of about 0.05 to 0.07 m/s^2, giving it a lower expected dispersal ability than the other *Elaphomyces* species. However, even spores as large as this could have high dispersal capacity; the model showed that for spores of

terminal velocity 0.08 m/s², over 40% of spores would still be in the air after 1000 m **[242]**. Kuparinen noted that, for small particles the size of fungal spores, even 100% increases in terminal velocity do not greatly diminish dispersal distance. In a spore key for airborne spores, there were ascospores of a similar size and shape to *Elaphomyces* spores: *Puccinia arachidis* uredospores, *Tilletia holci* and *T. barclayana*, and oospores of *Peronosclerospora sorghi* ***[239]***. *Elaphomyces*, therefore, would be as capable as other fungal spores at traveling long distances should they happen to become airborne.

Release height is also a significant contributor to dispersal distance. In our experiment, the spores were released 23.5 cm higher than the spore traps, and could have drifted up to a maximum height of 2.36 m, the height of the ceiling. The model of the effect of terminal velocity on dispersal was based on a release height of 9.8 m **[242]**. For particles of 0.04 m/s² released at a height of 6 m, 50% had settled at a distance of 200 m, and just over 30% remained airborne at 1000 m. This was the lowest height for which dispersal was modeled, and we hypothesize that most *Elaphomyces* would be released at much lower heights than this, as indeed are many fungi found in the airstream. They are also released at low heights — often as low as a few centimeters from the forest floor. Based on the simple size and shape of its spores, *Elaphomyces* cannot be considered to be disadvantaged in its capacity for air dispersal compared to typically air-dispersing fungi.

The final obstacles facing fungal spores that have reached the turbulent airstream are fluctuating temperatures and intense ultraviolet radiation from the sun. Species and strains vary in their vulnerability to radiation damage. The wheat rust *Puccinia striiformis* had 14—87% germination loss after a day of exposure to natural sunlight, while *P. graminis* and *P. recondita* experienced only 2—28% and 3—70% germination loss, respectively **[247]**. *Aspergillus niger* lost all germination ability after 4 days of continuous exposure to sunlight, while *Alternaria macrospora* had higher survivability until day 5, when it too was no longer viable**[248]**. *Alternaria solani*, *Uromyces phaseoli*, and *Peronospora tabacina* were killed after 4 days of sunlight exposure **[249]**. *Mycosphaerella fijiensis* spores exposed to UV light that imitated the spectrum of natural sunlight were no longer viable after only 6 hours **[250]**. The hostility of the dispersal route may explain why, though spores are plentiful in the atmosphere, trans-oceanic gene flow seems rare in fungi. Studies in the Mediterranean have found high concentrations of diverse fungal spores apparently crossing from Greece and Turkey to Israel **[240]**, and fungal spores from desert soils are frequently transported across oceans in dust clouds that can cross the Atlantic or Pacific in a 3-9 days **[251]**. For instance, though *Schizophyllum commune* spores were common in samples from the Caribbean, molecular population genetics did not indicate trans-oceanic crossings **[252]**.

Fungal spores may have adaptations that can allow their spores to better survive irradiation. Pigmentation and thick-walled spores have been documented to increase spore survival in several cases. Darker sclerotia of *Sclerotium rolfsii* had higher survival rates than paler sclerotia **[248]**. Aerial spores of *Trichoderma harzianum* had thick-walled, dark green spores that were more UV resistant than the thin-walled pale spores from submerged culture **[253]**. Aerial *Paecilomyces lilacinum* spores were also darker and more resistant to UV radiation than submerged spores, and had a spore rodlet layer associated with the pigment **[254]**. In these two cases, it was not known whether both structural layers and pigment contributed to the UV resistance. Darkly pigmented *Penicillium* spores trapped at 20 km in the atmosphere were viable, forming colonies in culture **[255]**. All species of *Elaphomyces* have dark brown to black pigmentation at maturity and thick cell walls. These features, which may also be necessary for surviving passage through the mammalian gut, would also give them a higher chance of surviving dispersal through air.

Another adaptation is to form clusters with multiple cells, whether simple aggregates of spores, or multi-celled sclerotia. Spore clumping allowed better survival for *Alternaria macrospora*, *Aspergillus niger*, and *Mycosphaerella pinodes*, as did the sclerotia of *S. rolfsii*, and survival increased linearly with the numbers of cells in a cluster **[248]**.This is interesting because, as we noted, the spores of *Calvatia* were frequently

clumped. *Calvatia* spores are lightly pigmented, and as such would have little chemical protection against UV radiation. This clumping could be due to the immaturity of the fruiting body, but if even completely mature *Calvatia* have clumps of spores, it could be an adaptation increasing the chance of survival during wind dispersal.

In conclusion, we find that *Elaphomyces* has a high capacity for dispersal through mammalian mycophagy and has the capacity for passive air dispersal. Its wide variety of mycophagists worldwide may be able to disperse it across inhospitable areas to prime habitat. Its powdery spores, which are avoided by its mycophagists, are of an appropriate size for long-distance air dispersal. Over short distances, it has similar dispersal patterns to a known passive air disperser, *Calvatia*. It would gain an additional advantage for long-distance dispersal if first carried into trees by its mycophagists, and instances of this behavior have been noted. While *Elaphomyces* may have the ability to travel to distant locations, it is unknown whether it can actually survive the journey and grow in new environments. Because of its thick spore wall and dark pigments, it may be able to survive exposure to the UV radiation in the air for a few days. However, it may not be able to germinate without first passing through the gut of its mycophagists. Further research on the survivability and germination requirements of *Elaphomyces* spores is necessary to fully understand its dispersal routes and patterns. As any truffle

with powdery spores could have the same ability, it would be ideal for truffle spore keys to be consulted along with spore atlases used in atmospheric studies.

Table 7. Published spore diameters for 54 *Elaphomyces* species.

Species #	Reference	*Elaphomyces species*	Spore Diameter
1	**[172]**	*timgroveii*	8—9
2	**[256]**	*appalachiensis*	7.5—9
3	**[148]**	*atropurpureus*	10
4	**[148]**	*citrinus*	8—10
5	**[257]**	*viridiseptum*	6—10
6	**[172]**	*chlorocarpus*	10—11
7	**[172]**	*rugosisporus*	10—12
8	**[172]**	*symeae*	12—13
9	**[172]**	*cooloolanus*	11—13
10	**[172]**	*pedicellaris*	11—13
11	**[148]**	*mutabilis*	13
12	**[148]**	*immutabilis*	7—15
13	**[148]**	*papillatus*	10—15
14	**[172]**	*aurantius*	14—16
15	**[258]**	*carbonaceus*	15—16.5
16	**[148]**	*aculeatus*	14—17
17	**[259]**	*nopporensis*	15—17.5
18	Kers	*striatosporus*	10—18
19	**[172]**	*coralloideus*	18—20
20	**[148]**	*leucosporus*	17—20
21	**[148]**	*reticulatus*	14—20
22	**[148]**	*echinatus*	20
23	**[148]**	*anthracinus*	17—21
24	**[148]**	*virgatosporus*	16—22
25	**[259]**	*asahimontanus*	13.5—22
26	**[259]**	*miyabeanus*	15—22
27	**[260]**	*guangdongensis*	17—22
28	**[260]**	*reticulosporus*	17—22

Species #	Reference	*E. sp.*	Spore Diameter
29	**[148]**	*leveillei*	20—22.5
30	**[261]**	*trappei*	18.5—23.5
31	**[148]**	*variegatus*	17—24
32	**[262]**	*subviscidus*	14—24
33	**[172]**	*queenslandicus*	24—25
34	**[148]**	*muricatus*	19—25
35	**[148]**	*verrucosus*	22—25
36	**[148]**	*cyanosporus*	23—25
37	**[260]**	*porcatosporus*	16—25
38	**[172]**	*laetiluteus*	23—26
39	**[259]**	*shimizuensis*	15—27
40	**[259]**	*neoasperulus*	17—27
41	**[148]**	*decipiens*	22—28
42	**[148]**	*persooni*	21—28
43	**[172]**	*austrogranulatus*	26—30
44	**[172]**	*suejoyaceae*	26—30
45	**[148]**	*granulatus (=cervinus)*	26—30
46	**[148]**	*morettii*	25—30
47	**[148]**	*foetidus*	24—30
48	**[259]**	*titibuensis*	25—30
49	**[148]**	*septatus*	28—32
50	**[259]**	*nikkoensis*	22.5—32.5
51	**[148]**	*asperulus*	29—34
52	**[259]**	*fragilisporus*	30—40
53	**[148]**	*maculatus*	35—50
54	**[172]**	*nothofagus*	43—55

Table 8. *Elaphomyces* spore deposition has significantly lower variance than *Calvatia* deposition beginning 20 cm from release point.

Distance (cm)	***F Ratio***	***p-value***
0	1.7801	0.3818
10	2.5571	0.1598
20	5.3247	0.0153*
50	7.2035	0.0048**
100	21.935	<0.0001***
200	31.369	<0.0001***
300	43.3747	<0.0001***
400	30.7155	<0.0001***
500	13.6381	0.0003***
600	8.6038	0.0024**
800	20.7306	<0.0001***
1000	33.9369	<0.0001***

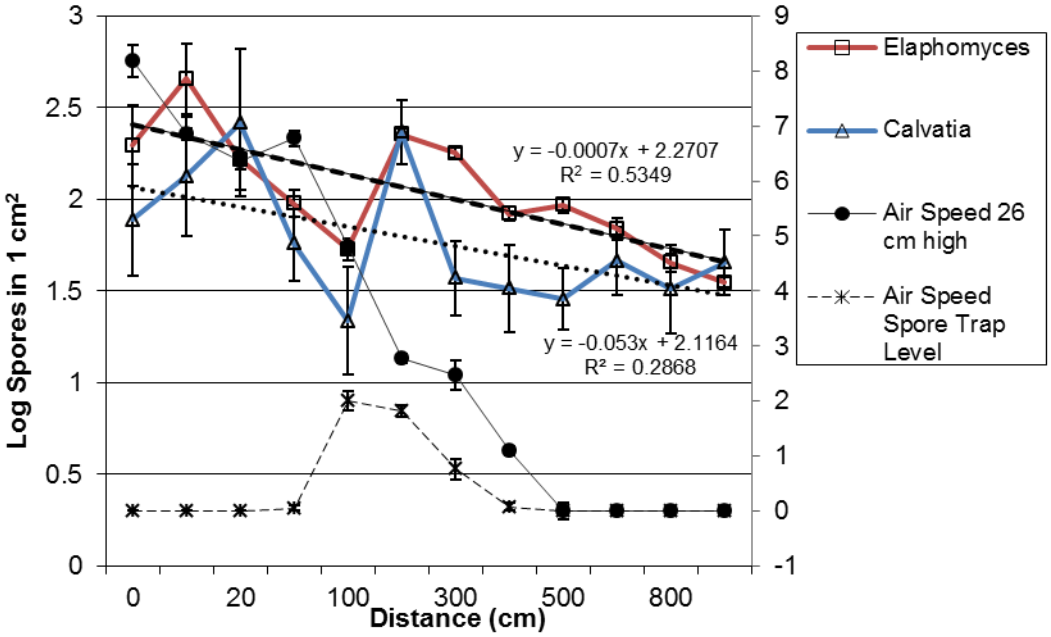

Figure 23. Spore deposition is similar for *Elaphomyces* and *Calvatia*.

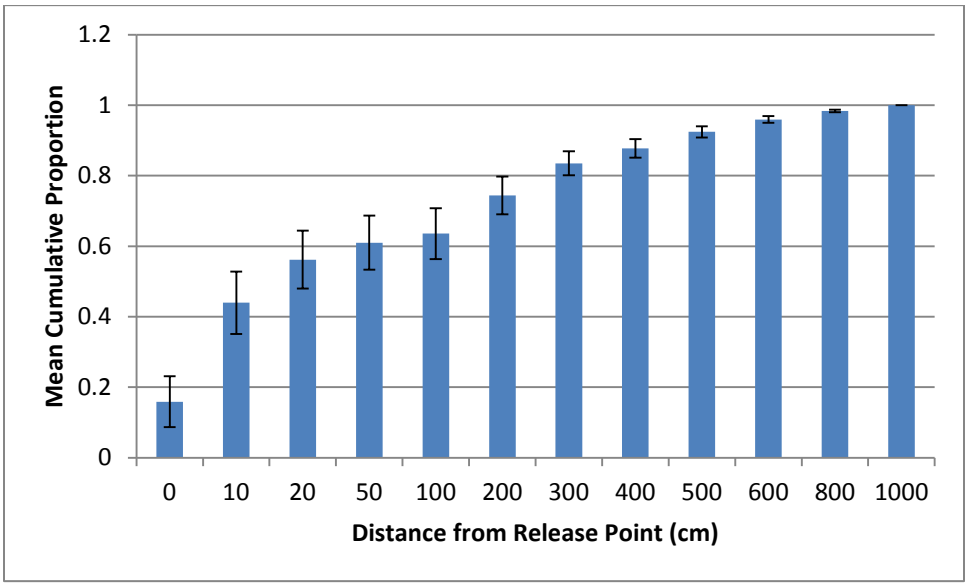

Figure 24. ***Elaphomyces*** **cumulative spore deposition shows 60% of captured spores are deposited within 1 meter of the release point. Bars show standard error.**

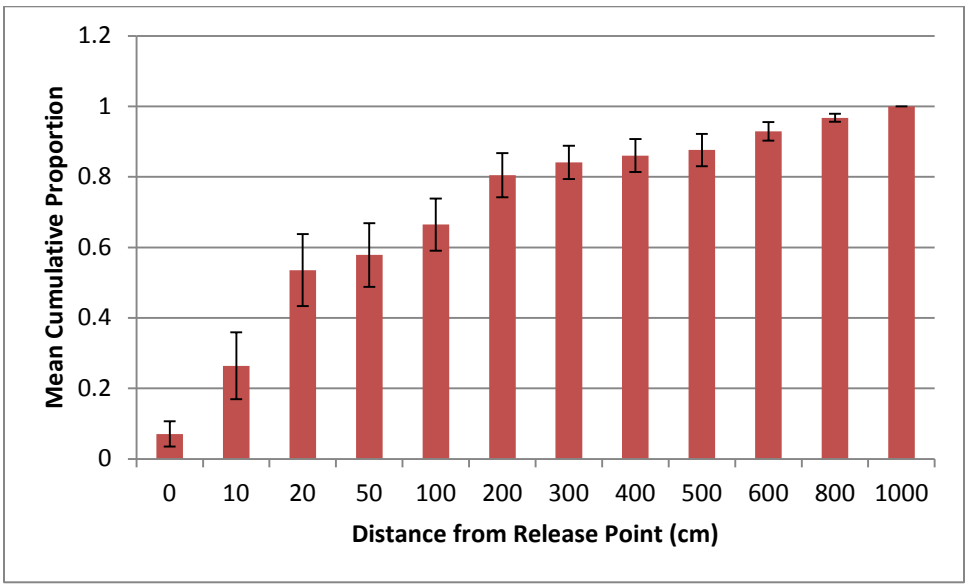

Figure 25. ***Calvatia cyathiformis*** **cumulative proportion of spore deposition shows over 60% of deposited spores fall within 1 m from the release point. Bars show standard error.**

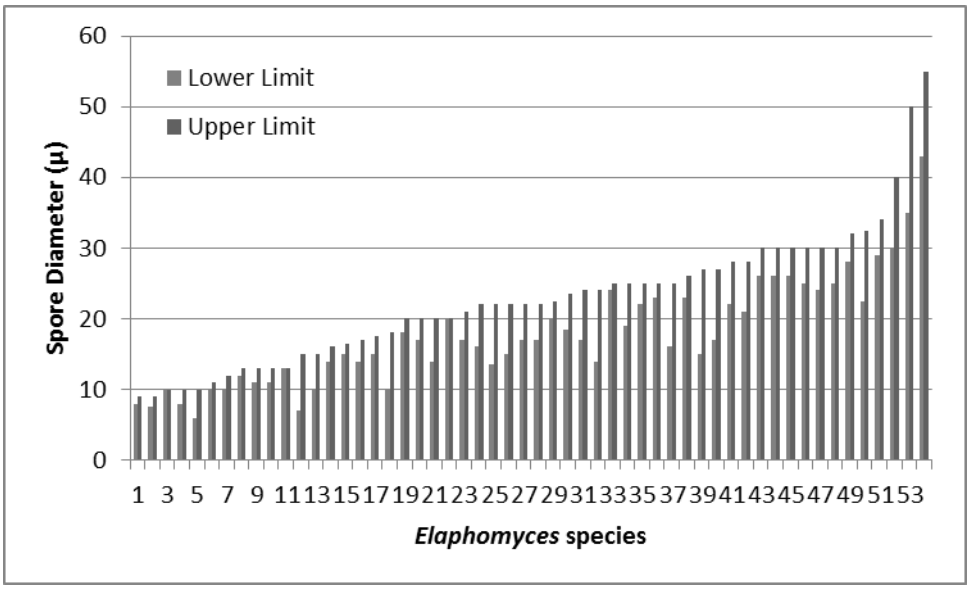

Figure 26. The majority of *Elaphomyces* spores are between 10 and 30 µm diameter.

Biography

I was born in South Bend, Indiana on April 5, 1982. I attended the University of Kansas and received a Bachelor of Science in Organismal Biology in 2004. I have published the following articles:

Hosaka K, Kasuya T, Reynolds HT, Sung GH. 2010. A new record of *Elaphomyces guangdongensis* (Elaphomycetaceae, Eurotiales, Fungi) from Taiwan. Bulletin of the National Museum of Nature and Science, series B 36: 107-115.

Reynolds HT, Currie CR. 2004. Pathogenicity of *Escovopsis weberi*: The parasite of the attine ant-microbe symbiosis directly consumes the ant-cultivated fungus. Mycologia 96: 956-959.

Since obtaining the bachelor's degree, I have received the following grants and fellowships:

External: Mycological Society of America Conference Travel Award – 2011

NSF East Asian Pacific Studies Institute, Japan – 2009

North American Truffle Society Student Grant-In-Aid – 2008

Mycological Society of America Conference Travel Award – 2007, 2011

Sigma Xi Grant-in-Aid of Research Award – 2006

Fulbright Student Award, France – 2004

Duke: Biology Department Semester Fellowship – 2010

Biology Department Grant-in-Aid – 2007, 2008, 2010

Duke Graduate Student Travel Award – 2008

Works Cited

1. Avise, J.C., *Phylogeography: the history and formation of species*2000, Cambridge, Mass.: Harvard University Press. 447.

2. Beheregaray, L.B., *Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere.* Molecular Ecology, 2008. **17**: p. 3754-3774.

3. Bass-Becking, *Geobiologie of inleiding tot de milieukunde.* 1934, The Hague, The Netherlands: WP Van Stockum & Zoon.

4. Buller, A.H., *Researches on Fungi*. Vol. II. 1922, London: Longmans, Green, and Co. 492.

5. Demoulin, V., *Phytogeography of the fungal genus Lycoperdon in relation to the opening of the Atlantic.* Nature, 1973. **242**: p. 123-125.

6. O'Donnell, K., E. Cigelnik, and H. Nirenberg, *Molecular systematics and phylogeography of the Gibberella fujikuroi species complex.* Mycologia, 1998. **90**: p. 465-493.

7. O'Donnell, K., C. Kistlutt, B. Tocke, and H. Casper, *Gene geneologies reveal global phylogeographic structure and reproductive isolation among lineages of Fusarium graminearum, the fungus causing wheat scab.* PNAS, 2000. **97**: p. 7905-7910.

8. Kasuga, T., T.J. White, G. Koenig, J. McEwen, A. Restrepo, E. Castaneda, C.D. Lacaz, E.M. Heins-Vaccari, R.S. De Freitas, R.M. Zancope-Oliveira, Z.Y. Qin, R. Negroni, D.A. Carter, Y. Mikami, M. Tamura, M.L. Taylor, G.F. Miller, N. Poonwan, and J.W. Taylor, *Phylogeography of the fungal pathogen Histoplasma capsulatum.* Molecular Ecology, 2003. **12**: p. 3383-3401.

9. Gomez, D.R., K.J. Evans, P.R. Harvey, J. Baker, J. Barton, M. Jourdan, L. Morin, S.R. Pennycook, and E.S. Scott, *Genetic diversity in the blackberry rust pathogen, Phragmidium violaceum, in Europe and Australasia as revealed by analysis of SAMPL.* Mycological Research, 2006. **110**: p. 423-430.

10. Reeb, V., P. Haugen, D. Bhattacharya, and F. Lutzoni, *Evolution of Pleopsidium (Lichenized ascomycota) S943 group I introns and the phylogeography of an intron-encoded putative homing endonuclease.* Journal of Molecular Evolution, 2007. **64**: p. 285-298.

11. Werth, S., H.H. Wagner, F. Gugerli, R. Holderegger, D. Csencsics, J.M. Kalwij, and C. Scheidegger, *Quantifying dispersal and establishment limitation in a population of an epiphytic lichen.* Ecology, 2006. **87**: p. 2037-2046.

12. Otalora, M.A.G., I. Martinez, G. Aragon, and M.C. Molina, *Phylogeography and divergence date estimates of a lichen species complex with a disjunct distribution pattern.* American Journal of Botany, 2010. **97**: p. 216-223.

13. Takamatsu, S. and S. Matsuda, *Estimation of molecular clocks for ITS and 28S rDNA in Erysiphales.* Mycoscience, 2004. **45**: p. 340-344.

14. Högberg, N., S. Kroken, G. Thor, and J.W. Taylor, *Reproductive mode and genetic variation suggest a North American origin of European Letharia vulpina.* Molecular Ecology, 2002. **11**: p. 1191-1196.

15. Buschbom, J. and G.M. Mueller, *Testing "species pair" hypotheses: Evolutionary processes in the lichen-forming species complex Porpidia flavocoerulescens and Porpidia melinodes.* Molecular Biology and Evolution, 2006. **23**: p. 574-586.

16. Buschbom, J., *Migration between continents: geographical structure and long-distance gene flow in Porpidia flavicunda (lichen-forming Ascomycota).* Molecular Ecology, 2007. **16**: p. 1835-46.

17. Halling, R.E., T.W. Osmundson, and M.A. Neves, *Pacific boletes: Implications for biogeographic relationships.* Mycological Research, 2008. **112**: p. 437-447.

18. Oda, T., C. Tanaka, and M. Tsuda, *Molecular phylogeny and biogeography of the widely distributed Amanita species, A-muscaria and A-pantherina.* Mycological Research, 2004. **108**: p. 885-896.

19. Hosaka, K., M.A. Castellano, and J.W. Spatafora, *Biogeography of Hysterangiales (Phallomycetidae, Basidiomycota).* Mycological Research, 2008. **112**: p. 448-462.

20. Mikheyev, A.S., T. Vo, and U.G. Mueller, *Phylogeography of post-Pleistocene population expansion in a fungus-gardening ant and its microbial mutualists.* Molecular Ecology, 2008. **17**: p. 4480-4488.

21. Murat, C., J. Diez, P. Luis, C. Delaruelle, C. Dupre, G. Chevalier, P. Bonfante, and F. Martin, *Polymorphism at the ribosomal DNA ITS and its relation to postglacial re-colonization routes of the Perigord truffle Tuber melanosporum.* New Phytologist, 2004. **164**: p. 401-411.

22. Zampieri, E., C. Murat, M. Cagnasso, P. Bonfante, and A. Mello, *Soil analysis reveals the presence of an extended mycelial network in a Tuber magnatum truffle-ground.* Fems Microbiology Ecology, 2010. **71**: p. 43-49.

23. Jeandroz, S., C. Murat, Y.J. Wang, P. Bonfante, and F. Le Tacon, *Molecular phylogeny and historical biogeography of the genus Tuber, the 'true truffles'.* Journal of Biogeography, 2008. **35**: p. 815-829.

24. Pringle, A., D. Baker, J. Platt, J. Wares, and J. Latge, *Cryptic speciation in the cosmopolitan and clonal human pathogenic fungus Aspergillus fumigatus.* Evolution, 2005. **59**: p. 1886-1899.

25. Latge, J.P., *Aspergillus fumigatus and aspergillosis.* Clinical Microbiology Reviews, 1999. **12**: p. 310-350.

26. James, T.Y., D. Porter, J.L. Hamrick, and R. Vilgalys, *Evidence for limited intercontinental gene flow in the cosmopolitan mushroom, Schizophyllum commune.* Evolution, 1999. **53**: p. 1665-1677.

27. Hibbett, D.S., *Shiitake mushrooms and molecular clocks: historical biogeography of Lentinula.* Journal of Biogeography, 2001. **28**: p. 231-241.

28. Zervakis, G.I., J.M. Moncalvo, and R. Vilgalys, *Molecular phylogeny, biogeography and speciation of the mushroom species Pleurotus cystidiosus and allied taxa.* Microbiology-Sgm, 2004. **150**: p. 715-726.

29. Gray, A., *Analogy between the flora of Japan and that of the United States.* American Journal of Science and Arts, 1846. **11**: p. 135-136.

30. Shen, Q., D.M. Geiser, and D.J. Royse, *Molecular phylogenetic analysis of Grifola frondosa (maitake) reveals a species partition separating eastern North American and Asian isolates.* Mycologia, 2002. **94**: p. 472-482.

31. Ko, K.S., Y.W. Lim, Y.H. Kim, and H.S. Jung, *Phylogeographic divergences of nuclear ITS sequences in Coprinus species sensu lato.* Mycological Research, 2001. **105**: p. 1519-1526.

32. Lickey, E.B., K.W. Hughes, and R.H. Petersen, *Biogeographical patterns in Artomyces pyxidatus.* Mycologia, 2002. **94**: p. 461-471.

33. Kauserud, H., I.B. Svegarden, C. Decock, and N. Hallenberg, *Hybridization among cryptic species of the cellar fungus Coniophora puteana (Basidiomycota).* Molecular Ecology, 2007. **16**: p. 389-399.

34. Ramsbottom, J., *Dry rot in ships.* The Essex Naturalist, 1937. **25**: p. 231–267.

35. Kauserud, H., I.B. Svegarden, G.P. Saetre, H. Knudsen, O. Stensrud, O. Schmidt, S. Doi, T. Sugiyama, and N. Hogberg, *Asian origin and rapid global spread of the destructive dry rot fungus Serpula lacrymans.* Molecular Ecology, 2007. **16**: p. 3350-3360.

36. Kauserud, H., N. Hogberg, H. Knudsen, S.A. Elborne, and T. Schumacher, *Molecular phylogenetics suggest a North American link between the anthropogenic dry rot fungus Serpula lacrymans and its wild relative S-himantioides.* Molecular Ecology, 2004. **13**: p. 3137-3146.

37. Riginos, C., K.E. Douglas, Y. Jin, D.F. Shanahan, and E.A. Treml, *Effects of geography and life history traits on genetic differentiation in benthic marine fishes.* Ecography, 2011. **34**: p. 566-575.

38. Hamrick, J.L. and M.J.W. Godt, *Effects of life history traits on genetic diversity in plant species.* Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, 1996. **351**: p. 1291-1298.

39. Wright, S., *Isolation by distance under diverse systems of mating.* Genetics, 1945. **30**: p. 571-572.

40. Wright, S., *The interpretation of population structure by F-statistics with special regard to systems of mating.* Evolution, 1965. **19**: p. 395-420.

41. Nei, M., *F-statistics and analysis of gene diversity in subdivided populations.* Annals of Human Genetics, 1977. **41**: p. 225-233.

42. Weir, B.S. and C.C. Cockerham, *Estimating F-statistics for the analysis of population structure.* Evolution, 1984. **38**: p. 1358-1370.

43. Excoffier, L., P.E. Smouse, and J.M. Quattro, *Analysis of molecular variance inferred from metric distances among DNA haplotypes - application to human mitochondrial-DNA restriction data.* Genetics, 1992. **131**: p. 479-491.

44. Slatkin, M., *A measure of population subdivision based on microsatellite allele frequencies.* Genetics, 1995. **139**: p. 457-462.

45. 9, J.v., *JMP*, 1989-2011, SAS Institute Inc.: Cary, NC.

46. Jost, L., *Gst and its relatives do not measure differentiation.* Molecular Ecology, 2008. **17**: p. 4015-4026.

47. Hedrick, P., *Perspective: variable loci and their interpretation in evolution and conservation.* Evolution, 1999. **53**: p. 313-318.

48. Charlesworth, B., *Measures of divergence between populations and the effect of forces that reduce variability.* Molecular Biology and Evolution, 1998. **15**: p. 538-543.

49. Meirmans, P., Hedrick, PW., *Assessing population structure: Fst and related measures.* Molecular Ecology Resources, 2011. **11**: p. 5-18.

50. Wright, S., *Evolution and the Genetics of Populations. Vol. 4. Variability Within and Among Natural Populations.*1978, Chicago: University of Chicago Press.

51. Nei, M., *Molecular Evolutionary Genetics*1987, New York: Columbia University Press.

52. Grubisha, L.C., S.E. Bergemann, and T.D. Bruns, *Host islands within the California Northern Channel Islands create fine-scale genetic structure in two sympatric species of the symbiotic ectomycorrhizal fungus Rhizopogon.* Molecular Ecology, 2007. **16**: p. 1811-22.

53. Rivas, G.-G., M.-F. Zapater, C. Abadie, and J. Carlier, *Founder effects and stochastic dispersal at the continental scale of the fungal pathogen of bananas Mycosphaerella fijiensis.* Molecular Ecology, 2004. **13**: p. 471-482.

54. Zamponi, L., Paffetti, D, Tegli, S, Lakomy, P, Capretti, P, *Genetic variation in Heterobasidion abietinum populations detected with the M13 minisatellite marker.* Forest Pathology, 2007. **37**: p. 321-328.

55. Liang, H.H., Z. Cheng, X.L. Yang, S. Li, Z.Q. Ding, T.S. Zhou, W.J. Zhang, and J.K. Chen, *Genetic diversity and structure of Cordyceps sinensis populations from extensive geographical regions in China as revealed by inter-simple sequence repeat markers.* Journal of Microbiology, 2008. **46**: p. 549-56.

56. Trappe, J., *Studies on Cenococcum graniforme. I. An efficient method for isolation from sclerotia.* Canadian Journal of Botany, 1969. **47**: p. 1389-1390.

57. LoBuglio, K., Taylor JW, *Recombination and genetic differentiationin the mycorrhizal fungus Cenococcum geophilum Fr. .* Mycologia, 2002. **94**: p. 772-780.

58. Xu, J.P., R.W. Kerrigan, A.S. Sonnenberg, P. Callac, P.A. Horgen, and J.B. Anderson, *Mitochondrial DNA variation in natural populations of the mushroom Agaricus bisporus.* Molecular Ecology, 1998. **7**: p. 19-33.

59. Xu, J., R.W. Kerrigan, P. Callac, P.A. Horgen, and J.B. Anderson, *Genetic structure of natural populations of Agaricus bisporus, the commercial button mushroom.* Journal of Heredity, 1997. **88**: p. 482-488.

60. Gannibal, P.B., S.S. Klemsdal, and M.M. Levitin, *AFLP analysis of Russian Alternaria tenuissima populations from wheat kernels and other hosts.* European Journal of Plant Pathology, 2007. **119**: p. 175-182.

61. Prospero, S., B. Lung-Escarmant, and C. Dutech, *Genetic structure of an expanding Armillaria root rot fungus (Armillaria ostoyae) population in a managed pine forest in southwestern France.* Molecular Ecology, 2008. **17**: p. 3366-3378.

62. Baumgartner, K., R. Travadon, J. Bruhn, and S.E. Bergemann, *Contrasting patterns of genetic diversity and population structure of Armillaria mellea sensu stricto in the eastern and western United States.* Phytopathology, 2010. **100**: p. 708-718.

63. Leuchtmann, A., Clay, K, *Isozyme evidence for host races of the fungus Atkinsonella hypoxylon (Clavicipitaceae) infecting the Danthonia (Poaceae) complex in the southern Appalachians.* American Journal of Botany, 1996. **83**: p. 1144-1152.

64. Coates, B.S., R.L. Hellmich, and L.C. Lewis, *Allelic variation of aBeauveria bassiana(Ascomycota: Hypocreales) minisatellite is independent of host range and geographic origin.* Genome, 2002. **45**: p. 125-132.

65. Parks, R., I. Carbone, J.P. Murphy, and C. Cowger, *Population genetic analysis of an Eastern U.S. wheat powdery mildew population reveals geographic subdivision and recent common ancestry with U.K. and Israeli populations.* Phytopathology, 2009. **99**: p. 840-849.

66. Marin, M., O. Preisig, B.D. Wingfield, T. Kirisits, and M.J. Wingfield, *Single sequence repeat markers reflect diversity and geographic barriers in Eurasian populations of the conifer pathogenCeratocystis polonica.* Forest Pathology, 2009. **39**: p. 249-265.

67. Morin, C., C. Breuil, and L. Bernier, *Genetic variability and structure of Canadian populations of the sapstain fungus Ceratocystis resinifera.* Phytopathology, 2004. **94**: p. 1323-1330.

68. Okori, P., P.R. Rubaihayo, A. Ekwamu, J. Fahleson, and C. Dixelius, *Genetic characterization of Cercospora sorghi from cultivated and wild sorghum and its relationship to other Cercospora fungi.* Phytopathology, 2004. **94**: p. 743-750.

69. Abang, M.M., R. Asiedu, P. Hoffmann, G.A. Wolf, H.D. Mignouna, and S. Winter, *Pathogenic and genetic variability among Colletotrichum gloeosporioides isolates from different yam hosts in the agroecological zones in Nigeria.* Journal of Phytopathology, 2006. **154**: p. 51-61.

70. Et-touil, K., L. Bernier, J. Beaulieu, J.A. Berube, A. Hopkin, and R.C. Hamelin, *Genetic structure of Cronartium ribicola populations in eastern Canada.* Phytopathology, 1999. **89**: p. 915-919.

71. Milgroom, M.G. and P. Cortesi, *Analysis of population structure of the chestnut blight fungus based on vegetative incompatibility genotypes.* Proceedings of the National Academy of Sciences of the United States of America, 1999. **96**: p. 10518-10523.

72. Yan, B., Z. Li, H. Huang, and L. Qin, *Genetic diversity and population differentiation of chestnut blight fungus, Cryphonectria parasitica, in China as revealed by RAPD.* Biochem Genet, 2007. **45**: p. 487-506.

73. Milgroom, M.G., K.R. Wang, Y. Zhou, S.E. Lipari, and S. Kaneko, *Intercontinental population structure of the chestnut blight fungus, Cryphonectria parasitica.* Mycologia, 1996. **88**: p. 179-190.

74. Breuillin, F., C. Dutech, and C. Robin, *Genetic diversity of the Chestnut blight fungus Cryphonectria parasitica in four French populations assessed by microsatellite markers.* Mycological Research, 2006. **110**: p. 288-296.

75. Johannesson, H., R. Vasiliauskas, A. Dahlberg, R. Penttila, and J. Stenlid, *Genetic differentiation in Eurasian populations of the postfire ascomycete Daldinia loculata.* Molecular Ecology, 2001. **10**: p. 1665-1677.

76. Parrent, J., *Population genetic structure of the polypore Datronia caperata in fragmented mangrove forests.* Mycological Research, 2004. **108**: p. 403-410.

77. Lihme, M., A.B. Jensen, and S. Rosendahl, *Local scale population genetic structure of Entomophthora muscae epidemics.* Fungal Ecology, 2009. **2**: p. 81-86.

78. Garcia, R.A., J.M.M. Zapater, B.G. Criado, and I. Zabalgogeazcoa, *Genetic structure of natural populations of the grass endophyte Epichloe festucae in semiarid grasslands.* Molecular Ecology, 2002. **11**: p. 355-364.

79. Wali, P.R., J.U. Ahlholm, M. Helander, and K. Saikkonen, *Occurrence and genetic structure of the systemic grass endophyte Epichloe festucae in fine fescue populations.* Microb Ecol, 2007. **53**: p. 20-29.

80. Cortesi, P., Mazzoleni A, Pizzatti C, Milgroom MG, *Genetic similarity of flag shoot and ascospore subpopulations of Erisyphe necator in Italy. .* Applied and Environmental Microbiology 2005. **71**: p. 7788-7791.

81. Högberg N, H.O., Stenlid J, *Population structure of the wood decay fungus Fomitopsis pinicola* Heredity 1999. **83**: p. 354-360.

82. Kauserud, H., *Genetic structure of Fennoscandian populations of the threatened wood-decay fungus Fomitopsis rosea (Basidiomycota).* Mycological Research, 2003. **107**: p. 155-163.

83. Gargouri, S., Bernier L, Hajlaoui MR, Marrakchi M. , *Genetic variability and population structure of the wheat foot rot fungus, Fusarium culmorum, in Tunisia. .* European Journal of Plant Pathology. 2003. **109**: p. 807-815.

84. Akinsanmi, O.A., D. Backhouse, S. Simpfendorfer, and S. Chakraborty, *Genetic diversity of Australian Fusarium graminearum and F. pseudograminearum.* Plant Pathology, 2006. **55**: p. 494-504.

85. Bentley, A.R., J.F. Leslie, E.C. Liew, L.W. Burgess, and B.A. Summerell, *Genetic structure of Fusarium pseudograminearum populations from the Australian grain belt.* Phytopathology, 2008. **98**: p. 250-255.

86. Britz, H., Coutinho TA, Wingfield BD, Marasas WFO, Wingfield MJ. , *Diversity and differentiation in two different populations of Gibberella circinata in South Africa. .* Plant Pathology, 2005. **54**: p. 46-52.

87. Roy, M., M.P. Dubois, M. Proffit, L. Vincenot, E. Desmarais, and M.A. Selosse, *Evidence from population genetics that the ectomycorrhizal basidiomycete Laccaria amethystina is an actual multihost symbiont.* Molecular Ecology, 2008. **17**: p. 2825-2838.

88. Gherbi, H., C. Delaruelle, M.A. Selosse, and F. Martin, *High genetic diversity in a population of the ectomycorrhizal basidiomycete Laccaria amethystina in a 150-year-old beech forest.* Molecular Ecology, 1999. **8**: p. 2003-2013.

89. Hayden, H.L., A.J. Cozijnsen, and B.J. Howlett, *Microsatellite and Minisatellite Analysis of Leptosphaeria maculans in Australia Reveals Regional Genetic Differentiation.* Phytopathology, 2007. **97**: p. 879-87.

90. Purwantara, A., *Genetic diversity of isolates of the Leptosphaeria maculans species complex from Australia, Europe and North America using amplified fragment length polymorphism analysis.* Mycological Research, 2000. **104**: p. 772-781.

91. Gout, L., M. Eckert, T. Rouxel, and M.H. Balesdent, *Genetic variability and distribution of mating type alleles in field populations of Leptosphaeria maculans from France.* Applied and Environmental Microbiology 2006. **72**: p. 185-91.

92. Huss, M., *Isozyme analysis of population structure and diversity in the puffball species Lycoperdon pyriforme* Mycologia, 1996 **88**: p. 977-985.

93. Abesha, E. and G.H. Caetano-Anollés, K., *Population genetics and spatial structure of the fairy ring fungus Marasmius oreades in a Norwegian sand dune ecosystem.* Mycologia, 2003. **95**: p. 1021-1031.

94. Bayon, C., M.H. Pei, C. Ruiz, T. Hunter, A. Karp, and I. Tubby, *Genetic structure and population dynamics of a heteroecious plant pathogen Melampsora larici-epitea in short-rotation coppice willow plantations.* Molecular Ecology, 2009. **18**: p. 3006-3019.

95. Pei MH, B.C., Ruiz C, Tubby I., *Population structure of poplar rust Melampsora larici-populina in the UK inferred from AFLP.* Plant Pathology 2007. **56**: p. 472-479.

96. Freed, S., F.-L. Jin, and S.-X. Ren, *Determination of genetic variability among the isolates of Metarhizium anisopliae var. anisopliae from different geographical origins.* World Journal of Microbiology and Biotechnology, 2010. **27**: p. 359-370.

97. Vercken, E., M.C. Fontaine, P. Gladieux, M.E. Hood, O. Jonot, and T. Giraud, *Glacial refugia in pathogens: European genetic structure of anther smut pathogens on Silene latifolia and Silene dioica.* PLoS Pathog, 2010. **6**: p. e1001229.

98. Van Putten, W.F., A. Biere, and J.M.M. Van Damme, *Host-related genetic differentiation in the anther smut fungus Microbotryum violaceum in sympatric, parapatric and allopatric populations of two host species Silene latifolia and S-dioica.* Journal of Evolutionary Biology, 2005. **18**: p. 203-212.

99. Fan, J.Y., L.Y. Guo, J.P. Xu, Y. Luo, and T.J. Michailides, *Genetic Diversity of Populations of Monilinia fructicola (Fungi, Ascomycota, Helotiales) from China.* Journal of Eukaryotic Microbiology, 2010. **57**: p. 206-212.

100. Boisselier-Dubayle MC, P.-B.J., Lambourdiere J. , *Genetic variability in wild populations of Mycena rosea. .* Mycological Research 1996. **100**: p. 753-758.

101. Schnieder, F., G. Koch, C. Jung, and J.A. Verreet, *Genotypic diversity of the wheat leaf blotch pathogen Mycosphaerella graminicola (anamorph) Septoria tritici in Germany.* European Journal of Plant Pathology, 2001. **107**: p. 285-290.

102. Zhang, Y.J., L.L. Xu, S. Zhang, X.Z. Liu, Z.Q. An, M. Wang, and Y.L. Guo, *Genetic diversity of Ophiocordyceps sinensis, a medicinal fungus endemic to the Tibetan Plateau: Implications for its evolution and conservation.* BMC Evolutionary Biology, 2009. **9**: p. 290-302.

103. Lee, S., R.C. Hamelin, D.L. Six, and C. Breuil, *Genetic Diversity and the Presence of Two Distinct Groups in Ophiostoma clavigerum Associated with Dendroctonus ponderosae in British Columbia and the Northern Rocky Mountains.* Phytopathology, 2007. **97**: p. 1177-85.

104. Comont, G., M.-F. Corio-Costet, P. Larignon, and F. Delmotte, *AFLP markers reveal two genetic groups in the French population of the grapevine fungal pathogen Phaeomoniella chlamydospora.* European Journal of Plant Pathology, 2010. **127**: p. 451-464.

105. Adhikari, T.B., S. Ali, R.R. Burlakoti, P.K. Singh, M. Mergoum, and S.B. Goodwin, *Genetic structure of Phaeosphaeria nodorum populations in the north-central and midwestern United States.* Phytopathology, 2008. **98**: p. 101-7.

106. Kauserud, H. and T. Schumacher, *Population structure of the endangered wood decay fungus Phellinus nigrolimitatus (Basidiomycota).* Canadian Journal of Botany, 2002. **80**: p. 597-606.

107. Franzén, I., R. Vasaitis, R. Penttila, and J. Stenlid, *Population genetics of the wood-decay fungus Phlebia centrifuga P. Karst. in fragmented and continuous habitats.* Molecular Ecology, 2007. **16**: p. 3326-33.

108. Dunn, A.R., M.G. Milgroom, J.C. Meitz, A. McLeod, W.E. Fry, M.T. McGrath, H.R. Dillard, and C.D. Smart, *Population Structure and Resistance to Mefenoxam ofPhytophthora capsiciin New York State.* Plant Disease, 2010. **94**: p. 1461-1468.

109. Lamour KJ, H.M., *Investigating the spatio-temporal genetic structure of Phytophthora capsici in Michigan. .* Phytopathology 2001. **91**: p. 973-980.

110. Linde, C., Drenth, A, Kemp, GHJ, Wingfield, MJ, von Broembsen, SL, *Population structure of Phytophthora cinnamomi in South Africa.* Phytopathology, 1997. **87**: p. 822-827.

111. Mascheretti, S., P.J. Croucher, M. Kozanitas, L. Baker, and M. Garbelotto, *Genetic epidemiology of the sudden oak death pathogen Phytophthora ramorum in California.* Molecular Ecology, 2009. **18**: p. 4577-4590.

112. Hitchcock, C.J., S.M. Chambers, and J.W. Cairney, *Genetic population structure of the ectomycorrhizal fungus Pisolithus microcarpus suggests high gene flow in south-eastern Australia.* Mycorrhiza, 2011. **21**: p. 131-137.

113. Urbanelli, S., V. Della Rosa, C. Fanelli, A.A. Fabbri, and M. Reverberi, *Genetic diversity and population structure of the Italian fungi belonging to the taxa Pleurotus eryngii (DC.:Fr.) Quel and P. ferulae (DC.:Fr.) Quel.* Heredity, 2003. **90**: p. 253-259.

114. Mboup, M., M. Leconte, A. Gautier, A.M. Wan, W. Chen, C. de Vallavieille-Pope, and J. Enjalbert, *Evidence of genetic recombination in wheat yellow rust populations of a Chinese oversummering area.* Fungal Genetics and Biology, 2009. **46**: p. 299-307.

115. Ali, S., M. Leconte, A.S. Walker, J. Enjalbert, and C. de Vallavieille-Pope, *Reduction in the sex ability of worldwide clonal populations of Puccinia striiformis f.sp. tritici.* Fungal Genetics and Biology, 2010. **47**: p. 828-838.

116. Kolmer, J.A. and M.E. Ordonez, *Genetic Differentiation of Puccinia triticina Populations in Central Asia and the Caucasus.* Phytopathology, 2007. **97**: p. 1141-1149.

117. Ordoñez, M.E., S.E. German, and J.A. Kolmer, *Genetic differentiation within the Puccinia triticina population in South America and comparison with the North American population suggests common ancestry and intercontinental migration.* Phytopathology, 2010. **100**: p. 376-83.

118. Majer, D., B.G. Lewis, and R. Mithen, *Genetic variation among field isolates of Pyrenopeziza brassicae.* Plant Pathology, 1998. **47**: p. 22-28.

119. Serenius, M., *Genetic variation, occurrence of mating types and different forms of Pyrenophora teres causing net blotch of barley in Finland.* Mycological Research, 2005. **109**: p. 809-817.

120. Bogacki, P., F.J. Keiper, and K.H. Oldach, *Genetic structure of South Australian Pyrenophora teres populations as revealed by microsatellite analyses.* Fungal Biology, 2010. **114**: p. 834-841.

121. Al-Sa'di, A.M., A. Drenth, M.L. Deadman, and E.A.B. Aitken, *Genetic diversity, aggressiveness and metalaxyl sensitivity of Pythium aphanidermatum populations infecting cucumber in Oman.* Plant Pathology, 2008. **57**: p. 45-56.

122. Chaijuckam, P., J.M. Baek, C.A. Greer, R.K. Webster, and R.M. Davis, *Population structure of Rhizoctonia oryzae-sativae in California rice fields.* Phytopathology, 2010. **100**: p. 502-510.

123. Ceresini, P.C., H.D. Shew, R.J. Vilgalys, and M.A. Cubeta, *Genetic diversity of Rhizoctonia solani AG-3 from potato and tobacco in North Carolina.* Mycologia, 2002. **94**: p. 437-449.

124. Kretzer, A.M., S. Dunham, R. Molina, and J.W. Spatafora, *Patterns of vegetative growth and gene flow in Rhizopogon vinicolor and R-vesiculosus (Boletales, Basidiomycota).* Molecular Ecology, 2005. **14**: p. 2259-2268.

125. Bouajila, A., M.M. Abang, S. Haouas, S. Udupa, S. Rezgui, M. Baum, and A. Yahyaoui, *Genetic diversity of Rhynchosporium secalis in Tunisia as revealed by pathotype, AFLP, and microsatellite analyses.* Mycopathologia, 2007. **163**: p. 281-294.

126. Bergemann, S.E., G.W. Douhan, M. Garbelotto, and S.L. Miller, *No evidence of population structure across three isolated subpopulations of Russula brevipes in an oak/pine woodland.* New Phytologist, 2006. **170**: p. 177-184.

127. Bergemann, S.E. and S.L. Miller, *Size, distribution, and persistence of genets in local populations of the late-stage ectomycorrhizal basidiomycete, Russula brevipes.* New Phytologist, 2002. **156**: p. 313-320.

128. Sexton, A.C., A.R. Whitten, and B.J. Howlett, *Population structure of Sclerotinia sclerotiorum in an Australian canola field at flowering and stem-infection stages of the disease cycle.* Genome, 2006. **49**: p. 1408-1415.

129. Lee, S.H. and S.M. Neate, *Population Genetic Structure of Septoria passerinii in Northern Great Plains Barley.* Phytopathology, 2007. **97**: p. 938-944.

130. Zhou, Z.H., M. Miwa, and T. Hogetsu, *Polymorphism of simple sequence repeats reveals gene flow within and between ectomycorrhizal Suillus grevillei populations.* New Phytologist, 2001. **149**: p. 339-348.

131. Muller, L.A.H., M. Lambaerts, J. Vangronsveld, and J.V. Colpaert, *AFLP-based assessment of the effects of environmental heavy metal pollution on the genetic structure of pioneer populations of Suillus luteus.* New Phytologist, 2004. **164**: p. 297-303.

132. Muller, L.A.H., J. Vangronsveld, and J.V. Colpaert, *Genetic structure of Suillus luteus populations in heavy metal polluted and nonpolluted habitats.* Molecular Ecology, 2007. **16**: p. 4728-4737.

133. Anderson, J.L. and C.A. Shearer, *Population genetics of the aquatic fungus Tetracladium marchalianum over space and time.* PLoS One, 2011. **6**: p. e15908.

134. Sha, T., J. Xu, M.G. Palanichamy, H.B. Zhang, T. Li, Z.W. Zhao, and Y.P. Zhang, *Genetic diversity of the endemic gourmet mushroom Thelephora ganbajun from south-western China.* Microbiology, 2008. **154**: p. 3460-3648.

135. Kauserud, H. and T. Schumacher, *Regional and local population structure of the pioneer wood-decay fungus Trichaptum abietinum.* Mycologia, 2003. **95**: p. 416-425.

136. Amend, A., M. Garbelotto, Z.D. Fang, and S. Keeley, *Isolation by landscape in populations of a prized edible mushroom Tricholoma matsutake.* Conservation Genetics, 2010. **11**: p. 795-802.

137. Lian, C., M. Narimatsu, K. Nara, and T. Hogetsu, *Tricholoma matsutake in a natural Pinus densiflora forest: correspondence between above- and below-ground genets, association with multiple host trees and alteration of existing ectomycorrhizal communities.* New Phytologist, 2006. **171**: p. 825-836.

138. Carriconde, F., M. Gardes, P. Jargeat, J. Heilmann-Clausen, B. Mouhamadou, and H. Gryta, *Population evidence of cryptic species and geographical structure in the cosmopolitan ectomycorrhizal fungus, Tricholoma scalpturatum.* Microb Ecol, 2008. **56**: p. 513-524.

139. Rubini, A., F. Paolocci, C. Riccioni, G.G. Vendramin, and S. Arcioni, *Genetic and phylogeographic structures of the symbiotic fungus Tuber magnatum.* Applied and Environmental Microbiology 2005. **71**: p. 6584-6589.

140. Riccioni, C., B. Belfiori, A. Rubini, V. Passeri, S. Arcioni, and F. Paolocci, *Tuber melanosporum outcrosses: analysis of the genetic diversity within and among its natural populations under this new scenario.* New Phytologist, 2008. **180**: p. 466-478.

141. Vergara, G., *Genetic variability of grey snow mould (Typhula incarnata).* Mycological Research, 2004. **108**: p. 1283-1290.

142. Barnes, C.W., L.J. Szabo, G. May, and J.V. Groth, *Inbreeding levels of two Ustilago maydis populations.* Mycologia, 2004. **96**: p. 1236-1244.

143. Tenzer, I. and C. Gessler, *Subdivision and genetic structure of four populations of Venturia inaequalis in Switzerland.* European Journal of Plant Pathology, 1997. **103**: p. 565-571.

144. Kasanen, R., *Migrational capacity of Fennoscandian populations of Venturia tremulae.* Mycological Research, 2004. **108**: p. 64-70.

145. L'Obel, M., *Icones Stirpium*. Vol. 2. 1591.

146. Bauhin, J., *Historia Plantarum Universalis.* 1650.

147. Linnaeus, C., *Species Plantarum*. Vol. 2. 1753, Stockholm: Impensis Laurentii Salvi.

148. Dodge, C., *The higher Plectascales.* Annales Mycologici, 1929. **29**: p. 145-184.

149. Cunningham, G., *The Gasteromycetes of Australasia. XV. The genera Mesophellia and Castoreum.* Linnean Society of New South Wales 1932. **57** p. 312-322.

150. Cunningham, G., *The Gasteromycetes of Australia and New Zealand.* 1944, Dunedin: John McInoe.

151. Zeller, S., *Representatives of the Mesophelliaceae in North America.* Mycologia 1944. **36**: p. 627-637.

152. Jülich, W., *Higher taxa of Basidiomycetes.* Bibliotheca Mycologia 1981. **85**: p. 1-45.

153. Hosaka, K., S.T. Bates, R.E. Beever, M.A. Castellano, W. Colgan, L.S. Dominguez, E.R. Nouhra, J. Geml, A.J. Giachini, S.R. Kenney, N.B. Simpson, J.W. Spatafora, and J.M. Trappe, *Molecular phylogenetics of the gomphoid-phalloid fungi with an establishment of the new subclass Phallomycetidae and two new orders.* Mycologia, 2006. **98**: p. 949-959.

154. Miller, O.K., T.W. Henkel, T.Y. James, and S.L. Miller, *Pseudotulostoma, a remarkable new volvate genus in the Elaphomycetaceae from Guyana.* Mycological Research, 2001. **105**: p. 1268-1272.

155. Eriksson, O., *Outline of the Ascomycota.* Myconet, 1999. **3**: p. 1-88.

156. LoBuglio, K.F., M.L. Berbee, and J.W. Taylor, *Phylogenetic origins of the asexual mycorrhizal symbiont Cenococcum geophilum Fr and other mycorrhizal fungi among the ascomycetes.* Molecular Phylogenetics and Evolution, 1996. **6**: p. 287-294.

157. Landvik, S., N. Shailer, and O. Eriksson, *SSU rDNA sequence support for a close relationship between the Elaphomycetales and the Eurotiales and Onygenales.* Mycoscience, 1996. **37**: p. 237-241.

158. Geiser, D.M., C. Gueidan, J. Miadlikowska, F. Lutzoni, F. Kauff, V. Hofstetter, E. Fraker, C.L. Schoch, L. Tibell, W.A. Untereiner, and A. Aptroot, *Eurotiomycetes: Eurotiomycetidae and Chaetothyriomycetidae.* Mycologia, 2006. **98**: p. 1053-1064.

159. Eriksson, O.E. and K. Winka, *Supraordinal taxa of Ascomycota.* Myconet 1997. **1**: p. 1-16.

160. Malloch, D. and R.F. Cain, *Trichocomataceae - Ascomycetes with Aspergillus, Paecilomyces, and Penicillium Imperfect States.* Canadian Journal of Botany-Revue Canadienne De Botanique, 1972. **50**: p. 2613-2628.

161. Park, H.G. and S.-C. Jong, *Molecular characterization of Monascus strains based on the D1/D2 regions of LSU rRNA genes.* Mycoscience, 2003. **44**: p. 25-32.

162. Stchigel, A.M., J. Cano, S.K. Abdullah, and J. Guarro, *New and interesting species of Monascus from soil, with a key to the known species.* Studies in Mycology, 2004. **50**: p. 299-306.

163. Stchigel, A.M. and J. Guarro, *A reassessment of cleistothecia as a taxonomic character.* Mycological Research, 2007. **111**: p. 1100-1115.

164. Apinis, A.E., *Dactylomyces and Thermoascus.* Transactions of the British Mycological Society, 1967. **50**: p. 573-582.

165. Malloch, D. and R.F. Cain, *The Trichocomaceae: ascomycetes with Aspergillus, Paecilomyces, and Penicillium imperfect states.* Canadian Journal of Botany, 1972. **50**: p. 2613-2628.

166. von Arx, J.A., *A re-evaluation of the Eurotiales.* Persoonia, 1987. **13**: p. 273-300.

167. Fennell, D.I., *Plectomycetes. Eurotiales*, in *The Fungi: an Advanced Treatise*, G.C. Ainsworth, F.K. Sparrow, and A.S. Sussman, Editors. 1973, Academic Press: New York. p. 45-68.

168. Berbee, M.L., A. Yoshimura, J. Sugiyama, and J.W. Taylor, *Is Penicillium monophyletic - an evaluation of phylogeny in the family Trichocomaceae from 18S, 5.8S and ITS ribosomal DNA-sequence data.* Mycologia, 1995. **87**: p. 210-222.

169. Ogawa, H., A. Yoshimura, and J. Sugiyama, *Polyphyletic origins of species of the anamorphic genus Geosmithia and the relationships of the cleistothecial genera: Evidence from 18S, 5S and 28S rDNA sequence analyses.* Mycologia, 1997. **89**: p. 756-771.

170. Luangsa-Ard, J.J., N.L. Hywel-Jones, and R.A. Samson, *The polyphyletic nature of Paecilomyces sensu lato based on 18S-generated rDNA phylogeny.* Mycologia, 2004. **96**: p. 773-780.

171. Peay, K.G., P.G. Kennedy, S.J. Davies, S. Tan, and T.D. Bruns, *Potential link between plant and fungal distributions in a dipterocarp rainforest: community and phylogenetic structure of tropical ectomycorrhizal fungi across a plant and soil ecotone.* New Phytologist, 2010. **185**: p. 529-542.

172. Castellano, M.A., J.M. Trappe, and K. Vernes, *Australian species of Elaphomyces (Elaphomycetaceae, Eurotiales, Ascomycota).* Australian Systematic Botany, 2011. **24**: p. 32-57.

173. Taylor, J.W. and M.L. Berbee, *Dating divergences in the Fungal Tree of Life: review and new analyses.* Mycologia, 2006. **98**: p. 838-849.

174. Hass, T., T.N. Taylor, and H. Kerp, *The oldest fossil ascomycetes.* Nature, 1999. **399**: p. 648-648.

175. Luo, Z.-X., C.-X. Yuan, Q.-J. Meng, and Q. Ji, *A Jurassic eutherian mammal and divergence of marsupials and placnetals.* Nature, 2010. **476**: p. 442-445.

176. Sung, G.H., G.O. Poinar, and J.W. Spatafora, *The oldest fossil evidence of animal parasitism by fungi supports a Cretaceous diversification of fungal-arthropod symbioses.* Molecular Phylogenetics and Evolution, 2008. **49**: p. 495-502.

177. Berbee, M.L. and J.W. Taylor, *Dating the Evolutionary Radiations of the True Fungi.* Canadian Journal of Botany-Revue Canadienne De Botanique, 1993. **71**: p. 1114-1127.

178. Nikoh, N. and T. Fukatsu, *Interkingdom host jumping underground: Phylogenetic analysis of entomoparasitic fungi of the genus Cordyceps.* Molecular Biology and Evolution, 2000. **17**: p. 629-638.

179. Edgar, R.C., *MUSCLE: multiple sequence alignment with high accuracy and high throughput.* Nucleic Acids Research, 2004. **32**: p. 1792-1797.

180. Maddison, W.P. and D.R. Maddison, *MacClade: analysis of phylogeny and character evolution*, 1992, Sinauer Associates: Sunderland, MA.

181. Swofford, D.L., *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods).* . 4 ed2003, Sunderland, MA: Sinauer Associates.

182. Miller, M.A., W. Pfeiffer, and T. Schwartz, *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*, in *Proceedings of the Gateway Computing Environments Workshop (GCE)*2010: New Orleans, LA. p. 1-8.

183. Zwickl, D.J., *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion.*, 2006, The University of Texas: Austin, TX.

184. Posada, D. and K.A. Crandall, *Modeltest: testing the model of DNA substition.* Bioinformatics, 1998. **14**: p. 817-818.

185. Huelsenbeck, J.P., F. Ronquist, R. Nielsen, and J.P. Bollback, *Bayesian inference of phylogeny and its impact on evolutionary biology.* Science, 2001. **294**: p. 2310-2314.

186. Rohnquist, F. and J.P. Huelsenbeck, *MRBAYES 3: Bayesian phylogenetic inference under mixed models.* Bioinformatics, 2003. **19**: p. 1572-1574.

187. Yu, Y. and A.J. Harris, *RASP (Reconstruct Ancestral State in Phylogenies)* 2011.

188. Drummond, A.J. and A. Rambaut, *BEAST: Bayesian evolutionary analysis by sampling trees.* BMC Evolutionary Biology, 2007. **7**: p. 214.

189. Perry, B.A., K. Hansen, and D.H. Pfister, *A phylogenetic overview of the family Pyronemataceae (Ascomycota, Pezizales).* Mycological Research, 2007. **111**: p. 549-571.

190. Orihara, T., F. Sawada, S. Ikeda, M. Yamato, C. Tanaka, N. Shimomura, M. Hashiya, and K. Iwase, *Taxonomic reconsideration of a sequestrate fungus, Octaviania columellifera, with the proposal of a new genus, Heliogaster, and its phylogenetic relationships in the Boletales.* Mycologia, 2010. **102**: p. 108-121.

191. McLoughlin, S., *The breakup history of Gondwana and its impact of pre-Cenozoic floristic provincialism.* Australian Journal of Botany, 2001. **49**: p. 271-300.

192. Springer, M.S., W.J. Murphy, E. Eizirik, and S.J. O'Brien, *Placental mammal diversification and the Cretaceous-Tertiary boundary.* Proceedings of the National Academy of Sciences of the United States of America, 2003. **100**: p. 1056-1061.

193. Keigwin, L.D.J., *Pliocene closing of the Isthmus of Panama, based on biostratigraphic evidence from nearby Pacific Ocean and Caribbean Sea cores.* Geology, 1978. **6**: p. 630-634.

194. Asai, I., H. Sato, and T. Nara, *Pseudotulostoma japonicum, comb. nov. (=Battarrea japonica), a species of the Eurotiales, Ascomycota.* Bulletin of the National Science Museum, Tokyo, 2004. **30**: p. 1-7.

195. Whitmore, T.C., *Wallace's Line: A Result of Plate Tectonics.* Annals of the Missouri Botanical Garden, 1982. **69**: p. 668-675.

196. Dingle, H., *The Australo-Papuan bird migration system: another consequence of Wallace's Line.* Emu, 2004. **104**: p. 95-108.

197. Van Steenis, C.G.G.J., *Plant-geography of east Malesia.* Botanical Journal of the Linnean Society, 1979. **79**: p. 97-178.

198. Bonito, G.M., A.P. Gryganskyi, J.M. Trappe, and R. Vilgalys, *A global meta-analysis of Tuber ITS rDNA sequences: species diversity, host associations and long-distance dispersal.* Molecular Ecology, 2010. **19**: p. 4994-5008.

199. Kinoshita, A., H. Sasaki, and K. Nara, *Phylogeny and diversity of Japanese truffles (Tuber spp.) inferred from sequences of four nuclear loci.* Mycologia, 2011. **103**: p. 779-794.

200. Sung, G.-H., N.L. Hywel-Jones, J.-M. Sung, J.J. Luangsa-Ard, B. Shrestha, and J.W. Spatafora, *Phylogenetic classification of Cordyceps and the clavicipitaceous fungi.* Studies in Mycology, 2006. **57**: p. 5-59.

201. Imai, S., *On a new species of Cordyceps parasitic on Elaphomyces in Japan.* Proceedings of the Imperial Academy of Japan 1934. **10**: p. 677-679.

202. Mains, E.B., *Species of Cordyceps parasitic on Elaphomyces.* Bulletin of the Torrey Botanical Club, 1957. **84**: p. 243-251.

203. Kobayasi, Y. and D. Shimizu, *Monographic studies of Cordyceps I. Groups parasitic on Elaphomyces.* Bulletin of the National Science Museum, Tokyo, 1960. **5**: p. 69-85.

204. Trewartha, G.T., *The Earth's Problem Climates.* 1981, Madison, WI: University of Wisconsin.

205. Köppen, W., *Das Geographische System der Klimate*, in *Handbuch der Klimatologie*, Köppen and Gieger, Editors. 1936, Gebrüder Borntraeger: Berlin.

206. Cork, S.G. and G.J. Kenagy, *Nutritional value of hypogeous fungus for a forest-dwelling ground squirrel.* Ecology, 1989. **70**: p. 577-586.

207. Johnson, C.N., *Interactions between mammals and ectomycorrhizal fungi.* Trends in ecology & evolution, 1996. **11**: p. 503-507.

208. Maser, C. and J.M. Trappe, *Fungal-small mammal relationships with emphasis on Oregon coniferous forests.* Ecology, 1978. **59**: p. 799-809.

209. Alsheikh, A.M. and J.M. Trappe, *Taxonomy of Phaeangium lefebvrei, a desert truffle eaten by birds.* Canadian Journal of Botany, 1983. **61**: p. 1919-1925.

210. Burdsall, H., *Operculate asci and puffing of ascospores in Geopora (Tuberales).* Mycologia, 1965. **57**: p. 485-488.

211. Meyer, M.D., M.P. North , and D.A. Kelt, *Fungi in the diets of northern flying squirrels and lodgepole chipmunks in the Sierra Nevada.* Canadian Journal of Zoology, 2005. **83**: p. 1581-1589.

212. Smith, A.H., H.V. Smith, and N.S. Weber, *How to Know the Non-Gilled Mushrooms.* 1981, Dubuque, IA: Wm. C. Brown Co.

213. States, J.S. and W. Gaud, *Ecology of hypogeous fungi associated with ponderosa pine I. patterns of distribution and sporocarp production in some Arizona forests.* 1997. **89**: p. 712-721.

214. Luoma, D.L., R.E. Frenkel, and J.M. Trappe, *Fruiting of hypogeous fungi in Oregon Douglas-fir forests: seasonal and habitat variation.* Mycologia, 1991. **83**: p. 335-353.

215. Flaherty, E.A., M. Ben-David, and W.P. Smith, *Diet and food availability: implications for foraging and dispersal of Prince of Wales northern flying squirrels across managed landscapes.* Journal of Mammalogy, 2010. **91**: p. 79-91.

216. Mitchell, D., *Spring and fall diet of the endangered West Virginia northern flying squirrel (Glaucomys sabrinus fuscus).* The American Midland Naturalist, 2001. **146**: p. 439-443.

217. Currah, R.S., E.A. Smreciu, T. Lehesvirta, M. Niemi, and K.W. Larsen, *Fungi in the winter diets of northern flying squirrels and red squirrels in the boreal mixedwood forest of northeastern Alberta.* Canadian Journal of Botany, 2000. **78**: p. 1514-1520.

218. Gabel, A., C. Ackerman, M. Gabel, E. Krueger, S. Weins, and L. Zierer, *Diet and habitat of northern flying squirrels (Glaucomys sabrinus) in the Black Hills of South Dakota.* Western North American Naturalist, 2010. **70**: p. 92-104.

219. Orrock, J.L., D. Farley, and J.F. Pagels, *Does fungus consumption by the woodland jumping mouse vary with habitat type or the abundance of other small mammals?* Canadian Journal of Zoology, 2003. **81**: p. 753-756.

220. Kataržytė, M. and E. Kutorga, *Small mammal mycophagy in hemiboreal forest communities of Lithuania.* Central European Journal of Biology, 2011. **6**: p. 446-456.

221. Johnson and C.N. Johnson, *Mycophagy and spore dispersal by a rat-kangaroo: consumption of ectomycorrhizal taxa in relation to their abundance.* Functional Ecology, 1994. **8**: p. 464-468.

222. Vernes, K., M. Castellano, and C.N. Johnson, *Effects of season and fire on the diversity of hypogeous fungi consumed by a tropical mycophagous marsupial.* Journal of Animal Ecology, 2001. **70**: p. 945-954.

223. Reddell , P., A.V. Spain, and M. Hopkins, *Dispersal of spores of mycorrhizal fungi in scats of native mammals in tropical forests of northeastern Australia.* Biotropica, 1997. **29**: p. 184-192.

224. Vernes, K. and T. Lebel, *Truffle consumption by New Guinea forest wallabies.* Fungal Ecology, 2011. **4**: p. 270-276.

225. Pokorny, B., S.A. Sayegh-Petkovsek, C. Ribaric-Lasnik, J. Vrtacnik, D.Z. Doganoc, and M. Adamic, *Fungi ingestion as an important factor influencing heavy metal intake in roe deer: evidence from faeces.* Science of the Total Environment, 2004. **324**: p. 223-234.

226. Hohmann, U. and D. Huckschlag, *Investigations on the radiocaesium contamination of wild boar (Sus scrofa) meat in Rhineland-Palatinate: a stomach content analysis.* European Journal of Wildlife Research, 2005. **51**: p. 263-270.

227. Johnson, C.N., *Nutritional ecology of a mycophagous marsupial in relation to production of hypogeous fungi.* Ecology, 1994. **75**: p. 2015-2021.

228. Claridge, A.W. and S.J. Cork, *Nutritional-value of hypogeal fungal sporocarps for the long-nosed potoroo (Potorous tridactylus), a forest-dwelling mycophagous marsupial.* Australian Journal of Zoology, 1994. **42**: p. 701-710.

229. Trappe, J. and C. Maser, *Germination of spores of Glomus macrocarpus (Endogonaceae) after passage through a rodent digestive tract.* Mycologia, 1976. **68**: p. 433-436.

230. Lamont, B.B., C.S. Ralph, and P.E. Christensen, *Mycophagous marsupials as dispersal agents for ectomycorrhizal fungi on Eucalyptus calophylla and Gastrolobium bilobum.* New Phytologist, 1985. **101**: p. 651-656.

231. Claridge, A.W., M.T. Tanton, J.H. Seebeck, S.J. Cork, and R.B. Cunningham, *Establishment of ectomycorrhizae on the roots of two species of Eucalyptus from fungal spores contained in the faeces of the long-nosed potoroo (Potorous tridactylus).* Australian Journal of Ecology, 1992. **17**: p. 207-217.

232. Ingold, C., *Dispersal in Fungi.* 1953, Oxford, England. The Clarendon Press. 208.

233. Trail, F., *Fungal cannons: explosive spore discharge in the Ascomycetes.* FEMS Microbiology Letters, 2007. **276**: p. 12-18.

234. Vernes, K. and N. Poirier, *Use of a robin's nest as a cache site for truffles by a red squirrel.* Northeastern Naturalist, 2007. **14**: p. 145-149.

235. Buller, A.H., *The red squirrel of North America as a mycophagist.* Transactions of the British Mycological Society, 1919. **6**: p. 355–362.

236. Ingold, C., *The gift of a truffle.* Bulletin of the British Mycological Society, 1973. **7**: p. 32.

237. Gellum, S.J. and E. Levetin, *The air spora close to a compost facility in Northeast Oklahoma: Part I -- spore trap sampling.* Aerobiologia, 2008. **24**: p. 3-12.

238. Oliveira, M., M.I. Amorim, E. Ferreira, L. Delgado, and I. Abreu, *Main airborne Ascomycota spores: characterization by culture, spore morphology, ribosomal DNA sequences and enzymatic analysis.* Applied Microbiology and Biotechnology, 2010. **86**: p. 1171-1181.

239. Lacey, M. and J. West, *The Air Spora: a Manual for Catching and Identifying Airborne Biological Particles.* 2006, Dordrecht, The Netherlands: Springer.

240. Waisel, Y., E. Ganor, V. Epshtein, A. Stupp, and A. Eshel, *Airborne pollen, spores, and dust across the East Mediteranean Sea.* Aerobiologia, 2008. **24**: p. 125-131.

241. Morales, J., F.J. González-Minero, M. Carrasco, V.M. Ogalla, and P. Candau, *Airborne basidiospores in the atmosphere of Seville (South Spain).* Aerobiologia, 2006. **22**: p. 127-134.

242. Kuparinen, A., T. Markkanen, H. Riikonen, and T. Vesala, *Modeling air-mediated dispersal of spores, pollen and seeds in forested areas.* Ecological Modeling, 2007. 177-188.

243. Tesmer, J. and M. Schnittler, *Sedimentation velocity of myxomycete spores.* Mycological Progress, 2007. **6**: p. 229-234.

244. Vogel, S., *Living in a physical world II: The bio-ballistics of small projectiles.* Journal of Biosciences, 2005. **30**: p. 167-175.

245. Vogel, S., *Living in a physical world III: Getting up to speed.* Journal of Biosciences, 2005. **30**: p. 303-312.

246. Roper, M., R.E. Pepper, M.P. Brenner, and A. Pringle, *Explosively launched spores of ascomycete fungi have drag-minimizing shapes.* PNAS, 2008. **105**: p. 20583-20588.

247. Maddison, A.C. and J.G. Manners, *Sunlight and viability of cereal rust uredospores.* Transactions of the British Mycological Society, 1972. **59**: p. 429-443.

248. Rotem, J. and H.J. Aust, *The effect of ultraviolet and solar radiation and temperature on survival of fungal propagules.* Journal of Phytopathology, 1991. **133**: p. 76-84.

249. Rotem, J., B. Wooding, and D.E. Aylor, *The role of solar radiation, especially ultraviolet, in the mortality of fungal spores.* Phytopathology, 1985. **75**: p. 510-514.

250. Parnell, M., P.J. Burt, and K. Wilson, *The influence of exposure to ultraviolet radiation in simulated sunlight on ascospores causing Black Sigatoka disease of banana and plantain.* International Journal of Biometeorology, 1998. **42**: p. 22-27.

251. Griffin, D., *Atmospheric movement of microorganisms in clouds of desert dust and implications for human health.* Clinical Microbiology Review, 2007. **20**: p. 459-477.

252. James, T.Y. and R. Vilgalys, *Abundance and diversity of Schizophyllum commune spore clouds in the Caribbean.* Molecular Ecology, 2001. **10**: p. 471-479.

253. Munoz, G.A., E. Agosin, M. Cotoras, R. San Martin, and D. Volpe, *Comparison of aerial and submerged spore properties for Trichoderma harzianum.* FEMS Microbiology Letters, 1995. **125**: p. 63-70.

254. Holland, R.J., T.S. Gunasekera, K.L. Williams, and K.M. Nevalainen, *Ultrastructure and properties of Paecilomyces lilacinus spores.* Canadian Journal of Microbiology, 2002. **48**: p. 879-885.

255. Griffin, D., *Terrestrial Microorganisms at an Altitude of 20,000 m in Earth's Atmosphere.* Aerobiologia, 2004. **20**: p. 135-140.

256. Linder, D.H., *A new species of Elaphomyces from the Great Smoky Mountain National Park.* Journal of the Elisha Mitchell Scientific Society, 1939. **55**: p. 131-133.

257. Trappe, J., Kimbrough, JW, *Elaphomyces viridiseptum, a new species from Florida.* Mycologia, 1972. **64**: p. 646-649.

258. Corner, E.J.H. and L.E. Hawker, *Hypogeous fungi from Malaya.* Trans Brit Mycol Soc, 1953. **36**: p. 125-137.

259. Kobayasi, Y., *On the Elaphomyces found in Japan.* Nagaoa, 1960. **7**: p. 35-50.

260. Zhang, B.-C., *Revision of Chinese species of Elaphomyces (Ascomycotina, Elaphomycetales).* Mycological Research, 1991. **95**: p. 973-985.

261. Moreno G, G.R., Montecchi A, *Hypogeous fungi from peninsular Spain.* Mycotaxon, 1991. **42**: p. 201-238.

262. Trappe, J. and G. Guzmán, *A newly determined species of Elaphomyces from Oregon, USA.* Madroño, 1971. **21**: p. 128-130.