MORTIERELLACEAE PHYLOGENOMICS AND TRIPARTITE PLANT-FUNGAL-BACTERIAL SYMBIOSIS OF MORTIERELLA ELONGATA

By

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ABSTRACT

MORTIERELLACEAE PHYLOGENOMICS AND TRIPARTITE PLANT-FUNGAL-BACTERIAL SYMBIOSIS OF MORTIERELLA ELONGATA

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Microbial promotion of plant growth has great potential to improve agricultural yields and protect plants against pathogens and/or abiotic stresses. Soil fungi in Mortierellaceae are non-mycorrhizal plant associates that frequently harbor bacterial endosymbionts. My research focused on resolving the Mortierellaceae phylogeny and on characterizing the effect of *Mortierella elongata* and its bacterial symbionts on *Arabidopsis thaliana* growth and molecular functioning.

Early efforts to classify Mortierellaceae were based on morphology, but phylogenetic studies with ribosomal DNA (rDNA) markers have demonstrated conflicting taxonomic groupings and polyphyletic genera. In this study, I applied two approaches: low coverage genome (LCG) sequencing and high-throughput targeted amplicon sequencing to generate multi-locus sequence data. I combined these datasets to generate a well-supported genome-based phylogeny having broad sampling depth from the amplicon dataset. Resolving the Mortierellaceae phylogeny into monophyletic groups led to the definition of 14 genera, 7 of which are newly proposed.

Mortierellaceae are broadly considered plant associates, but the underlying mechanisms of association are not well understood. In this study, I focused on the symbiosis between *M. elongata*, its endobacteria, and *A. thaliana*. I measured aerial plant growth and seed production and used transcriptomics to characterize differentially expressed plant genes (DEGs) while varying fungal treatments. *M. elongata* was shown to promote aerial plant growth and affect seed production independent of endobacteria. *A. thaliana* DEGs were related to hormone signaling, immune responses, root development, abiotic stress, and metabolism. These data suggest that the mechanism of plant-fungal symbiosis involves fungal manipulation and stimulation of the auxin, ethylene, and ROS response pathways. Future experiments are proposed that could test

these hypotheses and further characterize the fungal side of this symbiosis.

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KEY TO ABBREVIATIONS

PGP	Plant Growth Promotion
DPI	Days Post Inoculation
AM	Arbuscular Mycorrhizal
EM	Ectomycorrhizal
NM	Non-Mycorrhizal
BRE	*Burkholderia*-related Endobacteria
MRE	*Mycoplasma*-related Endobacteria, or Mollicute-related endobacteria
NRRL	Agricultural Research Service Culture Collection
CBS	Westerdijk Fungal Biodiversity Institute (Centraalbureau voor Schimmelcultures)
MRCA	most recent common ancestor
DEG	Differentially expressed gene
JA	Jasmonic acid
SA	Salicylic acid
IAA	indole-3-acetic acid
IBA	Indole-3-butyric acid
GA	Gibberellic acid
CK	cytokinin
BR	brassinosteroid
ABA	abscisic acid
ET	ethylene
PCR	polymerase chain reaction
rDNA	ribosomal DNA
ITS	Internal Transcribed Spacer
SSU	Small Subunit

LSU	Large Subunit
MLST	multi-locus sequence typing
LCG	low-coverage genome
BLAST	Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/)
RAxML	Randomized Axelerated Maximum Likelihood
EMM	estimated marginal mean
LM	linear model
LMM	linear mixed model
MEA	Malt Extract Agar
PDA	Potato Dextrose Agar
PNM	Plant Nutrient Medium
KM	Kaefer Medium
SDA	Sabouraud's Dextrose Agar
DI	deionized
TAE	Tris base Acetic acid and EDTA

CHAPTER 1. INTRODUCTION

Problem Statement

Microbial promotion of plant growth has great potential to improve agricultural yields and protect plants against pathogens and/or abiotic stresses, while also relieving economic and environmental costs of crop production (Li, Chen, et al. 2018; Bedini et al. 2018). Agriculturally important metrics pertaining to plant growth promotion include aerial biomass, root biomass, root architecture, seed number, seed size, and flowering time. One group of plant beneficial microbes is early-diverging filamentous fungi, which have been implicated in assisting plants in the colonization of land (Field et al. 2015). There are three main guilds of plant mutualistic fungi relevant to this study: arbuscular mycorrhizal (AM) fungi, ectomycorrhizal (EM) fungi, and non-mycorrhizal (NM) endophytic fungi. For the purpose of this study, NM root endophytes are defined as fungi that are found inside healthy plant roots but do not make any characteristic mycorrhizal structures. Most of these fungi are thought to promote plant growth primarily by providing water and mineral nutrients, and sometimes secondarily by precluding infection by pathogens and/or priming and regulating plant defense responses (Hooker, Jaizme-Vega, & Atkinson, 1994). However, the mechanisms of symbiosis can be very distinct between and within these functional guilds, largely because EM and NM associations represent convergent evolution on a phenotype, rather than a shared evolutionary mechanism of interaction (Tedersoo, May, & Smith 2010).

Mortierellaceae are early diverging soil fungi belonging to the subphylum Mortierellomycotina. They are closely related to Glomeromycotina (the AM fungi) and Mucoromycotina, some of which are EM fungi (James et al. 2006; Spatafora et al. 2016). Plant associations with *Mortierella* have been recorded since the early 1900s and these fungi are broadly considered NM plant associates (Stiles, 1915; Bisby, Timonin, & James, 1935). Mortierellaceae are commonly detected and isolated from soils, plant debris, insect guts, mosses and roots of living plant roots (Dixon-Stewart,

1932; Gams, 1977; Domsch et al. 1980), and have been found on every continent, including Antarctica (Gams, 1977; GBIF.org, 2019). However, the extent of the plant growth promotion (PGP) phenotype(s) and the underlying mechanism(s) of association are still not well understood. Moreover, the inability to resolve phylogenetic relationships within Mortierellaceae limits their classification and the ability to make inferences pertaining to species distributions and diversity, or the conservation of functional ecologies across Mortierellaceae species (Gams, 1977; Petkovits et al. 2011; Wagner et al. 2013).

Soil fungi in the Mortierellaceae also frequently harbor intracellular bacterial endosymbionts, making them an engaging research system for studying plant-fungal-bacterial symbioses. There are two lineages of endobacteria found in *Mortierella* species: *Mycoplasma*-related endobacteria (MRE) and *Burkholderia*-related endobacteria (BRE), which includes *Mycoavidus cysteinexigens* (Ohshima et al. 2016; Uehling et al. 2017; Desirò et al. 2018; Takashima et al. 2018). MRE are also found in Mucoromycotina, including EM species of Endogonales, and in the Glomeromycotina, which form AM (Bonfante & Desirò, 2017). The BRE in Mortierellaceae are closely related to the BRE in Glomeromycotina, *Ca.* Glomeribacter (Ohshima et al. 2016). Although the impact of Glomeromycotina MRE and BRE on the AM fungal-plant symbiosis has been characterized in one study, it remains unknown whether Mortierellomycotina endobacteria impact the Mortierellaceae-plant symbiosis (Lumini et al. 2007; Bonfante & Desirò, 2017).

Background

Mortierellaceae Phylogeny

The Mortierellaceae are a family of fungi whose diversity, global distribution, and phylogenetic structure remain poorly characterized (Nagy et al. 2011; Tedersoo et al. 2014). These challenges limit inferences of total Mortierellaceae diversity and species relationships, both of which are key to identifying functional groups or genetic patterns. This is especially important in the context of

plant symbioses, given that Mortierellomycotina is phylogenetically related to the AM fungi (Glomeromycotina) and to a lineage of EM fungi (Endogonales). There are well over 100 species in Mortierellaceae, which were estimated to have split from its most recent common ancestor (MRCA) with the Glomeromycotina 358-508 million years ago (Wagner et al. 2013; Uehling et al. 2017). It is possible that the MRCA were plant associated, a trait that may have been lost through their evolution. Alternatively, the trait for associating with plants may have been gained in some species from a MRCA that had shared potential for plant association, but was not necessarily dedicated to that lifestyle (Uehling et al. 2017; Bonfante & Venice, 2020). Resolution of the Mortierellaceae and subsequent plant growth promotion (PGP) bioassays of representative species is necessary to elucidate which of these hypotheses is better supported.

Early efforts to classify Mortierellaceae were based on macro- and micromorphology, including growth patterns, coenocytic hyphae, and asexual spore production (Gams, 1977). Most species within Mortierellaceae have macromorphological growth patterns on agar media and can produce three types of spores: asexual sporangiospores, asexual chlamydospores that can be produced terminally or intercalary, and sexual zygospores. One or more spore types may be absent in some species, such as *M. chlamydospora* which lacks sporangiospores and *M. parvispora* which lacks chlamydospores (Gams, 1977). Sexual reproduction is either heterothallic, where strains are required to out-cross with a compatible partner to mate, or homothallic, where a single strain possesses both mating types and is able to complete the sexual process without another individual. Macromorphology, micromorphology, and the production of all three spore types may vary considerably between growth media and conditions, which can complicate morphological species identifications (Petkovits et al. 2011).

Mortierellaceae species and their groupings were morphologically redefined throughout the mid and late 1900s by mycologists including Gams, Linnemann, Mil'ko, Zycha, and Turner (Gams, 1976; Domsch et al. 1980). By 1970, a total of 9 genera had been described in the family:

Mortierella Coemans 1863, *Carnoya* Dewèvre 1893, *Dissophora* Thaxter 1914, *Haplosporangium* Thaxter 1914, *Azygozygum* Chesters 1933, *Naumoviella* Novotelnova 1950, *Aquamortierella* Embree & Indoh 1967, *Echinosporangium* Malloch 1967, and *Actinomortierella* Chalabuda 1968. In 1976, the monotypic *Azygozygum chlamydosporum* was redefined as *Mortierella chlamydospora* (Plaats-Niterink et al. 1976). *Carnoya* and *Naumoviella* were also synonymized with *Mortierella* around this time, though few records exist for these changes, which have not yet been digitized or translated from the original German texts (Gams, 1977). From 1969-1977, Gams performed comprehensive revisions of Mortierellaceae species and genera (Gams 1976; Gams 1977). He combined *Actinomortierella* and *Haplosporangium* into the genus *Mortierella* and then divided *Mortierella* into two subgenera: *Micromucor* and *Mortierella*. Within *Mortierella* subgenus *Mortierella*, Gams recognized 9 sections and at least 73 species: *Alpina*, *Actinomortierella*, *Haplosporangium*, *Hygrophila*, *Schmuckeri*, *Simplex*, *Spinosa*, *Stylospora*, and *Mortierella* (Gams, 1977). This arrangement was the final major revision of *Mortierella* based on morphological characteristics.

In the early 1990s, with the advent of PCR and Sanger sequencing technologies, molecular systematics provided novel approaches that use variations in genome sequences to determine phylogenetic relationship between sampled taxa. Early phylogenies focused on rDNA and mitochondrial genes because they are ubiquitous among living organisms and have both highly conserved and hyper variable regions (Olsen & Woese, 1993). Highly conserved regions evolve very slowly and can be compared across extremely distantly related organisms, while the hyper variable regions generally evolve sufficiently to distinguish between species (Olsen & Woese, 1993). In fungi, the hyper variable region is the Internal Transcribed Spacer (ITS) region, between the highly conserved 18S (SSU) and 28S (LSU) coding regions (Schoch et al. 2012).

DNA sequence analyses have permitted the rearrangement and definition of several Mortierellaceae genera. *Mortierella* subgenus *Micromucor* was reclassified to belong within

Mucoromycota in the genus *Umbelopsis* (Meyer & Gams, 2003). *Mortierella* subgen. *Gamsiella* was elevated to generic status and *Echinosporangium* was renamed *Lobosporangium* to resolve a nomenclature conflict with a red alga also named *Echinosporangium* (Benny & Blackwell, 2004). *Echinochlamydosporium* was recently described as a novel Mortierellaceae genus basal to all previously described genera, but cultures are not readily available for corroborating studies (Jiang et al. 2011). The genus *Modicella* was reassigned from the Mucoromycotina to the Mortierellaceae (Smith et al. 2013). Therefore, the most current molecular-based Mortierellaceae classification divides species into seven recognized genera: *Lobosporangium*, *Dissophora*, *Mortierella*, *Modicella*, *Gamsiella*, *Aquamortierella*, and the enigmatic *Echinochlamydosporium* (Benny, 2009; Jiang et al. 2011; Wagner et al. 2013).

The first modern revision of the Mortierellaceae at the species level analyzed the ITS, SSU, and LSU rDNA regions across 85 strains representing 65 taxa (Petkovits et al. 2011). The authors established that the historic morphological classification system was largely unsupported by DNA sequence data and defined 12 new clades (Petkovits et al. 2011). The composition and general arrangement of the clades had strong statistical support, though the authors noted that placement of two clades (represented by *Mortierella strangulata* and *M. selenospora*, clades denoted /strangulata and /selenospora, respectively) did shift within the phylogenetic tree if ambiguously aligned sites in the ITS region were excluded from the analyses. It was also noted that several strains seemed to be misidentified. The authors concluded that species should be represented by multiple strains and/or identified on a genetic basis in order to increase confidence in their phylogenetic placement (Petkovits et al. 2011).

A second rDNA phylogenetic study shortly thereafter expanded the diversity of sequenced species to over 400 specimens, including 63 type strains (Wagner et al. 2013). The authors used morphology and sequence data to confirm the identities of included specimens. Recognizing that the ITS region is too divergent to align across the entire lineage without significant indel gaps,

they generated a preliminary tree using the LSU region to define clades within the Mortierellaceae. They then aligned and analyzed the ITS region within each clade to define finer relationships between species. Wagner et al. (2013) reorganized and combined several of the Petkovits et al (2011) clades into a total of seven rDNA-based clades. The study by Wagner et al. (2013) has remained the most comprehensive and useful revision of the Mortierellaceae.

Undiscovered fungi represent potential sources for biocontrol agents, pharmaceutical compounds, and agriculturally important plant symbionts (Hawksworth & Rossman, 1997). Detecting, isolating, and characterizing novel species is important to understanding the members of ecosystems, the complex interactions taking place, and how these ecological contexts drive the processes relevant to our interests. There is considerable variation in estimates of Mortierellaceae species diversity that remains unsampled (Hibbett & Glotzer, 2011; Nagy et al. 2011). Nagy et al. (2011) estimated the rate of novel species discovery in Mortierellaceae by comparing the total diversity of over 800 Mortierellaceae ITS sequences deposited to GenBank to the sequence diversity within 102 reference sequences from 78 described species. Since most of the sequence diversity in the GenBank dataset was already represented in culture and sequence repositories, they concluded that most Mortierellaceae diversity was already discovered and redetected, due to unsequenced type specimens. Nagy et al. (2011) estimated a total of approximately 127 species in the family. Given that 102 of the 125 currently accepted species in Mortierellaceae were described prior to 1980, and only 11 more between 1990 and 2011, this might seem to be a reasonable conclusion. However, this estimate was based only on well sampled areas, and did not take into account that vast regions of the world are still poorly sampled. Further, it should be noted that ITS and/or 28S rDNA regions have limited resolution at the species level, which may lead to underestimates of diversity (Nagy et al. 2011; Wagner et al. 2013). Hibbett & Glotzer (2011) countered the findings of Nagy et al. (2011), pointing out that type or authenticated material is unavailable for about half of the described Mortierellaceae species.

Further, Hibbett & Glotzer (2011) noted that most of the unidentifiable molecular operational taxonomic units (mOTUs) identified by Nagy et al. (2011) did not include sequences from cultures, having been generated from environmental metagenomic studies. In fact, there are currently close to 125 accepted Mortierellaceae species, and new species continue to be described at a steady, if not increasing, rate (Gams, 1977; Smith et al. 2013; Wagner et al. 2013; Degawa, 2014; Takashima, Degawa, Ohta & Narisawa, 2018). Some taxonomists caution that recent species descriptions may be established as redundant because the phylogenetic markers used to establish novelty of the culture sequence were not compared to a complete reference dataset (Wagner et al. 2013). Such oversights are entirely possible since a variety of rDNA markers are available and studies select among them for whichever is most suited to their study conditions (Bazzicalupo et al. 2013; Kohout et al. 2014). A comprehensive library of Mortierellaceae reference sequences for all possible described species, whether from type or authenticated strains, is not available yet.

Of the 125 currently described species, at least 119 are classified as belonging to the genus *Mortierella* (Smith et al. 2013; Wagner et al. 2013). This is predominantly because the other accepted genera were described based on morphological distinctions that are not shared with the novel species. Therefore, *Mortierella* has largely become a catch-all genus for anything that belongs in the Mortierellomycotina. Further, modern taxonomic studies have established that *Mortierella* is polyphyletic with respect to the other genera in the family. One option for resolving this polyphyly by collapsing all Mortierellaceae species and genera into *Mortierella*, in order to circumvent the extensive revision of species and genus descriptions necessary to fully resolve species relationships and establish monophyletic genera (Petkovits et al. 2011). However, this solution would exacerbate the already poor resolution of species in this genus by rendering the genus-level identification equally as informative as the sub-phylum level classification and is generally not endorsed (Petkovits et al. 2011; Wagner et al. 2013).

DNA sequencing and phylogenetic methods have the ability to provide a framework that can guide classification and taxonomy decisions (Petkovits et al. 2011; Wagner et al. 2013). However, it has become clear that neither ribosomal data nor morphological characterizations are sufficient by themselves to resolve phylogenetic relationships within Mortierellaceae (Petkovits et al. 2011; Wagner et al. 2013). Thus, additional non-ribosomal markers are needed to identify monophyletic clades and describe novel genera to increase genus-level taxonomic resolution.

Criteria for choosing non-ribosomal (nuclear) phylogenetic markers include genes that are single-copy and not under selective pressure; they should also contain sufficient sequence variation to make phylogenetic inferences. Identification of nuclear markers has historically been done manually, starting from protein sequence and characteristics, as in the case of RPB1 (Jokerst, Weeks, Zehring, & Greenleaf, 1989; Sidow & Thomas, 1994). Even with the advent of genome sequencing, discovery and evaluation of novel nuclear markers has been a largely manual process (Blair, Coffey, Park, Geiser, & Kang, 2008). There has been at least one effort to automate the discovery and evaluation of nuclear markers, a program called DIscoMark, which analyzes orthologous gene datasets to identify candidate loci (Detering, Rutschmann, Simon, Fredslund, & Monaghan, 2016). Both approaches are dependent on the availability of high-quality input genomes, of which there were only three for Mortierellaceae species at the start of the present work.

Another approach used to resolve phylogenies relies upon genomic data to perform comparative phylogenomics and thus circumvent the challenges of amplicon sequencing of individual markers (Spatafora et al. 2016; Zhang et al. 2017). There are two approaches for genome sequencing: high-coverage *de novo* genome sequencing and low-coverage genome (LCG) sequencing. High-coverage genomes allow higher quality assembly and annotation (Sims et al. 2014). The LCG approach recovers less data and lower confidence assemblies, which must be guided by a reference *de novo* genome. These two approaches represent a tradeoff between

data and confidence vs. per-sample cost and sample throughput. The *de novo* sequencing approach was recently used to perform a comprehensive molecular review of zygomyceteous fungi, wherein 192 protein coding genes were used to resolve phylum-level relationships between 43 taxa across 7 phyla in the Kingdom Fungi and 3 outgroup taxa (Spatafora et al. 2016). The LCG approach has been applied successfully in insects and olive tree systems, from both low and high quality specimens and genome coverage between 0.5-30X (Olofsson et al. 2019; Zhang et al. 2019). These LCG phylogenomic approaches have relied on first identifying existing phylogenetic markers in assembled whole genome sequence data and mining them from the LCG dataset, rather than discovering novel markers. Olofsson et al. (2019) also demonstrated the capability of an LCG approach to extract phylogenetic information from degraded herbarium specimens with extremely low coverage (<0.5X), which encourages LCG sequencing of fungal herbarium specimens (Olofsson et al. 2019).

These studies together suggest that estimations of Mortierellaceae species diversity and phylogeny remain uncertain, largely due to confused and inadequate morphological definitions, inadequate phylogenetic markers for molecular identification, and a dearth of reference sequences for type material. The genus *Mortierella* is polyphyletic with respect to the other Mortierellaceae genera and has become a catch-all genus for novel Mortierellaceae species. Further taxonomic revisions likely need to include non-ribosomal phylogenetic markers. A combination of high- and low-coverage genome sequencing may be suitable for recovery of sequence information from both fresh cultures and degraded herbarium specimens. Phylogenomics may serve as a framework for amplicon-based studies and identification of candidate non-ribosomal markers. The application of non-ribosomal marker discovery for amplicon-based phylogenetics and low-coverage genome sequencing for phylogenomics to resolving the Mortierellaceae phylogeny is discussed further in Chapter 2.

Plant-Fungal Symbiosis

Plant microbiomes are dynamic and complex, and consist of bacteria, archaea, fungi, protists, and viruses. The effect that each member of the microbiome has on the host plant occurs on a spectrum from beneficial to pathogenic, and can shift along that spectrum depending on interactions with other members of the microbiome or abiotic changes in the plant environment (Zeilinger et al. 2015). Microbial protection against biotic and/or abiotic stresses and promotion of plant growth has great potential to naturally improve agricultural yields while also relieving economic and environmental costs of crop production (Rodriguez et al. 2008). Understanding the mechanisms of plant-microbe symbiosis could provide insight into what triggers shifts in the microbial behavior toward the plant. It could also enable us to also adjust land management, plant breeding, and agricultural practices to capitalize on beneficial plant interactions.

Plant-associated fungi have extensive mycelial networks which efficiently scavenge and transport minerals, nutrients and water to host plants. There are three main guilds of plant mutualistic fungi relevant to this study: arbuscular mycorrhizal fungi (AM), ectomycorrhizal (EM) fungi, and non-mycorrhizal (NM) root endophytic fungi. Both AM and EM fungi promote plant growth primarily by providing water and mineral nutrients and often secondarily by niche occupation precluding infection by pathogens (Hooker et al. 1994). However, the signaling mechanisms and fungal symbiotic structures are very distinct between these two functional guilds. The term NM fungal endophyte encompasses an extremely diverse group of fungi, including representatives from Ascomycota, Basidiomycota, and Mucoromycota. Focusing on root endophytes narrows the diversity considerably within each phylum, but the range of species, lifestyles, and mechanisms of plant association are still extremely broad. In all of these associations, the fungus receives or derives nutrients from the plant host. In mycorrhizal associations, plants actively transfer photosynthates to the fungus. NM fungi may also penetrate into the root and receive photosynthates, as with the mycorrhizae, but NM fungi are also able to

degrade and utilize general root exudates and sloughed off plant root cells (Buée et al. 2009).

As mentioned above, plant-microbe interactions are complex and can often involve multiple symbionts. Fungal endobacteria are known to have a crucial role in how their host fungus interacts with plant partners in many tripartite symbioses (Vannini et al. 2016). In plant pathogen *Rhizopus microsporus*, endobacteria are necessary for fungal sporulation and for production of a toxin that kills plant cells, releasing nutrients to the fungus (Partida-Martinez & Hertweck, 2005; Lackner et al. 2011). In AM fungus *Gigaspora margarita*, endobacteria *Candidatus* Glomeribacter provides additional defenses to environmental stress, as demonstrated by increased carbonylation of proteins in response to oxidative stress, which accumulate in cured AM fungi and are transmitted to host plant roots (Salvioli et al. 2016; Vannini et al. 2016). Endobacteria are also found in some EM fungi, but their impact on those plant-fungal associations is still unknown.

Ectomycorrhizal (EM) Fungi

Ectomycorrhizal associations are classically defined on the basis of symbiotic morphology (Tedersoo & Brundrett, 2017). The first characteristic is clusters of short, highly branched lateral plant roots wrapped in a thick fungal mantle. The second is the Hartig net, a web of fungal hyphae seen in the cross-section of the short, lateral EM root, where the hyphae have penetrated around and between, but not into root epidermal and cortex cells. Recent studies have expanded this definition to include phylogenetic relatedness to other EM fungi and/or demonstrably mutualistic, since some EM associations have poorly formed EM structures (Tedersoo & Brundrett, 2017).

Only about 2% of known plant species are capable of supporting an EM symbiosis; these are predominantly woody shrub and tree species (Tedersoo et al. 2010). Most commercial and academic research interest in EM fungi is focused on applications in forestry and agriculture, *e.g.* habitat restoration, Christmas tree farms, and timer plantations (Peterson et al. 1984; Jeffries & Rhodes, 1987). However, in addition to the benefits that EM fungi confer to plants, many EM fungi are also studied and farmed for production of their fruiting bodies, e.g., truffles, porcini

mushrooms, and chanterelles (Hall et al. 2003).

EM symbiosis has arisen and persisted through convergent evolution of both the plant and fungal partners. In fungi, EM capacity has evolved over 60 times, arising from saprotrophic fungi mostly in Basidiomycota, but also Ascomycota and Mucoromycotina, with current estimates of diversity at 20,000-25,000 species (Tedersoo et al. 2010). In plants, it is estimated that 250–300 genera and 6000–7000 species are consistently EM hosts (Tedersoo & Brundrett, 2017). Most EM plants seem to have evolved EM capacity from a pre-existing AM capacity, though there are some exceptions to this generalization (Tedersoo & Brundrett, 2017). Each plant host may interact with hundreds of EM fungi throughout their lifecycle, possibly selecting for different fungal partners at each life stage and/or season (Courty et al. 2010). Each individual fungus can also interact with many different plant hosts simultaneously, serving as a means of plant-plant communication, though the range of compatible plant host species for each fungal species is generally limited (Bruns et al. 2002; Courty et al. 2010). EM fungi can live independent of their plant hosts as saprotrophs and are therefore usually culturable and amenable to experimental manipulation, though they generally grow slowly (Jeffries & Rhodes, 1987). However, EM plant hosts have long generation times, more intense growth requirements, and larger genomes than many model plants, such as the Brassicaceae (Tedersoo et al. 2010).

In addition to helping to physically exclude pathogens from roots, EM fungi benefit plants by obtaining and transferring minerals and simple organic nutrients to plant roots. They scavenge these nutrients by secreting enzymes from their hyphae and absorbing the digested products. There are differences in the key enzyme functions of different EM fungal lineages and species therein (Tedersoo et al. 2012). It has been suggested that these differences are also due to differences in the substrates and environmental conditions in which each EM fungal lineage or species evolved (Courty et al. 2010). This environmental specificity, combined with the high abundance of indigenous EM fungi in soils, means that it is often difficult to “transplant” EM fungi

onto established plants, rather is often more effective to simply rely on the extant fungi when relocating plants, an issue particularly challenging for truffle growers that are focused on growing a single EM species rather than the whole community.

The signals exchanged in EM fungi symbioses are highly variable between different combinations of plants and fungi (Garcia et al. 2015). This has restricted the ability to translate research into the mechanism of EM symbiosis from one system to another. However, some commonalities can be observed. First, it appears to be critical that the EM fungus has lost most of the cell-wall degrading enzymes commonly found in saprotrophic fungi, particularly cellulases, to avoid production of plant cell damage-associated compounds that trigger the plant immune system (Clear & Hom, 2019). The initiation of EM symbiosis appears to involve fungal detection of plant root exudates (often flavonoids in particular) (Daguerre et al. 2017; Clear & Hom, 2019). In some EM fungi, the fungus responds to plant stimulus by producing a class of volatile organic compounds called sesquiterpenes that induce characteristic root branching independent of cell contact, others are known to produce auxins (Daguerre et al. 2017). In order to increase plant cell plasticity and promote hyphal penetration between plant root cells, EM fungi secrete glucanases, chitinases, aquaporins, and small secreted effector proteins (SSPs) (Clear & Hom, 2019). However, the identity of the crucial SSPs appears to vary between species and only one has been well characterized (Clear & Hom, 2019). A well characterized SSP is MiSSP7, produced by *Laccaria bicolor* during the early stages of symbiosis with *Populus trichocarpa*. This mycorrhizal secreted protein blocks jasmonic acid (JA) defense signaling and thereby promotes fungal colonization (Plett et al. 2014). However, there are over 10,000 EM symbiotic genes in the *L. bicolor* genome, few of which have functional characterizations (Kaur & Reddy, 2019). MiSSP7 has no homologs in close relatives, thus, while the underlying mechanisms may be conserved (*e.g.* use of SSPs), the specifics appear to vary between fungal species and plant hosts.

Several species of EM fungi Endogonales have been confirmed to host obligate *Mycoplasma-*

related endobacteria (MRE), both from microscopic examination and by 16S rDNA sequencing. MRE genomes have been recovered from the sequenced metagenomes of some colonized Endogonales species. However, these MRE genomes are very difficult to analyze or characterize, particularly as a single fungal strain may host multiple, relatively diverse MRE populations (Bonfante & Desirò, 2017). The impact of MRE on EM fungal hosts is still unknown (Bonfante & Venice, 2020).

Arbuscular Mycorrhizal (AM) Fungi

Similar to EM fungi, AM fungi are typically defined morphologically. Unlike EM fungi, AM fungi penetrate plant cortical cells and form arbuscules, which are highly branched/coiled fungal structures within the plant cell. AM fungi do not produce fruiting bodies, but they do form very large spores containing hundreds of nuclei. The benefit of AM fungi to plants has long been of interest and use in agriculture to improve plant survivability, growth, and disease resistance, even though the primary mechanisms of interaction have only recently been formally described (Jeffries & Rhodes, 1987; Hooker et al. 1994; Gutjahr & Parniske, 2013).

In contrast to the convergent evolution of EM symbiosis, AM fungi compose a monophyletic lineage (Glomeromycotina), which are estimated to have arisen between 358-558 million years ago (Uehling et al. 2017). The AM fungi are an ancient plant-fungal association and have been implicated in assisting plants in the colonization of land (Taylor et al. 1995). AM fungi are obligate biotrophs and spores only germinate after detecting exudates from a nearby plant root and they have limited resources with which to grow to the plant root. Unlike the EM fungi, AM fungi have low host specificity and have been found in about 80% of vascular plant species and some non-vascular plants (Smith & Read, 2010). AM fungal spores have hundreds of nuclei, which in combination with the requirement for a living plant host, make experimental manipulation of AM fungi very difficult.

Arbuscular mycorrhizal fungi associate with many common model plants, including legumes

used to study legume-rhizobia symbioses. It was discovered very early on that many of the plant symbiotic genes necessary for legume-rhizobia symbiosis were also necessary for successful AM symbiosis (Duc et al. 1989). Moreover, the obligate biotrophism of AM fungi was useful for characterizing the early stages of symbiotic signaling. AM fungal spores germinate when they detect constitutively produced plant root hormones called strigolactones (Gutjahr & Parniske, 2013). Prior to contact with the plant root, the fungus responds to the plant root by producing and secreting a mixture of chitooligosaccharides, referred to as Myc factors. These Myc factors stimulate plant symbiotic genes by much the same mechanism as Nod factors in the legume-rhizobia symbiosis (Gutjahr & Parniske, 2013). Indeed, many of the plant genes involved in initiating AM symbiosis are also involved in legume-rhizobia symbiosis, though the common proteins are part of distinct plant signaling receptor protein complexes (Genre & Russo, 2016). The Myc factors trigger a signaling cascade from the receptor protein complex that initiates transduction of the AM signal between plant cells via calcium spiking. AM fungi also secrete an effector protein (SP7) that suppresses plant ethylene signaling (Mukherjee & Ané, 2011). Ethylene has been found to inhibit the root architectural changes required for AM colonization. Once established, individual arbuscules persist in root cells for 24-72 hours, before the plant initiates senescence and turnover of the arbuscule. The mechanism for this plant regulation of AM symbiosis is unknown, but is predicted to be a mechanism by which plants can select for and reward AM partners providing the most nutrients to the plant, particularly nitrogen and phosphorus (Gutjahr & Parniske, 2013).

There are two lineages of obligate endobacteria found to live within tissues of AM fungi: *Mycoplasma*-related endobacteria (MRE) and *Burkholderia*-related endobacteria (BRE). The MRE in AM fungi are closely related to those found in the EM Endogonales. The effect of MRE on the metabolism and plant symbiosis of their fungal hosts is unknown, but the relationship appears to be facultative for the fungus, which can complete its life cycle without either

endobacteria. Both endobacteria are transmitted vertically by bacterial vesicles into AM spores (Bianciotto et al. 2004). One study examined the effect of BRE *Ca.* Glomeribacter gigasporum on the fitness and plant association of its fungal host *Gigaspora margarita* (Lumini et al. 2007). The authors found that *G. margarita* strains cured of their BRE were still able to colonize plant roots, but were significantly impaired in the pre-symbiotic distance that germinating spores could traverse to make contact with roots, likely due to reduced lipid and protein reserves compared to BRE-colonized spores (Lumini et al. 2007). This suggests that AM fungal BRE do not impact the AM symbiosis directly, other than increasing pre-symbiotic fungal fitness.

Non-Mycorrhizal (NM) Root Endophytic Fungi

For the purpose of this study, NM root endophytes are defined as fungi found inside living, healthy plant roots that do not make any characteristic mycorrhizal structures. Similar to EM fungi, NM root associated fungi have evolved in Mucoromycota, Ascomycota, and Basidiomycota. Many NM fungi have a broad host range, most importantly including plants that cannot support other mycorrhizal symbioses, such as Brassicaceae (Tester et al. 1987, Buée et al. 2009). In general, NM fungi live in soil as saprotrophs and associate with plants opportunistically as plants recruit and select soil microbes to colonize the rhizosphere by tailoring their root exudates (Zeilinger et al. 2016). In some cases, such as *Fusarium oxysporum*, different isolates within a species may be plant beneficial or plant pathogenic, which is thought to be controlled by biosynthetic gene clusters on supernumerary chromosomes (Validov et al. 2011; van Dam et a, 2017; Hoogendoorn et al. 2018).

The primary benefits of fungal saprotrophs to plants are nutrient provision, secretion of competitive antifungal compounds, consumption of root exudates that would attract pathogens, and physical exclusion of pathogens (Buée et al. 2009). It has been suggested that plant root exudates stimulate the growth and activity of saprotrophic NM fungi, which then also degrade complex organic compounds in the surrounding soil, some of which may be transferred to or

directly absorbed by the plant (Subke et al. 2004). It is also possible that NM fungi interact with mycorrhizal fungi to indirectly associate with plants (Buée et al. 2009). Some NM endophytes are also mycophiles and feed on would-be fungal competitors and plant pathogens, as in the case of many *Trichoderma* spp. (Harman et al. 2004). Very few NM associations have been studied closely, but these fungi represent potential sources of biocontrol agents, producers of novel pharmaceutical compounds, and scavengers of plant nutrients.

The most thoroughly characterized plant beneficial NM fungus is *Serendipita (=Piriformospora) indica*, a Basidiomycete, which has unusually strong plant growth promoting (PGP) activity on a wide variety of plants, such as tobacco, *Arabidopsis thaliana*, barley, and legumes (Peškan-Berghöfer et al. 2004). *S. indica* has been shown to confer resistance to pathogens and abiotic stress by priming the plant immune system, transmitting nutrients, and manipulating plant hormone signaling (del Barrio-Duque et al. 2019).

The benefits conferred by *S. indica* in pure-culture experimental conditions are similar to those of AM fungi, but with the benefit of an axenically culturable fungus (del Barrio-Duque et al. 2019). Interestingly, *S. indica* was found to have no PGP effect on mycorrhizae-deificent pea and soybean mutants, which implicates a symbiotic mechanism similar to that of the AM fungi (Varma et al. 2001). Prior to contact with the root, *S. indica* secretes effectors that stimulate a systemic stress and defense response (Vahabi et al. 2015). In general, once hyphae make contact with the root, this response is shut off and the plant shifts over to a mutualistic interaction (Vahabi et al. 2015). However, the timing of this shift and the role of plant hormones jasmonic acids (JA) and gibberellic acid (GA) differ considerably between plant species (Liu et al. 2019). Similar to AM fungi, *S. indica* colonizes root cells progressively along the growing root length and over time the colonized cells are turned over (Jacobs et al. 2011). However, unlike the AM fungi, where the root cell survives this turnover, *S. indica* kills the colonized plant cells to release and absorb the cell contents (Jacobs et al. 2011). Arabidopsis secretes defensive proteins that regulate the extent of

S. indica colonization, balancing the benefit and cost of the symbiosis (Thürich et al. 2018).

While the mechanism of *S. indica*-plant interaction may be highly variable, significant improvement of plant health, growth, and performance is universal under research conditions (Lie et al. 2019). Unfortunately, *S. indica* is not commonly found in soils, necessitating inoculating fields with non-native microbes, which has ethical and ecological implications (Rabiey et al. 2017). Therefore, it could be highly beneficial to explore globally cosmopolitan NM fungi and determine how to promote their selection and plant association using plant genotype and land management.

Mortierellaceae-Plant Symbiosis

Mortierellaceae have been isolated from living, healthy plant roots for over 100 years and from roots and soils across multiple continents and habitats (Stiles, 1915; Bisby et al. 1935; Domsch et al. 1980). Some Mortierellaceae species appear to be globally cosmopolitan. Because the Mortierellomycotina are closely related to both AM fungi and the Endogonales, research into the mechanism of PGP activity may provide insight into the evolution of plant symbiosis in this highly diverse phylum. Moreover, Mortierellaceae are easily isolated and cultured and can be studied on both mycorrhizal and non-mycorrhizal plants.

Many Mortierellaceae species host both MRE and BRE. The lineage of BRE associated with Mortierellaceae, named *Mycoavidus cysteinexigens*, are distinct from, but form a sister clade to *Ca.* Glomeribacter found within Glomeromycotina (Ohshima et al. 2016; Uehling et al. 2017). Multiple phylotypes of both lineages have been found in different fungal isolates and species (Desirò et al. 2018; Takashima et al. 2018). Metabolic studies with isolates of *M. elongata* which have been “cured” of their endobacteria through the use of antibiotic passaging, indicate that the endobacteria restrict fungal growth rates and upregulate cellular respiration (Uehling et al. 2017). Moreover, preliminary data show a strong effect of the endobacteria on the lipid and secondary metabolite profiles of *Mortierella* isolates (Uehling et al. 2017). Genome sequence analysis of *Mortierella* and BRE has also shown that *Mortierella* lacks genes to synthesize many secondary

metabolites and that this is potentially complemented by the endobacteria (Uehling et al. 2017). It is uncertain whether endobacteria confer a functional advantage to Mortierellaceae. It is possible that any functional advantage may be restricted to environmental conditions that have not yet been replicated in experimental conditions. Until the present study, Mortierellaceae-plant interaction studies did not include endobacteria as a factor.

In both controlled research conditions and in field soils, *Mortierella* species have demonstrated a wide variety of benefits to their host plants, which can include protection against nematodes, pathogenic fungi, and abiotic stresses, as well as plant growth promotion under controlled conditions (Al-Shammari et al. 2013; Wani et al. 2017; Shemshura et al. 2018; Johnson et al. 2019). In addition, *Mortierella* species also interact with other fungi and bacteria in the root microbiome, some of which have been isolated and shown to work cooperatively with *Mortierella* (Moreno et al. 2016; Tamayo-Velez & Osorio, 2017; Liao et al. 2019; Uehling et al. 2019).

Root-knot nematodes cause severe damage to agricultural plants by feeding on plant roots and vectoring both bacterial and viral plant diseases (DiLegge et al. 2019). *Mortierella alpina* inhibits the hatching of root-knot nematode *Meloidogyne javanica* eggs and reduced the severity of the nematode pathogenicity on tomato plants *in vivo*, which significantly increased plant growth compared to the uninoculated, nematode infected controls (Al-Shammari et al. 2013). The closely related *Mortierella globalpina* had a similar inhibitory effect on *Meloidogyne chitwoodi*, also tested on tomato (DiLegge et al. 2019). Interestingly, the latter study compared tomato plant growth in control, nematode-only, fungus-only, and nematode+fungus treatments and found that any treatment including the fungus (*Mortierella globalpina*) had increased root and shoot biomass (DiLegge et al. 2019). This demonstrates that *M. globalpina*, and potentially *M. alpina*, promote plant growth and protect plants from nematode pathogens.

Recent research into natural biocontrol of phytopathogenic fungi has found that *M. alpina* produces a suite of mycotoxic compounds (Shemshura et al. 2018). Culture exudates and

extracted lipids from *M. alpina* were found to inhibit the growth of three plant pathogenic fungi: *Purpureocillium lilacinum*, *Fusarium tricinctum*, and *F. oxysporum* (Shemshura et al. 2018). Arachidonic acid composed up to 40% of the lipids and 11% of the total dry biomass produced by *M. alpina*. Purified arachidonic acid strongly inhibited the growth of all three pathogens and also inhibited their production of mycotoxins that diffuse into the food products of infected crops and cause severe diseases in mammals (Shemshura et al. 2018). This finding is particularly important when taken in combination with another study that found exogenously applied phytohormones increased the production of arachidonic acid by *M. alpina* (Zhang et al. 2019). The screened plant hormones included cytokinins (6-benzyl adenine (BA) & furfuryl adenine (KT)), auxin, gibberellin, and abscisic acid. Each hormone had different stimulatory effects and required an optimized concentration. However, Zhang et al. (2019) also noted that combining both cytokinins had no synergy and cytokinin+auxin inhibited *M. alpina* growth and production of arachidonic acid. This suggests a potential mechanism by which plants can employ *M. alpina* to produce metabolites to protect themselves against pathogenic fungi and simultaneously regulate *M. alpina* colonization of the rhizosphere. A study of *M. alpina* on saffron crocus also demonstrated agriculturally important corm rot disease protection via arachidonic acid and disease effect mitigation through the jasmonic acid pathway (Wani et al. 2017).

Mortierellaceae interact cooperatively with other microbiome species in the plant rhizosphere. Liao et al. (2019) found that *M. elongata* structures the *Populus* fungal rhizobiome selected from forest soil, slightly reducing the activity of AM fungi and increasing that of EM fungi and other NM root endophytes. *M. elongata* also promoted *Populus* growth in a cultivar-dependent manner (Liao et al. 2019). Transcriptome analysis indicated that *M. elongata* inoculation resulted in up-regulation of *Populus* genes related to lipid signaling, nutrient uptake, and growth promotion. Liao et al. (2019) aslo found alteration of gene expression related to gibberellin, jasmonic acid, salicylic acid, and ethylene signaling, suggesting that *M. elongata* manipulated plant defense responses.

Another study of *M. elongata* and the *Populus* microbiome identified a free-living *Burkholderia* strain that exchanges metabolites with *M. elongata* in a multi-phased interaction that increases the growth of both partners (Uehling et al. 2019). It is yet unknown whether this association occurs naturally in the *Populus* rhizosphere and/or affects the *M. elongata*-*Populus* interaction. Two studies co-inoculated a phosphate-solubilizing *Mortierella* sp. with AM fungus *Rhizoglomus fasciculatum* onto four different species of nursery trees (Moreno et al. 2016; Tamayo-Velez & Osorio, 2017). The first study found that while *R. fasciculatum* did increase growth of three tree species 16-37% compared to uninoculated controls, co-inoculation with *Mortierella* sp. increased growth promotion to 108-116% compared to the uninoculated controls (Moreno et al. 2016). The second study examined the growth of avocado with applications of *R. fasciculatum* and *Mortierella* sp. individually and together (Tamayo-Velez & Osorio, 2017). They found that neither fungus significantly increased plant growth individually, but co-inoculation increased plant height, biomass, and phosphorous content (Tamayo-Velez & Osorio, 2017).

Ozimek et al. (2018) studied the microbiome of winter wheat to identify important winter-active plant associates. Two such symbionts were identified as *M. verticillata* & *M. antarctica*. *M. verticillata* produced high levels of auxin indoleacetic acid (IAA) when supplied with the precursor compound tryptophan and increased wheat root and shoot fresh weight by 40% (Ozimek et al. 2018). *M. antarctica* produced IAA independent of tryptophan amendment and increased root fresh weight by 40% and shoot fresh weight by 24%. Both strains were found to produce gibberellic acid and *M. antarctica* produced ACC (1-aminocyclopropane-1-carboxylate) deaminase, which degrades ethylene to ammonia, simultaneously reducing the concentration of a growth-inhibitory hormone and providing a nitrogen source to the plant root (Ozimek et al. 2018).

Two studies recently explored symbiosis between Arabidopsis and *M. hyalina* (Johnson et al. 2019; Meents et al. 2019). Johnson et al. (2019) found that *M. hyalina* increases Arabidopsis aerial growth and inhibits *Alternaria brassicae* disease development. They found that *M. hyalina*

exudes induced calcium spiking in Arabidopsis roots, much as AM fungi induce during early symbiotic signaling with legumes (Genre & Russo, 2016; Johnson et al. 2019). An Arabidopsis mutant deficient in the calcium spiking response was also insensitive to *M. hyalina* disease protection and could not regulate *M. hyalina* colonization (Johnson et al. 2019). Meents et al. (2019) found that *M. hyalina* produces IAA in pure culture and colonized Arabidopsis roots had increased IAA levels, but that auxin-responsive Arabidopsis genes were not differentially expressed compared to uninoculated Arabidopsis roots. However, Meents et al. (2019) did observe a significant increase in jasmonates in colonized roots after 1 day of interaction, which was not produced by *M. hyalina* in pure culture. This elevated phytohormone level was not observed by Johnson et al. (2019) in Arabidopsis roots 12 days post inoculation (DPI), suggesting that the elevated hormone levels are important for establishing, but not maintaining the Arabidopsis-*M. hyalina* symbiosis. Both studies also quantified ABA levels in Arabidopsis roots with and without *M. hyalina* and together found that ABA was unaffected at 1 DPI but reduced at 12 DPI (Johnson et al. 2019; Meents et al. 2019). Salicylic acid concentrations were not different in colonized roots at either stage of interaction, which the authors concluded was consistent with high IAA levels and antagonism between the IAA and SA signaling pathways (Johnson et al. 2019; Meents et al. 2019).

Li et al. (2018) explored the effect of organic fertilizer applications on the maize rhizobiome and found that the abundance of *M. elongata* significantly increased in response to organic amendments. The authors isolated *M. elongata* from the rhizosphere and inoculated unfertilized maize seedlings in bulk soil (Li et al. 2018). They found that plants inoculated with *M. elongata* had increased growth, increased phosphate, IAA and ABA concentrations, and an altered rhizosphere community. They suggested that *M. elongata* promoted plant growth by solubilizing and supplying phosphate to the plant, suppressing plant stress and defense responses and optimizing root architecture (Li et al. 2018).

In summary, Mortierellaceae is a highly diverse, globally distributed fungal lineage. Some species are known to have PGP activity, and a variety of mechanisms have been proposed. These fungi have a broad host range, including non-mycorrhizal plants, and are easily cultured, allowing for simplified research design compared to mycorrhizal fungi. *M. elongata* has been detected on all continents except Antarctica, precluding the need to inoculate agricultural or forest ecosystems with a non-native microbe. Moreover, *M. elongata* frequently hosts both BRE and MRE and has been shown to interact with free living soil microbiota (Desirò et al. 2018; Takashima et al. 2018; Uehling et al. 2019). These characteristics make *M. elongata* an excellent model system for studying tripartite plant-fungal- bacterial interactions, as will be explored in Chapter 3.

Research Focus

The goals of this dissertation research were to:

1. *Resolve the phylogeny of the Mortierellaceae*

Hypotheses: (H1) There are phylogenetically informative single-copy loci conserved across the Mortierellaceae for which family-specific primers can be developed from the genome sequences of a few species. (H2) Low-coverage genome sequencing of species across the Mortierellaceae will yield sufficient genetic loci to construct a highly supported Mortierellaceae phylogeny. (H3) Combining the LCG and amplicon datasets will provide sufficient data across sufficient species diversity to resolve the Mortierellaceae phylogeny.

2. *Confirm and characterize the symbiosis phenotype.*

Hypotheses: (H4) *Mortierella elongata* will promote the growth and seed production of *Arabidopsis thaliana*. (H5) *M. elongata* BRE and MRE impact the fungal-plant symbiosis.

3. *Elucidate the genetic basis of Mortierella-plant association.*

Hypotheses: (H6) Plant and fungal genes are differentially expressed in co-culture as

compared to individual cultures. (H7) Genes that are differentially expressed during symbiosis are involved in maintaining a stable plant-fungal symbiotic interaction.

Value of this research

Existing ribosomal markers are insufficient to classify Mortierellaceae isolates to the species level. Identification of superior phylogenetic loci would improve identification of Mortierellaceae isolates both from pure culture and in environmental studies, which would clarify species diversity and geographical distribution. The genus *Mortierella* currently contains nearly all of the recognized species in the Mortierellomycotina. This renders the current genus-level taxonomy equally as informative as the subphylum-level classification. Defining novel genera will improve the informative value of genus-level identification for novel species. As traits and ecological functions are determined for representative members of each group, conserved features might become apparent as definitive of that taxonomic group. This will enable inferences about the ecological function of new species or isolates classified within those groups. Finally, as the functions of each group become defined, the evolution of those traits within Mortierellaceae and the fungal tree of life can be studied.

Many studies have explored the impact of a variety of Mortierellaceae species in different environmental and experimental conditions. However, the role of Mortierellaceae bacterial endosymbionts in the plant-fungal symbiosis is unexplored. The Mortierellaceae are emerging as an extremely tractable research system. The *Mortierella* species reported to be plant-beneficial are distributed across several Mortierellaceae clades. It is valuable to understand the mechanism of interaction in each of these representative species to determine whether the Mortierellaceae have a conserved mechanism of plant association and how each functional group interacts with plants. If the Mortierellaceae do have a conserved approach, it can be compared to that of the Glomeromycotina and Endogonales to infer characteristics of their most recent common ancestor.

CHAPTER 2. RESOLVING THE MORTIERELLACEAE PHYLOGENY THROUGH SYNTHESIS OF MULTI-GENE PHYLOGENETICS AND PHYLOGENOMICS

Authors & Contributions

1. Natalie Vandepol - primer design & validation, MGP PCR, MGP sequence analysis, & writing
2. Julian Liber - microscopy, photography, species & genus description, isolate troubleshooting
3. Alessandro Desirò - DNA extraction & strain isolations
4. Hyunsoo Na - LCG sequencing
5. Megan Kennedy - LCG sequencing
6. Kerrie Barry - LCG sequencing coordination
7. Igor V Grigoriev - LCG sequencing coordination
8. Andy Miller - metadata for shared strains, proofreading, taxonomy & nomenclature
9. Kerry O'Donnell - idea, strains, research support, MGP sequencing, & proofreading
10. Jason Stajich - idea, software development, Low Coverage genome sequence assembly, annotation, analysis & writing, data deposition.
11. Gregory Bonito - idea, research support, & writing

Introduction

Early diverging fungi belonging to Mortierellomycotina are diverse in ecology and species richness and are classified within a single order (Mortierellales) and as belonging to a single family (Mortierellaceae). Phylogenetically, this lineage is closely related to arbuscular mycorrhizal fungi (Glomeromycotina) and Mucoromycotina, and are among the earliest diverging lineages of fungi to have independently evolved differentiated macroscopic fruiting body structures, in the form of ~1cm sporocarps (Smith et al. 2013; Spatafora et al. 2016; Chang et al. 2019). Several species of *Mortierella* are prolific producers of polyunsaturated fatty acids and have relevance to nutraceutical industries and bioenergy research (Goyzueta et al. 2019). Mortierellaceae are

commonly detected and isolated from soils, plant debris, insect guts, mosses and living plant roots (Dixon-Stewart, 1932; Gams, 1977; Domsch et al. 1980), and have been found on every continent, including Antarctica (Gams, 1977; GBIF.org, 2019). Yet, the inability to resolve phylogenetic relationships within Mortierellaceae has limited inferences pertaining to species distributions, diversity, and functional ecology of Mortierellaceae (Gams, 1977; Petkovits et al. 2011; Wagner et al. 2013).

Early efforts to classify Mortierellaceae were based on macro- and micromorphology, including colony growth patterns, hyphal branching, and spore production (Gams, 1977). Most species within Mortierellaceae have distinct macromorphological growth patterns on agar media, with colonies forming rounded to slightly pointed rosette “petals”, although some species grow in simple rings and others are completely devoid of visible growth rings (**Fig. 2.1**). Mortierellaceae fungi can produce three types of spores: asexual sporangiospores born in sporangia, asexual chlamydospores that can be terminal or intercalary, and sexual zygospores (**Fig. 2.2**). One or more spore types may be absent in some species, such as *M. chlamydospora* which lacks sporangiospores and *M. parvispora* which lacks chlamydospores (Gams, 1977). In some species, chlamydospores may be decorated with spines or other ornamentations and are referred to as stylospores (Chien, Kuhlman, and Gams 1974). Both heterothallism and homothallism have been observed in Mortierellaceae and sexuality varies by species, although mating is not commonly observed. Macromorphology, micromorphology, and the production of all three spore types may vary considerably between growth media and conditions, which can complicate morphological species identifications. (**Fig. 2.3**).

Species and their groupings were repeatedly redefined in the mid 1900s, which was concluded by Gams in 1977, who divided the lineage into two subgenera: *Micromucor* and *Mortierella*. Within *Mortierella* subgenus *Mortierella*, Gams recognized 9 sections: *Alpina*, *Actinomortierella*, *Haplosporangium*, *Hygrophila*, *Schmuckeri*, *Simplex*, *Spinosa*, *Stylospora*, and *Mortierella*, the last of which contained the type genus and species for the Mortierellaceae,

Mortierella polycephala (Gams, 1977). *Micromucor* was later reclassified to belong within Mucoromycota in the genus *Umbelopsis* (Meyer & Gams, 2003). Additional genera, *Gamsiella*, *Dissophora*, *Modicella*, and *Lobosporangium* were subsequently described and accepted as Mortierellaceae, but remained polyphyletic with respect to *Mortierella* (Thaxter, 1914; Benjamin, 1978; Benny & Blackwell, 2004; Petkovits et al. 2011; Smith et al. 2013). The genus *Haplosporangium* was retired by Gams (1977) and all included species, including the type species *H. bisporale*, have been transferred to *Mortierella*, in various clades. The first modern sequencing-based revision of the Mortierellaceae established that the morphological classification system was largely unsupported and defined 12 new clades (Petkovits et al. 2011). Only sect. *Schmuckeri*, sect. *Actinomortierella*, and sect. *Mortierella* were retained in the new clades. A second rDNA sequencing effort expanded the diversity of sequenced species and reorganized and combined several of the Petkovits et al (2011) clades into a total of seven clades: selenospora, verticillata-humilis, lignicola, dissophora, capitata, alpina, and gamsii (Wagner et al. 2013).

Currently, there are close to 125 accepted Mortierellaceae species, and new species continue to be formally described each year (**Table 2.1**). Estimates based on environmental sequencing predict there to be more than 170 Mortierellaceae species worldwide, indicating at least one quarter of the species in this family remain to be described (Gams, 1977; Benny, 2009; Nagy et al. 2011; Smith et al. 2013; Wagner et al. 2013; Degawa, 2014; Takashima et al. 2018). The current Mortierellaceae classification divides species into six genera: *Aquamortierella, Dissophora*, *Gamsiella*, *Lobosporangium*, *Mortierella*, and *Modicella* (Benny, 2009). However, most of these genera are monotypic or ditypic and nearly all of the species in the family are classified as belonging to the polyphyletic genus *Mortierella* (Smith et al. 2013; Wagner et al. 2013).

Broad sampling of taxa, combined with robust phylogenetic analysis, and detailed morphological examinations, will underlie a phylogenetically-informed revision of Mortierellaceae classification and taxonomy. However, ribosomal data are unable to resolve phylogenetic

relationships within Mortierellaceae (Wagner et al. 2013). That is because the Internal Transcribed Spacer (ITS) rDNA is too divergent to align across the family for phylogenetic reconstruction, though it can be useful as a DNA barcode for classifying isolates to ITS-based clades and is sometimes sufficient for species determinations. Conversely, large subunit (LSU) and small subunit (SSU) rDNA regions are too highly conserved to sufficiently resolve higher order phylogenetic relationships (Wagner et al. 2013). Thus, additional non-ribosomal markers are needed in order to identify monophyletic genera and increase genus-level taxonomic resolution.

In this study we applied two parallel approaches to resolve the Mortierellaceae phylogeny. In the first approach, we generated low-coverage genomes (LCG) from a phylogenetically diverse set of ingroup taxa to generate a robust and fully resolved phylogeny, which is a relatively new approach for phylogenomics (Olofsson et al. 2019; Zhang et al. 2019). We tested whether the LCG approach was suitable for fungi as a high-throughput method of genome sequencing to efficiently recover sequence data for phylogenomics. In the second approach, we developed and tested an automated pipeline to identify multiple lineage-specific markers for phylogenetic applications from a small number of representative genomes, and used multiplexed targeted amplicon sequencing to generate multi-gene sequence data across several hundred taxa. This allowed for an improved taxon sampling in terms of breadth and depth of as compared to the LCG approach. We then combined the concordant LCG and multi-gene phylogenetic (MGP) datasets to generate a resolved phylogeny and taxonomy for Mortierellaceae.

Materials & Methods

Sampling, Isolation, & Culture Conditions

Diverse isolates were obtained from established culture collections including the Agricultural Research Service Culture Collection (NRRL) and Westerdijk Fungal Biodiversity Institute (CBS - Centraalbureau voor Schimmelcultures) and from collaborators to broaden geographic and

biodiversity sampling. Fresh isolates for this study were also obtained from roots, soils and plant substrates collected across Australia, Fiji, New Zealand, Uganda, and the United States (**Table 2.2**). Isolates were obtained using three methods: soil baiting, shrimp baiting, and soil dilution plating or swabbing (Finkelstein, 2013; Nampally et al. 2015). Soil baiting involved placing substrates in squares of water agar (10 g/L BactoAgar (Difco)) supplemented with antibiotics (*i.e.* streptomycin, chloramphenicol) on the lid of an inverted water agar dish. Aerial hyphae able to colonize the upper plate were then transferred to new 1% malt extract agar (MEA: 10 g/L Malt extract, 1 g/L Yeast extract, and 10 g/L BactoAgar (Difco)) plates. Shrimp baiting refers to incubating soils with shrimp exoskeletons, which have been washed and sterilized (Nampally et al. 2015). This substrate is enriched in chitin and selects for chitinolytic fungi, which includes many *Mortierella* species (Nampally et al. 2015). After 1-week, colonized exoskeletons were surface sterilized with 3% hydrogen peroxide for 1 minute, quenched with sterile water, and plated on squares of MEA or 1.2% potato dextrose agar (PDA: 12 g/L Potato dextrose broth and 10 g/L BactoAgar (Difco) on the lid of an inverted petri dish. Isolates whose macromorphology were consistent with *Mortierella* spp. were collected from the edge of growing colonies and transferred to new PDA or MEA plates until the cultures appeared to be pure. Soil dilution or swabbing involved either serial dilutions (1:100 and 1:1000) of soil in DI water plated on Saborauds Dextrose Agar (SDA (Thermo Scientific), or swabs streaked onto SDA. Individual isolates were picked at 1 day and 7 days and transferred to SDA.

In total, 318 isolates were studied with the aim of resolving the Mortierellaceae phylogeny (**Table 2.2**). We included 59 strains from the ARS Culture Collection (NRRL) and 4 from the CBS strain repository selected to increase geographic diversity in our dataset. Sixteen of these were type strains. These included 21 isolates from across the United States, 12 from Europe, 3 from India, 2 from Mexico, 2 from Antarctica, and 1 each from Australia, Canada, Colombia, New Zealand, and Russia. We were unable to obtain metadata for 15 NRRL strains. In total, the NRRL and CBS isolates comprised 36 previously identified species and 5 strains unidentified to species.

Preliminary Isolate Identification

To generate preliminary isolate identifications, DNA was extracted from mycelium using an alkaline extraction buffer (see Appendix D for details). We PCR-amplified the ITS region and the 5' portion of the LSU using the universal fungal primers ITS1-F and LR3 (Vigalys & Hester, 1990; Gardes & Bruns, 1993). PCR products were separated by gel electrophoresis on a 1% agarose TAE gel containing ethidium bromide and visualized under ultraviolet illumination. The sizes of DNA fragments were estimated using a 100-bp ladder (ThermoFisher). Products with bands of the expected size were purified and template DNA was used in 10μL sequencing reactions with BigDye® Terminator v3.1 (Applied Biosystems), using the primers ITS1-F and LR3 (Vigalys & Hester, 1990; Gardes & Bruns, 1993). Sequences were generated on an Applied Biosystems 3730XL high throughput capillary sequencer at the Michigan State University Research Technology Support Facility Genomics Core. Sequences were *de novo* assembled with Geneious 8.1.3 and analyzed using the NCBI BLASTn tool (Johnson et al. 2008). Preliminary identifications were assigned based on sequence similarities and E-values. In the case of multiple equally high-quality BLAST hits to multiple Mortierellaceae species, the isolate was designated at the genus level (e.g. '*Mortierella* sp.'), with an indication of the clade to which it likely belonged as defined by Wagner et al. (2013).

Genomic DNA Extraction

To prepare high-quality genomic DNA, isolates were grown in liquid 1% malt extract broth culture for 1-2 weeks. Mycelium was harvested by vacuum filtration and genomic DNA was extracted following a CTAB-based chloroform extraction protocol (Doyle, 1991). DNA quality and concentration were estimated by gel electrophoresis and NanoDrop.

Low Coverage Genome (LCG) Library Preparation & Sequencing

Plate-based DNA library preparation for Illumina sequencing was performed on the PerkinElmer Sciclone NGS robotic liquid handling system using Kapa Biosystems library preparation kit. 200ng of sample DNA was sheared to 600bp using a Covaris LE220 focused-

ultrasonicator. The sheared DNA fragments were size-selected by double-SPRI and the selected fragments were end-repaired, A-tailed, and ligated with Illumina compatible sequencing adaptors from IDT containing a unique molecular index barcode for each sample library. The prepared libraries were quantified using KAPA Biosystem's next-generation sequencing library qPCR kit and run on a Roche LightCycler 480 real-time PCR instrument. The quantified libraries were then multiplexed with other libraries, and the pool of libraries was then prepared for sequencing on the Illumina HiSeq sequencing platform utilizing a TruSeq paired-end cluster kit, v4, and Illumina's cBot instrument to generate a clustered flow cell for sequencing. Sequencing of the flow cell was performed on the Illumina HiSeq 2500 sequencer using HiSeq TruSeq SBS sequencing kits, v4, following a 2x100 indexed run recipe.

Low-Coverage Genome (LCG) Sequence Analysis

Automated genome assembly was performed with the tool Automatic Assembly For The Fungi (AAFTF) which performs read trimming and filtering against PhiX and other contaminants using BBMap v38.16 followed by genome assembly with SPAdes v3.13.1 (Bankevich et al. 2012; Bushnell, 2014; Stajich & Palmer, 2018; Stajich & Palmer, 2019). Assemblies were further cleaned of vector sequences, screened for contaminant bacteria with sourmash using database Genbank Microbes 2018.03.29 (Pierce et al. 2019). Duplicated small contigs were removed using minimap2 v2.17 alignment of contigs smaller than the assembly N50 (Li, 2018). Contigs were further polished for the total Illumina read set with Pilon v1.10 and sorted by length and renamed (Walker et al. 2014). Each set of paired-end sequence reads for an isolate was automatically processed with AAFTF to produce a polished, vector screened, de-duplicated, polished, and sorted genome assembly.

Genomes were annotated with funannotate v1.7.0, which used a combination of evidence from *ab initio* gene prediction and protein alignments to produce a predicted gene set (Palmer & Stajich, 2017; Love et al. 2019). For each genome, the funannotate prediction step was run and allowed to train the augustus v3.3.2 gene predictor with BUSCO aligned core genes from the

fungi_odb9 data set (Stanke et al. 2006; Palmer & Stajich, 2017). Genemark.hmm-ES was run in self-training mode to produce additional predictions (Ter-Hovhannisyan et al. 2008). These *ab initio* predictions were combined with exon locations inferred by aligning proteins from the SwissProt database to the genome first with BLASTX v2.2.31+, followed by exonerate v2.4.0 to produce splice site-aware alignments. These data combined into consensus gene models for each genome using EVidenceModeler (NCBI & Camacho, 2008; Slater & Birney, 2005; Haas et al. 2008). All these analysis steps are run as part of the funannotate 'predict' procedure. The predicted gene models in each genome were further annotated with putative functional information using eggNOG v1.03, CAZY, MEROPs, and Pfam databases searched with HMMER and DIAMOND (Eddy, 1998; Buchfink, Xie, & Huson, 2015). These annotated draft genomes were deposited in NCBI GenBank along with the primary Illumina sequences in NCBI SRA (**Table 2.3**).

PHYling methods for genome analysis

To examine the phylogenetic relationships of the strains using these sequenced, assembled, and annotated genomes, the PHYling pipeline was applied to a set of conserved, typically single-copy markers that were previously developed (Spatafora et al. 2016; Beaudet et al. 2017; Stajich 2020). The predicted protein set from each strain was searched for each of the 434 single-copy markers in the "JGI_1086" set, which was developed from Joint Genome Institute's orthologous clusters from genomes in 2015. The best hits from each strain for each marker were aligned to the original HMM using hmmalign and the resulting alignments were trimmed with trimAL (Eddy, 1998; Capella-Gutiérrez, Silla-Martínez, & Gabaldón, 2009). Codon alignments were generated by back translating the protein alignments using the input coding sequences for each gene with the script bp_mrtrans.pl from BioPerl (Stajich et al. 2002) incorporated into PHYling. These individual alignments were concatenated together by PHYling using the script combine_multiseq_aln.py, recording the start/end of the alignment into a partition file. The concatenated protein and codon alignments were each used for phylogenetic analyses, initially in

FastTree and later in RAxML (Price, Dehal, & Arkin, 2010; JTT substitution model, Stamatakis, 2014). In addition, individual gene alignments were subjected to phylogenetic analyses to estimate gene trees. All the gene trees were combined and processed with ASTRAL v5.14.3 to infer a coalescent species tree from the individual gene trees (Mirarab et al. 2014).

Multi-Gene Phylogenetic (MGP) Primer Design & Validation

We used a series of custom Python scripts to extract all exon sequences from the annotated *de novo* genome of *Mortierella elongata* AG77 (Uehling et al. 2017; https://github.com/natalie-vandepol/mortierellaceae_mlst). We then conducted a BLAST search for these exon sequences in the *de novo M. alpina* B6842 and *M. verticillata* NRRL6337 genomes (Wang et al. 2011; Spatafora et al. 2016). We filtered the results for high-identity, single-copy hits and used MUSCLE to align the sequences (Edgar, 2014). From these, loci were selected based upon sequence identity and primers were manually designed using MEGA6 and OligoCalc (Kibbe, 2007; Tamura et al. 2013). Primer sequences were tested *in silico* with iPCRess against several *Mortierella*, *Umbelopsis,* and *Mucor* genomes to estimate the likelihood of off-target amplification (Slater, 2007; **Table 2.4**).

Primer sets were selected for testing based on melting temperature compatibility. Primer sets were tested using genomic DNA from a panel of *Mortierella* isolates and DreamTaq Master Mix (MM) (ThermoFisher Scientific). PCR products were visualized through gel electrophoresis, as previously described, using a 1.2% agarose gel. Product sizes were estimated using a 100-bp ladder (ThermoFisher). Amplicons were Sanger sequenced and aligned in Mesquite 3.6. Thirteen loci showed primer specificity, robust amplification, and good alignment of sequences across the panel of Mortierellaceae diversity (**Table 2.4**). These loci, together with the fungal ribosomal primers ITS1-F and LR5, were tested for multiplex compatibility (Hopple & Vilgalys, 1994). Four sets of primer pairs were identified for multiplexed amplification of 3-4 loci in a single reaction. These sets were composed as follows: 1) RPB1, EF1a, 615, and 1870; 2) 370, 4955, and 10927; 3) 5401, 4121, and ITS1-F/LR5; and 4) 5512, 2175, 5491, and 2451.

MGP Multiplex Amplification, Library Preparation, & Sequencing

Multiplex PCR was performed using Platinum Multiplex PCR Master Mix (Applied Biosystems). 2.5µl aliquots of the products were mixed with loading dye and subjected to electrophoresis on a 1.2% agarose gel and DNA was visualized by ethidium bromide staining. PCR products and their sizes were estimated based on a 100bp ladder (ThermoFisher). Primer sets that failed to amplify in the MultiPlex reactions were amplified individually with DreamTaq MM and screened using gel electrophoresis. Amplicons were pooled and sent to NCAUR (USDA-ARS, Peoria, IL) for library preparation using the Nextera DNA Library Preparation and Nextera Index Kits (Illumina) and sequenced on a MiSeq platform instrument.

MGP Sequence Analysis

Demultiplexed paired-end sequences were filtered for PhiX using the filter_phix tool in the USEARCH pipeline and assembled using SPAdes v3.7.1 (Edgar, 2010; Bankevich et al. 2012). The resulting contigs were identified to locus through a BLAST search against the genome sequences used for primer design and representative fungal and bacterial ribosomal sequences. A custom python script parsed the BLAST results to group contigs by locus. To minimize missing data, we defined “full-length” sequences as being at least 80% of the expected length for the locus. The count of full and partial length sequences for each sample and locus are summarized in **Table 2.5**. Loci were selected for further analysis based on the frequency of duplicate full-length sequences by comparing the total number of sequences for that locus to the number of samples (hereafter isolates represented by DNA sequence data) with at least one sequence. Loci with a sequence:sample ratio higher than 1.2:1 were excluded from the dataset, since this degree of over-representation would require extensive manual analysis to resolve, if possible, and could represent genuine paralogs or gene duplication events rather than barcode migration or cross-contamination between samples (**Table 2.5**). Resolution of duplicate sequences in the case of a gene duplication or paralogs would likely result in different copies being retained for a given sample and the locus still being unusable.

The filtered contigs were aligned using MUSCLE and trimmed to conserved regions in Mesquite 3.6 (Edgar, 2004; Maddison and Maddison, 2007). Additional loci were excluded if they had distinct sequence variants that could not be aligned. For the remaining 6 loci that were not excluded for the reasons described above, informative regions were identified using Gblocks 0.19b and analyzed with PartitionFinder 2 to determine the appropriate nucleotide substitution model (Castresana, 2000; Lanfear, Frandsen, Wright, Senfeld, & Calcott, 2016). Phylogenetic trees for each marker were generated with RAxML using the CIPRES gateway (RAxML-HPC2 on XSEDE (8.2.12)) (Ronquist & Huelsenbeck, 2003; Miller, Pfeiffer, & Schwartz, 2010; Stamatakis, 2014).

Taxonomic identification of isolates was verified through BLAST searches of ITS sequences and considered valid if the best hits were to the expected or closely related species. When the best hits were consistently to species in a different ITS-based clade, the isolate was indicated as "*Mortierella* sp.". Isolates were removed from the dataset if sequence data was only present for one locus or if they consisted of multiple copies for the majority of the loci. The remaining isolates were screened for multiple copies of any locus. In cases of multiple copies, if one sequence was clearly consistent in phylogenetic placement compared to other loci, that sequence copy was assumed to be the orthologue and was kept, and any paralogues were deleted. Otherwise, all sequences for that locus were removed for that sample.

To improve the data matrix by increasing the number and diversity of isolates having four or more of the six loci, missing loci of several isolates were individually amplified with DreamTaq, screened via agarose gel electrophoresis, and Sanger sequenced. In addition, when genome sequences were available for a sample with missing loci, sequences from close relatives were used in a BLAST search against the raw genome sequencing reads to search for the target loci. This process was also used to construct MGP loci for LCG samples that had not been included in the MGP dataset. Matching reads were assembled and aligned to the query sequence in Geneious.

Three outgroup taxa were selected from published genomes available on JGI Mycocosm Portal: *Mucor circinelloides* CBS277, *Umbelopsis ramanniana AG#*, and *Lichtheimia corymbifera* FSU9682. The MGP loci were identified in these genomes using BLAST analyses in the MycoCosm portal and the sequences downloaded for inclusion in the alignments.

After finalizing the dataset and alignments, Gblocks 0.91b and PartitionFinder 2 were used to exclude characters (type = DNA, allowed gaps = with half) and identify the optimal model of evolution for phylogenetics for each locus (GTR+G+I) (Castresana, 2000; Lanfear et al. 2016). SequenceMatrix 1.8 was used to generate a concatenated nucleotide matrix of the 6 loci (Vaidya, Lohman, & Meier, 2011). Four different phylogenetic tree building approaches were carried out. These included both constrained and unconstrained RAxML analyses of the concatenated matrix, a partially constrained MrBayes analysis of the concatenated matrix, and RAxML analyses of constrained and unconstrained single-gene alignments. A custom R script was used to prune the LCG tree to remove isolates not included in our concatenated and single gene datasets. The pruned genome tree provided a guide tree to constrain RAxML phylogenetic analyses. Key nodes were used to define partial constraints for MrBayes phylogenetic analyses. We did explore using ASTRAL to generate a consensus phylogeny as a counterpoint to our concatenated matrix analyses (Mirarab et al. 2014). However, we elected to not use this approach for our six gene dataset since this program was intended for datasets of several hundred genes.

Results

Geographic and Biodiversity Sampling

We improved geographic sampling of Mortierellaceae with our own isolates obtained from soils collected in Italy (1), Australia (32), Fiji (1), New Zealand (8), Uganda (13), and across the United States (53). These new isolates account for 110 cultures representing 14 previously described species and 44 strains that could not be resolved to species, 26 of which represent 4 putatively novel species. In most cases, isolates that could not be identified to species by ITS

sequence could still be assigned to one of the ITS-based clades established by Wagner et al. (2013).

An additional 145 isolates were contributed by collaborators. Of those, 141 were from the United States, 1 from Argentina, and 3 where metadata was missing. The 145 contributed isolates represented 25 previously described species and 46 isolates that could not be identified to species.

The LCG dataset included 73 strains representing 28 described species and 21 isolates unidentified to species by ITS sequence analysis. Representatives were included from all seven ITS-based clades defined by Wagner et al. (2013) and 11 of the 12 clades defined by Petkovits et al. (2011), the exception being the strangulata clade.

For the MGP approach, we PCR-amplified and sequenced the initial 13 loci across 332 isolates. Thirty-two isolates were excluded on the basis of quality filtering as described in detail later. These 32 excluded isolates are not reported in the sampling and metadata to avoid confusion about included versus excluded diversity, but are listed in **Table 2.6**. We added 14 LCG isolates by mining the genomes for the MGP loci. Therefore, the final MGP dataset contained 314 isolates that represent 48 distinct Mortierellaceae taxa and three outgroup species (**Table 2.2**). In this dataset, the gamsii, verticillata-humilis, and alpina clades were over-represented. Indeed, these are often the most commonly isolated Mortierellaceae. In addition, we specifically oversampled *M. elongata* and *M. alpina* isolates from disparate geographic regions in an attempt to better resolve these species complexes. In total, we included 117 isolates representing 11 of the 22 described species and one putative novel species from the gamsii clade, 69 isolates representing 7 of the 8 described species from the verticillata-humilis clade, and 70 isolates representing 7 of the 10 described species from the alpina clade. By comparison, the lignicola, dissophora, capitata, and selenospora clades were relatively undersampled. In the lignicola clade, we included 13 isolates representing 4 of the 8 described species, and 7 that were not previously identified to species. From the dissophora clade, we included 22 isolates representing 5 of the 7

described species and three putative novel species. For the capitata clade, 9 isolates represented 4 of the 9 described species and one unidentified species. For the selenospora clade, our dataset included 16 isolates, which represent 6 of the 12 described species. This study is the first modern phylogenetic analysis to include *Modicella*, represented by *M. reniformis* MES-2146. For the 81 described Mortierellaceae species that were not included in this study, we have summarized the current classification and, when possible, estimated their likely placement in our proposed classification based on high ITS sequence similarity to the species included in this study (**Table 2.1**).

The Low Coverage Genome Approach

Molecular Results

For two samples, the sequencing coverage was too low to assemble and be included in the study. One sample was found to be contaminated. On average, Illumina sequencing returned 588 (316-1217) GB of sequence data per isolate, which were assembled into ~3,700 (1,100-12,500) contigs (**Table 2.3**). The average depth of genome coverage was 14.8X (8.5X-38.5X). Genome annotation identified an average of ~12,000 predicted proteins (7,338-16,572) in each genome. Our search for the 434 phylogenetic markers in the annotated protein set for each isolate identified between 354-419 markers in each genome (**Table 2.3**). Altogether there were a total of 109,439 characters in the concatenated LCG nucleotide matrix, which has been uploaded to TreeBase (submission 25806).

LCG Dataset & Phylogeny

There are 9 main branches in the LCG tree (**Fig. 2.4**). The wolfii-capitata clade is sister to the rest of the lineages and includes the taxa *Mortierella ambigua*, *M.* aff. *ambigua*, and *M. wolfii*. The next diverging lineage is *M. selenospora*. The third branch contains all representatives of the verticillata-humilis clade (17 isolates, 7 species). The single *M. cystojenkinii* isolate appears as a monotypic lineage. The fifth branch contains all included representatives of the gamsii clade (19 isolates, 7 described species) and a putative novel species that does not cluster with any known

taxon in either ITS or phylogenomic analyses, represented by *Mortierella* sp. GBAus35, NVP41, and AD031. The sixth branch includes all representatives of the alpina clade (11 isolates, 2 species), and is divided into two subgroups containing predominantly *M. polycephala* and *M. alpina*, respectively, the former of which is the type species for *Mortierella*. The seventh branch is represented by the lignicola clade and the eight branch is the monotypic *Lobosporangium transversale*. The ninth and final branch contains *Gamsiella multidivaricata*, *Modicella reniformis*, *Dissophora ornata*, *Mortierella globulifera*, and several putative novel species that are sister to *Modicella*.

The Multi-Gene Phylogenetics Approach

Primer Design

From the three reference genomes, 1269 exons met initial quality criteria related to length, copy number, and sequence similarity. Of those, 130 were classified as suitable candidates for primer design due to their internal moderate variability flanked at each end by highly conserved areas. Further analysis yielded 74 primer sets meeting target amplicon length, GC content, primer length, ambiguity, and self-compatibility criteria (**Table 2.4**). Of those, 55 passed *in silico* PCR. As a positive control for this locus selection process, we checked our primer sets for exons from RNA polymerase subunit B (*RPB1*) and elongation factor 1-alpha (*EF1a*), which have been used successfully as fungal phylogenetic markers. We found both *RPB1* and *EF1a* among the 55 primer sets that had passed *in silico* PCR. We selected 22 loci, including *RPB1* and *EF1a*, for *in vitro* testing based on similar annealing temperatures (**Table 2.4**). Thirteen were selected for continued use based on consistent amplification across a panel of Mortierellaceae isolates and alignment of the amplicon sequences.

Molecular Results

Illumina sequencing generated a total of 711.5 GB of sequence data (2.8 million reads) that were demultiplexed into our initial 333 samples. Sequences were assembled into 7905 contigs across 329 isolates for a total of 7.6 GB unfiltered, assembled contigs having an average

coverage of about 100x. Three isolates and the PCR negative control had little or no sequence data, could not be assembled, and were excluded from further analyses.

Filtering Loci, Sequences, and Isolates

Further quality control steps were taken to assess the consistency and validity of each locus as a phylogenetic marker. Loci 4121 (hypothetical protein, predicted amino acid transporter), 2175 (CTP synthase), and 615 (chitin synthase) were found to exceed the 120% sequence over-representation cutoff described in the methods and were therefore excluded (**Tables 2.4** & **2.5**). Loci 10927 (hypothetical protein, class V myosin motor head), 5401 (adenosylmethionine-8-amino-7-oxononanoate transaminase, biotin/cofactor biosynthesis), and 5491 (delta-12 fatty acid desaturase) each had two very distinct sequence variants that could not be aligned, so these loci were excluded from further analysis. Locus 370 (acyl-CoA oxidase) was found to be a member of a gene family, with no one homologue consistently amplified across sampled taxa. Therefore, this locus was excluded from further analysis.

Some isolates had more than one full-length sequence for a given locus, referred to here as duplicates (**Tables 2.5** & **2.7**). In most cases, we attribute this to cross-contamination of isolates during post-PCR sample handling. Duplicate sequences were resolved by identifying where the sample was placed phylogenetically by other loci, and then determining which of the duplicate sequences were congruent. We resolved 2 of 5 duplicates in locus 1870 (xanthine dehydrogenase), 1 of 1 in locus 2451 (calcium-translocating P-type ATPase), 4 of 7 in locus 4955 (hypothetical protein, DNA replication licensing factor), 2 of 5 in locus *EF1a* (elongation factor 1-alpha), and 11 of 13 in locus *RPB1* (RNA polymerase II subunit RPB1). Locus 5512 (glycosyltransferase Family 21 protein) had no duplicates.

Thirty-two isolates were completely excluded from further analysis for various reasons detailed here and summarised in **Table 2.6**. Four isolates were completely excluded from the dataset because all but one locus had duplicate sequences, therefore no trends could be used to resolve duplicated sequences. Eleven isolates were removed for having zero full length

sequences or only ITS sequences. Five isolates were removed because only one locus amplified and there was no corroboration from other loci to verify the placement of the sample. Two isolates were excluded because they were contaminated by *Umbelopsis* and the loci were strongly incongruent. Five additional isolates were removed because placement according to the non-ribosomal loci radically disagreed with the species identification by ITS sequence. Four isolates were found to be duplicated in our dataset, due to having been assigned new isolate ID numbers after having been shared between labs. One isolate was excluded because the only loci for which it had sequences were deleted loci. We mined reference genomes and the unassembled LCG dataset for MGP loci and were able to fill in several "holes" in the dataset from loci that had failed to amplify/sequence. We also added 15 new isolates that were not included in the initial MGP dataset by successfully recovering at least two full-length MGP loci from genome sequences.

Primer Performance

Many isolates did not have all 6 loci represented in the final dataset. This was particularly true for isolates that were not closely related to the species from which the primers were designed. We attribute this result to sequence mismatch preventing the primer from binding, hereafter referred to as "primer mismatch", rather than absence of the target genes. Sequence reads for all of the loci were detected in genome sequences of isolates across all clades of the Mortierellaceae, although incomplete sequencing often meant that the assembled reads did not meet the minimum sequence length and therefore could not be included in our analyses (**Table 2.8**). The effect of primer mismatch varied by loci (e.g., 1870, 5512 had lower recovery; *EF1a*, *RPB1* had higher recovery), as seen by inconsistent percentages of isolates amplified across the clades. In contrast, locus 2451 amplified poorly across all clades.

Unconstrained MGP Phylogeny

The MGP dataset included 314 isolates representing 48 unique Mortierellaceae taxa and three outgroup species. These included 69 of the 73 Mortierellaceae LCG isolates, the exceptions being *Mortierella wolfii* NRRL 66265, *Mortierella* sp. GBAus43, *M. chlamydospora* NRRL 2769,

and *Mortierella* sp. NRRL A-10996. The final 6 gene concatenated matrix contained a total of 8181 characters, which has been uploaded to TreeBase (submission 25806). No strong incongruence was observed between the unconstrained MGP phylogeny and the LCG tree, but some weak incongruences were evident (**Fig. 2.5**). For instance, in the MGP phylogeny, the wolfii-capitata clade was split into several basal branches. The verticillata-humilis, gamsii, and alpina clades were still internally cohesive and consistently placed along the backbone. However, the alpina clade was split between two separate branches. *Mortierella dichotoma* was not represented in the LCG dataset. Our analyses place it apart from the other "clade 1" species, as defined by Wagner et al. (2013), where it comprised a monotypic branch between the verticillata-humilis and cystojenkinii clades. The MGP phylogeny also placed the selenospora clade within the second alpina branch and *M. parvispora* and *M. beljakovae* were grouped with *M. dichotoma*, rather than the rest of the lignicola clade. The lignicola clade, *Gamsiella*, *Dissophora*, *Modicella*, *Lobosporangium*, and the novel group detected in this study were still clustered together. All together, the internal group structure was generally consistent with the LCG analysis, although *Lobosporangium* was placed within the lignicola clade, possibly due to long branch attraction with *Mortierella* sp. NRRL3175. There were several weakly supported nodes (40-71%) along the backbone of the unconstrained MGP phylogeny, indicating that (similar to rDNA) the MGP loci were generally able to resolve relationships within, but not between, the major groups.

LCG-Constrained MGP Phylogeny

Since the unconstrained MGP phylogeny was unable to resolve the backbone placement of the major clade of Mortierellaceae, we synthesized our datasets to leverage the high confidence of the LCG phylogeny with the sampling depth of the MGP dataset. To obtain a constraint tree, we "pruned" the LCG tree to remove the 4 isolates not represented in the MGP dataset. Since MrBayes does not accept a constraint tree as a direct input, we defined major nodes of the LCG tree as partial constraints. However, the resulting tree topology was clearly inconsistent with the genome tree, though somewhat consistent with the unconstrained RAxML tree (**Fig. 2.6**). We

then generated an LCG-constrained RAxML phylogeny from the concatenated MGP matrix. This phylogeny had strong support (74-100% at major nodes), was congruent with the LCG phylogeny, and will be used henceforth as our best estimate of the Mortierellaceae phylogeny (**Fig. 2.7**).

Resolving the Mortierellaceae phylogeny into monophyletic lineages resulted in a total of 14 clades. (**Fig. 2.7**). To help stabilize Mortierellaceae taxonomy we have resurrected *Actinomortierella* and erected seven novel monophyletic genera to accommodate supported clades: *Benniella, Entomortierella*, *Gryganskiella*, *Linnemannia*, *Lunasporangiospora*, *Necromortierella*, and *Podila*.

We erected the genus *Linnemannia* to include the monophyletic gamsii clade, which contains the *L. elongata* complex, *L. gamsii*, *L. amoeboidea* and related species. *L. elongata* isolates do not appear to cluster by geographic origin, indicating that *L. elongata* may be cosmopolitan globally. Different genotypes of isolates within the *L. elongata* complex could not be distinguished using the ITS sequence data, and even with a global sampling provide poor resolution of the species within this complex. *L. gamsii* isolates were split between two branches: one monophyletic and the other shared with *L. zychae*, *L. exigua*, and *L. acrotona*. Lastly, *L. amoeboidea* originally clustered with *M. alpina* in ITS-based studies but consistently was resolved in *Linnemannia* in our analyses (Wagner et al, 2013; Petkovits et al. 2011).

Since *M. polycephala* is the type species for *Mortierella*, the genus *Mortierella* is conserved for the alpina clade, which was split into two main groups. The first included *M. polycephala*, *M. bisporalis*, *M. reticulata*, and *M. indohii*. The other branch was composed of the *M. alpina* complex and *M. antarctica*.

Podila includes species within the verticillata-humilis clade, which contains *P. verticillata*, *P. minutissima*, and related species. *Podila* species have historically been difficult to resolve or identify by ITS analysis, as the ITS sequences of species within this group usually share 98-99% identity. Although *P. horticola* and *P. minutissima* ITS sequences share 99% identity, they were distinguished in our analyses.

The deeply diverged clade in the Mortierellaceae phylogeny that includes *Lobosporangium*, *Dissophora*, *Gamsiella*, *Modicella*, and the newly erected genera *Entomortierella* and *Benniella* also has the highest diversity of ecologies and morphologies in the family. *Entomortierella* includes the lignicola clade and *E. parvispora*. *Lobosporangium* was placed between *Entomortierella* and the deepest branch with *Gamsiella, Dissophora, Modicella*, and *Benniella*.

The MGP Loci as Phylogenetic Markers

In this study, we found *EF1a* to be the least informative of the tested loci, as even the constrained gene tree had extremely low support (1-50% at most major nodes) and many misplaced taxa (e.g., *Mortierella alpina* KOD1046 grouped with *Podila* and *M. alpina* NRRL 6302 grouped with *Entomortierella*, both separate from the dominant *M. alpina* cluster). However, *EF1a* and *RPB1* had the highest recovery rate across all isolates, including for outgroup taxa. *RPB1* was the best performing individual locus of the MGP dataset, both in terms of its consistent amplification and ability to distinguish species. For example, the ITS1f-LR5 sequences of *Linnemannia elongata* NVP64 and *L. gamsii* NVP61 shared 98% similarity and 97% similarity with *L. hyalina*. However, *RPB1* sequences of these species showed 85-96% interspecific sequence similarity, with intraspecific sequence similarity usually 98-99% (**Tables 2.9** & **2.10**). The other loci provided additional resolution of species within other genera. For example, locus 2451 provided the best resolution of species in *Podila*, with generally 96-99% sequence similarity within species and 89-94% similarity between species. *P. epicladia* and *P. minutissima* shared the highest sequence similarity (96-98% similarity within, 95-96% similarity between). For more reliable separation of these two species, locus 5512 could be used, where *P. epicladia* and *P. minutissima* were each 98-99% similar within species and 96% similar between species.

Taxonomy

The proposed classification for Mortierellaceae follows general principles promoted in the Hibbett et al. (2007) phylogenetic classification of Kingdom Fungi and followed by Spatafora et

al. (2016) in their reclassification of zygomyceteous fungi. Below are the accepted and proposed genera, each in alphabetical order, a brief discussion of each genus, and the species included in this study. Following each genus description are any comb. nov. descriptions for each species transferred to that genus. Species characteristics, synonyms, basionyms, and MycoBank numbers are also summarized in **Table 2.11**.

Accepted Genera

Actinomortierella Chalab. 1968

≡ *Mortierella* Coem., Bulletin de l'Académie Royale des Sciences de Belgique Classe des Sciences 15: 536 (1863)

≡ *Carnoya* Dewèvre, Grevillea 22 (101): 4 (1893) [MB#20101]

≡ *Naumoviella* Novot., Chung-kuo Ti Chen-chun [Fungi of China]: 155 (1950) [MB#20362]

MycoBank No: MB#20012

Type species: *Actinomortierella capitata* (Marchal) Vandepol & Bonito

Description: Phylogenetically basal within the Mortierellaceae. Sporangiophores have an apical inflation in the uppermost portion from which short branches arise. The main sporangiophore and branches may or may not form terminal sporangia. Sporangiospores are globose to ellipsoid; chlamydospores are absent.

Habitat: Isolates of this genus have been reported from Fiji, India, New Zealand, across North America, and the United Kingdom. *A. ambigua* and *A. capitata* are most commonly isolated from soil and dung. *Actinomortierella.* sp. aff *ambigua* BC1065 was isolated from decaying wood and fungivorous millipedes (Macias et al. 2019). In contrast, *A. wolfii* is generally isolated from compost, decaying hay and the lungs of diseased animals, usually cattle, where it causes mycotic pneumonia and abortion. *A. wolfii* is the only Mortierellaceae species known to be pathogenic to mammals and thus grows well at unusually high temperatures (Seviour, Cooper, and Skilbeck 1987).

Species in this study:

Actinomortierella ambigua (B.S. Mehrotra) Vandepol & Bonito **comb. nov.**

MycoBank No.: MB#835795

Type specimen: M-80

Basionym: *Mortierella ambigua* B.S. Mehrotra (1963)

Actinomortierella capitata (Marchal) Vandepol & Bonito **comb. nov.**

MycoBank No.: MB#835796

Type specimen: No type known

Basionym: *Mortierella capitata* Marchal, Bull. Soc. R. Bot. Belg.: 134 (1891)

Synonym: *Mortierella vesiculosa* Mehrotra, Baijal & B.R. Mehrotra (1963); *Carnoya capitata* (Marchal) Dewèvre (1893)

Actinomortierella wolfii (B.S. Mehrotra & Baijal) Vandepol & Bonito **comb. nov.**

MycoBank No.: MB#835797

Type specimen: M-82

Basionym: *Mortierella wolfii* B.S. Mehrotra & Baijal, Mycopathologia et Mycologia Applicata 20 (1-2): 51 (1963)

Notes: *Mortierella capitata* Marchal (1891) was reassigned to *Carnoya* by Dewèvre (1893) and then transferred to *Actinomortierella* by Chalab (1968) along with several other Mortierellaceae species. The genus *Actinomortierella* was subsequently reduced to a subsection of *Mortierella* by Gams (1977). Since all but one of the species formerly in *Actinomortierella* were clustered together in our analyses (the exception being *Lunasporangiospora chienii* as *Actinomortierella umbellata*), we are resurrecting *Actinomortierella*. However, while *Actinomortierella* was validly described by Chalabuda in Griby Roda Mortierella (1968), the novel combinations for *A. ambigua*, *A. capitata*, and *A. wolfii* were in violation of Article 41.5 of the Shenzhen Code. Therefore, we provide valid combinations for each of these species.

Aquamortierella Embree & Indoh 1967

MycoBank No: MB#20047

Type species: *Aquamortierella elegans* Embree & Indoh

Description: Hyphae highly branched. Sporangiospores uniquely reniform (kidney-shaped) to allantoid (sausage-shaped). Zygospores unknown and chlamydospores not mentioned. Suggested to be the only known member of the Mucorales, wherein the Mortierellaceae were then presumed to belong, to normally form sporangia and discharge spores under water.

Habitat: Initially isolated from midge larvae in a freshwater stream in New Zealand. It was also found in Japan.

Notes: No living material of this taxon currently exists.

Dissophora Thaxter 1914

MycoBank No: MB#20187

Type species: *Dissophora decumbens* Thaxter

Description: Fertile, septate aerial stolons are abruptly differentiated from fine vegetative hyphae. Sporangiophores arise as buds from these stolons in intervals behind the advancing apex. *D. globulifera* comb. nov., was not originally described in this genus, but it does produce sporangiophores from aerial stolons, in accordance with the original diagnostic characteristic. Unlike the other *Dissophora* species, *D. globulifera* sporangiophores appear as outgrowths from the bases of older sporangiophores, forming “tufts”, rather than singly along the length of the fertile aerial stolon.

Habitat: All *Dissophora* species have been isolated from forest litter and soil. *D. globulifera* has also been isolated from agricultural soil. *D. decumbens* isolates are from North America, *D. ornata* isolates are from South America, and *D. globulifera* has been isolated from Europe and Japan.

Species in this study:

Dissophora decumbens Thaxt. (1914) [MB#160412]

Dissophora globulifera (Rostr.) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833727

Type specimen: MBT#8101 (Neotype)

Basionym: *Mortierella globulifera* O. Rostr., Dansk botanisk Arkiv 2 (5): 2 (1916)

Synonym: *Mortierella ericetorum* Linnem. (1953)

Dissophora ornata (Gams) Gams [MB#135572]

≡ *Mortierella ornata* W. Gams (1983)

Gamsiella Benny & M. Blackwell 2004

≡ *Mortierella* subgen. Gamsiella R.K. Benj., Aliso 9: 157 (1978) [MB#530804]

MycoBank No: MB#28820

Type species: *Gamsiella multidivaricata* (R.K. Benj.) Benny & Blackwell

Description: This genus was originally monotypic and defined based on the sporangiophore morphology of *G. multidivaricta*: “branched aerial hyphae form intercalary, lateral enlargements which become several times successively di- or tridivaricately branched, the ultimate branches forming two-spored sporangia on slender, elongate, attenuated pedicels” (Benjamin, 1978). *G. stylospora*, does not form sporangiospores, instead making stylospores. As Dixon-Stewart described (1932) “Stylospores very well developed on Czapek's medium, borne on fine aerial upright hyphae. No sporangia have been seen”.

Habitat: Isolates have been reported from soil, decaying wood, and dung in Australia and Russia.

Species in this study:

Gamsiella multidivaricata (R.K. Benj.) Benny & Blackwell [MB#488121]

≡ *Mortierella multidivaricata* R.K. Benj. (1978)

Gamsiella stylospora (Dixon-Stew) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833728

Type specimen: CBS 211.32 [MBT#8202]

Basionym: *Mortierella stylospora* Dixon-Stew., Transactions of the British Mycological Society 17 (3): 218 (1932)

Lobosporangium M. Blackwell & Benny 2004

MycoBank No: MB#28819

Type species: *Lobosporangium transversale* (Malloch) Blackwell & Benny

Description: Sporangiophores are branched, sporangia transversely elongate with 1-5 spines at the apex. Sporangiospores are irregularly shaped. Zygospores are absent.

Habitat: The type strain of this monotypic genus was isolated from Nevada soil in 1964. It has only been reported since from soils in Texas and the Sonoran Desert.

Species in this study:

Lobosporangium transversale (Malloch) Blackwell & Benny [MB#488122]

≡ *Echinosporangium transversale* Malloch [as 'transversalis'] (1967)

Modicella Kanouse 1936

MycoBank No: MB#20336

Type species: *Modicella malleola* (Harkn.) Gerd. & Trappe

Description: Species in this genus are the only Mortierellaceae known to produce macroscopic fruiting bodies, in the form of small, whitish, round sporocarps. Spores can be germinated on artificial media and grown axenically. They are morphologically Mortierellaceae-like in their acolumellate sporangium and garlic-like odor that is similar to that of other Mortierellaceae species.

Habitat: *Modicella* specimens are found growing saprotrophically on soils and decaying plant matter. *M. malleola* has been recorded in Europe, New Zealand, North America, and Taiwan, whereas *M. reniformis* has only been found in South America.

Species in this study:

Modicella reniformis (Bres.) Gerd. & Trappe [MB#317772]

≡ *Endogone reniformis* Bres. (1896)

Mortierella Coemans 1863

MycoBank No: MB#20345

Type species: *Mortierella polycephala* Coemans

Description: Sporangiospores are absent in some species, when present they can range from smooth and ellipsoid to reticulated and/or irregular, depending on the species. Chlamydospores, where present, are scarce and either smooth or spiny. When known, zygospore production is by heterothallic mating, though some homothallic *M. polycephala* isolates have been reported (de Hoog et al. 2000).

Habitat: Most *Mortierella* species prefer to grow at cooler temperatures. Several species of *Mortierella* are known to be mycoparasitic to varying degrees. The type species of this genus, *M. polycephala*, was originally isolated from a mushroom. One strain in the present study was isolated from the surface of a truffle. *M. bisporalis* is a facultative biotrophic mycophile that first competes with its host for substrate, then causes lysis of the host mycelium and penetrates the host to live biotrophically (Rudakov, 1978). *Mortierella alpina* has also been shown to parasitize oospores of members of Oomycota (Phylum Heterokontophyta) (Willoughby, 1988).

Species in this study:

Mortierella alpina Peyronel (1913) [MB#170280]

≡ *Mortierella oblatispora* W. Gams & G.J. Bollen (?)

≡ *Mortierella acuminata* Linnem. (1941)

≡ *Mortierella renispora* Dixon-Stew.(1932)

≡ *Mortierella monospora* Linnem. (1936)

≡ *Mortierella thaxteri* Björl. (1941)

Mortierella antarctica Linnem. (1969) [MB#317880]

Mortierella bisporalis (Thaxt.) Björl. (1936) [MB#258541]

≡ *Haplosporangium bisporale* Thaxt. (1914)

Mortierella indohii C.Y. Chien (1974) [MB#317900]

Mortierella polycephala Coem. (1863) [MB#145769]

≡ *Mortierella vantieghemi* Bachm. (1900)

≡ *Mortierella vantieghemii* Bachm. (1900)

≡ *Mortierella raphani* Dauphin (1908)

≡ *Mortierella vantieghemi var. raphani* (Dauphin) Linnem. (1941)

Mortierella reticulata Tiegh. & G. Le Monn. (1873) [MB#236117]

Novel Genera

Benniella Vandepol & Bonito, **gen. nov.**

MycoBank No: MB#833778

Etymology: In honor of Gerald Benny, an American mycologist who has dedicated his career to the study of zygomyceteous fungi. Dr. Benny made significant contributions to Mortierellaceae taxonomy, among others. This included establishing the subphylum Mortierellomycotina, elevating *Gamsiella* to generic level, and renaming *Echinosporangium* as *Lobosporangium*.

Type species: *Benniella erionia* Liber & Bonito

Description: Colonies on MEA or PDA are pure white to off white in color and do not produce rosette or colony patterns when young. With age, some slight growth rings may develop in the mycelium along the agar surface. Aerial mycelium is very abundant, over 1 cm thick. Hyphae are sterile, without observed sporangiophores or zygospores.

Habitat: These fungi have been isolated from dried soils collected in the United States (Indiana and Ohio), Australia, and Uganda. Isolates were baited from soils using sterilized shrimp exoskeletons.

Benniella erionia Liber & Bonito, **sp. nov.**

MycoBank No: MB#833779

Etymology: “erionia” - from the Greek “erion” meaning “wool”. This describes the appearance of the mycelium as unpatterned, light colored, and wooly.

Type specimen: Australia, Western Australia, Camballan, sub-humid upland forest woodlands dominated by *Eucalyptus marginata* and *Corymbia calophylla*. 24 Sept. 2014, G.M. Bonito, FLAS-F-66497 (holotype) [MBT#392648].

Description: Colonies on MEA are pure white, and do not produce rosettes or patterning. Hyphae are sterile, without observed sporangiophores or zygospores, and are 3.63 ± 0.09 µm (mean ± SEM) in diameter. Terminal structures borne on axillary hyphae are swollen and irregularly branched, and in older cultures, these become darkened and resemble chains of spherical chlamydospores, each spore 10.7 ± 1.89 µm. Growth rates on PDA ½ + YE are 6.6-9.5 µm/min (min and max of 3 replicates) at room temperature (RT), and 5.0-8.5 µm/min at 30°C. On MEA + YE, growth rates are 4.9-8.4 µm/min at RT, and 4.9-5.2 µm/min at 30°C.

Habitat: The type specimen (isolate GBAus27B) was cultured from soils collected in woodland of *Eucalyptus marginata* and *Corymbia calophylla* in Australia, on sandy gravels on low divides in the subhumid zones. Isolates were baited from soils using sterilized arthropod exoskeletons. The other *B. erionia* isolate in this study, INSO1-46B2, was isolated from soybean field soil in Indiana, USA.

Entomortierella Vandepol & Bonito, **gen. nov.**

MycoBank No: MB#833613

Etymology: entomon- (insect) refers to the insect association common to the species in this genus.

Type species: *Entomortierella lignicola* (Martin; Gams & Moreau) Vandepol & Bonito Description: Most of the species produce sporangiospores, usually globose and smooth, but spiny in the case of *E. lignicola*. *Entomortierella beljackovae*, *E. chlamydospora*, and *E. echinosphaera* produce chlamydospores, the latter two of which are usually spiny. Almost all of the species are known to produce zygospores, but are divided between hetero- and homothallic sexual lifestyles.

Habitat: Species in this genus appear to be arthropod and/or worm associates, as they are commonly isolated from ant pellets, termite nests, and vermicompost. They are also frequently isolated from soil, roots, and rotting plant matter.

Species in this study:

Entomortierella beljakovae (Milko) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833729

Type specimen: CBS 123.72 [MBT#8042]

Basionym: *Mortierella beljakovae* Milko (1973)

Entomortierella chlamydospora (Chesters) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835762

Type specimen: MBT#8049 (Syntype)

Basionym: *Azygozygum chlamydosporum* Chesters, Transactions of the British Mycological Society 18 (3): 213 (1933)

Synonyms: *Mortierella chlamydospora* (Chesters) Plaats-Niterink (1976)

Entomortierella echinosphaera (Plaats-Niterink) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835750

Type specimen: CBS575.75 (Holotype)

Basionym: *Mortierella echinosphaera* Plaats-Niterink, Persoonia 9 (1): 91 (1976)

Entomortierella lignicola (G.W. Martin) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835763

Type specimen: CBS 207.37 [MBT#15136]

Basionym: *Haplosporangium lignicola* G.W. Martin (1941)

Synonyms: *Mortierella lignicola* (G.W. Martin) W. Gams & R. Moreau (1960); *Mortierella sepedonioides* Linnem. (1941)

Entomortierella parvispora (Linnem.) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835751

Type specimen: MBT#8163 (Syntype)

Basionym: *Mortierella parvispora* Linnem., Pflanzenforschung 23: 53 (1941)

Synonyms: *Mortierella gracilis* Linnem. (1941)

Gryganskiella Vandepol, Stajich & Bonito, **gen. nov.**

MycoBank No: MB#833857

Etymology: In honor of Andrii Gryanskyi, a Ukrainian-American mycologist, for his contributions in research, training and genomics of fungi in Mucoromycota.

Type species: *Gryganskiella cystojenkinii* (W. Gams & Veenb.-Rijks) Vandepol & Bonito

Description: Sporangiospores are smooth and elliptical to cylindrical. Chlamydospores are lightly pigmented, light brown or ochre/orange. While this characteristic is not unique to this genus, it is conserved within this group.

Habitat: These species have been reported from agricultural soil & moss in the Netherlands & South America.

Species in this study:

Gryganskiella cystojenkinii (Gams & Veenb.-Rijks) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833858

Type specimen: CBS 456.71 [MBT#8054]

Basionym: *Mortierella cystojenkinii* W. Gams & Veenb.-Rijks, Persoonia 9 (1): 137 (1976)

Gryganskiella fimbricystis (Gams) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833859

Type specimen: CBS 943.70 [MBT#8084]

Basionym: *Mortierella fimbricystis* W. Gams, Persoonia 9 (1): 138 (1976)

Linnemannia Vandepol & Bonito, **gen. nov.**

MycoBank No: MB#833612

Etymology: In honor of Germaine Linnemann, a German mycologist who contributed many Mortierellaceae species descriptions and early hypotheses on their evolutionary relationships.

Type species: *Linnemannia hyalina* (Harz; Gams) Vandepol & Bonito

Description: Nearly all known species of *Linnemannia* produce sporangiospores, with the exception of *L. acrotona*. When produced, sporangiospores are usually ellipsoid, but can also be spherical to cylindrical. Production of chlamydospores is irregular between species. When produced, most chlamydospores are various shades of brown. *L. amoeboidea* makes irregular amoeba-like chlamydospores. The species for which the sexual reproductive mode is known are heterothallic.

Habitat: This genus contains some of the most widely distributed Mortierellaceae species. *L. elongata*, *L. hyalina* and *L. gamsii*, are especially common in neutral or calcareous soils. Most of the species in this genus are isolated from soils and are usually associated with plant rhizospheres or decaying plant matter.

Species in this study:

Linnemannia acrotona (Gams) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833769

Type specimen: CBS 386.71 [MBT#8005]

Basionym: *Mortierella acrotona* W. Gams, Persoonia 9 (1): 133 (1976)

Linnemannia amoeboidea (Gams) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833770

Type specimen: CBS 889.72 [MBT#8022]

Basionym: *Mortierella amoeboidea* W. Gams, Persoonia 9 (1): 116 (1976)

Linnemannia camargensis (Gams & Moreau) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835745

Type specimen: CBS 221.58 [MBT#18848]

Basionym: *Mortierella camargensis* W. Gams & R. Moreau, Annales Scientifiques Université Besançon 3: 103 (1960)

Synonyms: *Haplosporangium gracile* Nicot (1957)

Linnemannia elongata (Linnem.) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833768

Type specimen: MBT#140592

Basionym: *Mortierella elongata* Linnem., Pflanzenforschung 23: 43 (1941)

Synonyms: *Mortierella debilis* E. Wolf (1954)

Linnemannia exigua (Linnem.) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835752

Type specimen: MBT#140594

Basionym: *Mortierella exigua* Linnem., Pflanzenforschung 23: 44 (1941)

Synonyms: *Mortierella indica* B.S. Mehrotra (1960); *Mortierella sterilis* B.S. Mehrotra & B.R. Mehrotra (1964)

Linnemannia gamsii (Milko) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835747

Type specimen: CBS 749.68 [MBT#8087]

Basionym: *Mortierella gamsii* Milko, Opredelitel mukoralnykh gribov [Key to the identification of Mucorales]: 76 (1974)

Synonyms: *Mortierella spinosa* Linnem. (1936)

Linnemannia hyalina (Gams) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833682

Type specimen: MBT#56360 (Isotype)

Basionym: *Mortierella hyalina* (Harz) W. Gams, Nova Hedwigia 18: 13 (1970)

Synonyms: *Hydrophora hyalina* Harz (1871); *Mortierella candelabrum var. minor* Grove (1885); *Mortierella hygrophila* Linnem. (1941); *Mortierella hygrophila var. minuta* Linnem. (1941)

Linnemannia nantahalensis (C.Y. Chien) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835746

Type specimen: CBS 610.70 [MBT#8154]

Basionym: *Mortierella nantahalensis* C.Y. Chien, Mycologia 63 (4): 826 (1971)

Linnemannia schmuckeri (Linnem.) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835748

Type specimen: MBT#8193 (Syntype)

Basionym: *Mortierella schmuckeri* Linnem., Archiv für Mikrobiologie 30: 263 (1958)

Linnemannia sclerotiella (Milko) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835749

Type specimen: CBS 529.68 [MBT#8195]

Basionym: *Mortierella sclerotiella* Milko, Novosti Sistematiki Nizshikh Rastenii 4: 160 (1967)

Linnemannia zychae (Linnem.) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835753

Type specimen: CBS 316.52 [MBT#8235]

Basionym: *Mortierella zychae* Linnem., Pflanzenforschung 23: 46 (1941)

Synonyms: *Mortierella brachyrhiza* E. Wolf (1954); *Mortierella zychae var. japonica* J.Y. Lee (1972); *Mortierella zychae var. simplex* Linnem. (1941)

Lunasporangiospora Vandepol & Bonito, **gen. nov.**

MycoBank No: MB#833611

Etymology: luna- (crescent) refers to the lunate sporangiospores unique to the two species in this genus.

Type species: *Lunasporangiospora chienii* (P.M. Kirk) Vandepol & Bonito

Description: Sporangiospores are smooth and characteristically lunate. Chlamydospores are terminal and scarce in *L. selenospora* and absent in *L. chienii*. Mating and zygospores are unknown in both species.

Habitat: Isolates of this genus have been reported from mushroom compost and forest soil from North America, Europe, and Asia.

Species in this study:

Lunasporangiospora chienii (P.M. Kirk) Vandepol & Bonito, **comb. nov.**

MycoBank No: MB#833681

Type specimen: CBS 124.71 [MBT#8211]

Basionym: *Mortierella chienii* P.M. Kirk, Index Fungorum 2: 1 (2012)

Synonyms: *Mortierella umbellata* C.Y. Chien (1972); *Actinomortierella umbellata* (C.Y. Chien) Chalab. (1973)

Lunasporangiospora selenospora (W. Gams) Vandepol & Bonito, **comb. nov.**

MycoBank No: MB#833724

Type specimen: CBS 811.68 [MBT#8196]

Basionym: *Mortierella selenospora* W. Gams, Persoonia 9 (1): 128 (1976)

Necromortierella Vandepol & Bonito, **gen. nov.**

MycoBank No: MB#833725

Etymology: necro- (death) refers to the necrotrophic mycophilic lifestyle of the type species, in that it kills and consumes the cells of other fungi.

Type species: *Necromortierella dichotoma* (Linnem. ex W. Gams) Vandepol & Bonito

Description: Sporangiophore narrow, tapering quickly to a narrower apex with irregular dichotomous branching. Sporangiospores are ellipsoidal to cylindrical. Chlamydospores are elongated or irregular.

Habitat: *N. dichotoma* is the only known necrotrophic mycophile (kills fungal cells and feeds saprotrophically on the dead tissue) in Mortierellaceae. This species has only been reported from mouse dung in Germany.

Notes: There may be additional species in this genus that were not included in this study. Additional work must be done to determine whether related species are also necrotrophic mycophiles.

Species in this study:

Necromortierella dichotoma (Gams) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833726

Type specimen: MBT#8056 (Syntype)

Basionym: *Mortierella dichotoma* W. Gams, Persoonia 9 (1): 128 (1976)

Podila Stajich, Vandepol & Bonito, **gen. nov.**

MycoBank No: MB#833766

Etymology: In honor of Gopi Podila (1957-2010), an Indian American biologist who advanced the fields of plant-microbe interactions, plant genetics and biotechnology in bioenergy crops. In particular, Podila researched the genetic basis of the poplar microbiome and metabolome.

Type species: *Podila minutissima* (Tiegh.) Vandepol & Bonito

Description: All species produce sporangiospores, though with variable morphologies: globose to fusoid, smooth to spinulose or verrucose. Chlamydospores are absent in some species and scarce or unknown in the others. When known, zygospore morphology ranges from naked to smooth and globose and mating is usually heterothallic, though at least one species is homothallic.

Habitat: Species of *Podila* are frequently isolated from forest and agricultural soil, compost, dung, and municipal waste. *P. minutissima* has been isolated from *Populus* roots (Bonito et al. 2016). They have been reported from Europe, New Zealand, and North America. *P. minutissima* is a semi-saprotrophic mycophile (saprotrophically consumes dead fungal tissue) it is possible that additional species in this genus are also mycophilic/mycoparasitic to some degree (Rudakov, 1978).

Species in this study:

Podila clonocysits (Gams) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835720

Type specimen: CBS 357.76 [MBT#8053]

Basionym: *Mortierella clonocysits* W. Gams, Persoonia 9 (1): 132 (1976)

Podila epicladia (Gams) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835721

Type specimen: CBS 355.76 [MBT#8074]

Basionym: *Mortierella epicladia* W. Gams & Emden, Persoonia 9 (1): 133 (1976)

Podila epigama (Gams & Domsch) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835722

Type specimen: CBS 489.70 [MBT#8076]

Basionym: *Mortierella epigama* W. Gams & Domsch, Transactions of the British Mycological Society 58 (1): 11 (1972)

Podila horticola (Linnem.) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835723

Type specimen: MBT#8105 (Syntype)

Basionym: *Mortierella horticola* Linnem., Pflanzenforschung 23: 21 (1941)

Podila humilis (Linnem. ex W. Gams) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835724

Type specimen: MBT#8109 (Syntype)

Basionym: *Mortierella humilis* Linnem. ex W. Gams, Beitrag zu einer Flora der Mucorineae Marburgs, Diss. (1963)

Podila minutissima (Tiegh.) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#833767

Type specimen: no type known

Basionym: *Mortierella minutissima* Tiegh., Annales des Sciences Naturelles Botanique 4: 385 (1878)

Synonym: *Mortierella minutissima var. dubia* Linnem. (1941)

Podila verticillata (Linnem.) Vandepol & Bonito **comb. nov.**

MycoBank No: MB#835725

Type specimen: MBT#140598

Basionym: *Mortierella verticillata* Linnem., Pflanzenforschung 23: 22 (1941)

Synonyms: *Mortierella marburgensis* Linnem. (1936); *Haplosporangium fasciculatum* Nicot (1957); *H. attenuatissimum* F.J. Chen (1992)

Discussion

To our knowledge, this study provides the most extensive and in-depth sampling of Mortierellaceae diversity to date, that extends to new isolates from Africa, Australia, and the United States, where several novel species and lineages were discovered. We also included

Modicella, sampled from a sporocarp, in the first application of low coverage genome sequencing to large-scale fungal phylogenetic systematics (Petkovits et al. 2011; Smith et al. 2013; Wagner et al. 2013). We further developed and tested a pipeline for identifying non-ribosomal phylogenetic markers. By combining these approaches, we were successful in resolving the phylogeny of Mortierellaceae to provide a phylogenetic-based framework for their taxonomy.

There is considerable uncertainty concerning Mortierellaceae species diversity that remains to be sampled (Hibbett & Glotzer, 2011; Nagy et al. 2011). Nagy et al. (2011) estimated the rate of novel species discovery in Mortierellaceae by comparing the diversity represented in sequence repositories to diversity within putatively novel sequences and those of described species. They concluded that most Mortierellaceae diversity was already discovered and they estimated a total of approximately 126 species in the family. Given that 102 of the 125 currently accepted species in Mortierellaceae were described prior to 1980, and only 9 more between 1990 and 2000, this might seem to be a reasonable conclusion (**Tables 2.1** & **2.11**). However, taking into account that vast regions of the world are still unsampled, and the limited resolution of ITS and/or 28S rDNA regions in metabarcoding diversity studies, this estimate may be low. There are several examples of distinct species of *Mortierella* that have very similar ITS sequence similarity, e.g. *Podila horticola* and *P. minutissima*. The rate of species discovery in Mortierellaceae has increased in the last decade, with at least seven new species being described in the family between 2011 and 2019 from Poland, Japan, Taiwan, and Korea (Hibbett & Glotzer, 2011; Ariyawansa et al. 2015; Li et al. 2016; **Table 2.1**). Moreover, our sampling efforts in Africa and Australia for this study yielded multiple novel species an entirely novel lineage at the generic level. From our deep sampling efforts in Illinois caves, we recovered 119 isolates. These represented 8 genera and 9 under-represented species, which include isolates of *L. amoeboidea* and *N. dichotoma*. These species are rarely isolated and the collection of new strains is inherently valuable to understanding the ecology, genetics, and distribution of these fungi (Gams, 1977). For the purposes of exploring

the diversity and distribution of Mortierellaceae, we expect more species to be discovered in undersampled locations such as South America, Africa, and Asia.

We found low coverage genome sequencing is a relatively cost-effective means of generating a high-confidence fungal phylogeny. Further, it requires fewer assumptions, and less upstream handling and preparation time than traditional genome sequencing or multi-locus sequence typing, as only a single high quality, high concentration DNA preparation is needed. The approximately 15X sequencing coverage we achieved was sufficient to recover several hundred orthologous genes for our phylogenomic analyses. Occasional misassembly of target loci necessitated mining MGP loci from the raw genome reads, rather than the assembled contigs. Nonetheless, we were still able to detect and recover full length MGP loci from 15 isolates entirely from genome sequences. The LCG approach has been applied successfully in other systems as well, including insects and olive trees, with both low and high quality specimens and genome coverage between 0.5-30X (Olofsson et al. 2019; Zhang et al. 2019). All LCG phylogenomic approaches have relied on first identifying existing phylogenetic markers in assembled whole genome sequence data. Olofsson et al. (2019) built phylogenies with two classical markers and compared these to phylogenies based on SNPs obtained from additional orthologous gene sets. They also demonstrated the capability of an LCG approach to extract phylogenetic information from degraded herbarium specimens with extremely low coverage (<0.5X), which encourages LCG sequencing of fungal herbarium specimens as well (Olofsson et al. 2019). The approach used by Zhang et al. (2019) was more similar to the LCG approach described here, including the breadth of phylogenetic diversity represented in their Hexapoda dataset, but with higher genome coverage than our dataset (20x-30x vs. 15X) and with fewer genomes (21 vs. 73).

Non-ribosomal (nuclear) phylogenetic markers should be single-copy genes that are not under selective pressure and contain sufficient sequence variation to make phylogenetic inferences. Identification of nuclear markers has historically been done manually, starting from protein sequence and characteristics, as in the case of *RPB1* (Jokerst et al. 1989; Sidow & Thomas,

1994). Even with the advent of genome sequencing, discovery and evaluation of novel nuclear markers has been a largely manual process (Blair et al. 2008). There has been at least one other effort to automate the discovery and evaluation of nuclear markers, a program called DIscoMark, which uses a similar approach to the unbiased MGP locus identification method developed here but starting with orthologous genes instead of raw genomes (Deteringet al. 2016). Both approaches are dependent on the availability of high-quality input genomes. In this study, our pipeline did successfully identify single-copy loci, some of which were phylogenetically informative that we used to improve DNA-based species identification. *Elongation Factor 1 alpha* (EF1α) and *RNA polymerase II large subunit* (RPB1) have previously been used as phylogenetic markers in Fungi (James et al. 2006; Stockinger et al. 2014). The six MGP markers were sufficient to sort Mortierellaceae species into clades and provide structure at the species level, however, the 6 loci were insufficient to resolve the higher-level organization of clades along the Mortierellaceae backbone. The limited number of high-quality genomes available for the locus selection pipeline made it difficult to screen loci and primers *in silico* for off-target amplification or gene paralogs prior to *in vivo* use. Additional reference genomes would also inform the primer design process, reducing primer mismatch, locus bias and off-target amplification.

The main trade-off between the LCG and MGP approaches is sampling depth versus breadth. The high capacity of Illumina sequencing platforms meant that there was a minimum sample number needed for the MGP approach to be cost-effective. Therefore, we were able to include “lower priority/higher risk” isolates than in the genome sequencing project, including a high proportion of isolates that could not be identified by ITS sequence data. However, even with multiplex PCRs, there was significantly more sample handling and bench time required for the MGP protocol compared to the LCG. In light of these issues, we suggest that the LCG approach is a superior method for resolving the broad phylogeny of such a diverse lineage. By combining LCG and MGP approaches, we were able to resolve higher-level phylogenetic relationships using

the LCG-derived data, while improving sampling depth and breadth with the MGP approach to place taxa and improve diversity sampling within the phylogeny.

This study upheld the majority of the modern rDNA clades defined by Petkovits et al. (2011) and Wagner et al. (2013) (**Table 2.12**). To resolve the polyphyly of *Mortierella*, we have erected seven novel genera in Mortierellaceae. These include *Podila* (verticillata-humilis clade), *Mortierella* (alpina-polycephala clade), and *Linnemannia* (gamsii clade) (Petkovits et al. 2011; Wagner et al. 2013). However, ITS-clades 1 (selenospora-parvispora), and 5 (strangulata & wolfii) as described by Wagner et al. (2013) are not supported. *Lunasporangiospora*, *Actinomortierella*, *Gryganskiella*, and *Necromortierella* more closely correspond to the selenospora, wolfii, and parvispora clades as described by Petkovits et al. (2011). The MGP dataset places *E. parvispora*, originally part of selenospora-parvispora, in the newly erected genus *Entomortierella* with the retained lignicola clade. The LCG dataset places *Actinomortierella* species (Petkovits /wolfii) at the base of the tree, apart from *Lobosporangium transversale* (strangulata clade), which is still near the middle of the phylogeny. The composition of clade 4 (globulifera, angusta, and mutabilis) was retained, although the species were resolved as separate genera, *Dissophora* and *Gamsiella*. The monophyletic genus *Mortierella* has two main subgroups, the previously defined alpina and polycephala clades (Petkovits et al. 2011). These two clades were widely separated in the Bayesian analyses of Petkovits et al. (2011) and distinct groups within clade 6 as defined by Wagner et al. (2013).

Some of the new genera described here have loosely conserved ecological niches (**Table 2.11**). For example, taxa now classified as *Entomortierella* have almost all been isolated from insect nests or bodies (Gams, 1977; Watanabe et al. 1998). Several members of the re-defined genus *Mortierella* are known to be mycophilic and/or have been isolated from mushrooms and truffles (Domsch et al. 1980). These associations are not unique to these genera, as demonstrated by *Actinomortierella capitata*, *Actinomortierella* aff. *ambigua*, and *Necromortierella dichotoma*, but genus-level conservation may represent specialization and evolution of an

ancestral trait (Gams, 1977; Macias et al. 2019). While much remains unknown about the ecological function of most Mortierellaceae, these trends inspire some additional confidence in the groupings defined by our phylogenetic analyses.

Existing ITS-based species identifications, or lack thereof due to highly similar ITS sequences, are not fully resolved by this study, as this will require the inclusion of type specimens to confidently identify correct ITS classifications. This is most notable in *Podila*, *Mortierella*, and *Linnemannia* due to extensive sampling, high species number, and ITS sequence similarity. Rather, we provide a genus-level framework that will empower future studies to thoroughly resolve individual genera.

Conclusions

Previous research has estimated that the majority of Mortierellaceae diversity has already been discovered and may reside in culture collections (Nagy et al. 2011). However, our research reveals novel species and genera in both thoroughly sampled and historically undersampled regions, including Michigan, USA and Uganda, respectively. Based on these results, we believe that there is a need for continued geographic sampling efforts to identify new species and to establish the ranges and ecological niches of recognized species of Mortierellaceae, including *L. elongata* (Ozimek et al. 2018; Liao et al. 2019).

While greatly improved by our study, ecological data to accompany sequence data are still scarce for Mortierellaceae. One of the valuable contributions of this work is the curation of reference sequences with updated taxonomy, supported by multiple independent loci, that will be integrated into NCBI and UNITE reference sequence database. These vouchered sequence data could also be used to seed non-ITS reference databases. Together, these data will improve the ability to accurately identify taxa and novel species and thereby improve understanding of the diversity and ecology of these fungi. Further consolidation of global geographic and environmental records of Mortierellaceae isolates would help resolve the range and ecology of these species.

We recommend future efforts prioritize sequencing of non-ribosomal markers from type isolates, additional culture collections, and isolates from under-sampled regions.

Figures & Tables

Figure 2.1 – Divseristy of Mortierellaceae macromorphologies

a) *Mortierella* sp. JL58 on MEA+YE, 11 days; b-d) *Mortierella* sp. JL29, AP5, and JL1 on MEA+YE, 11 days; e) *Mortierella elongata* NVP64- on PDA/2+YE, 6 days; f-h) *M. alpina* NVP153, JL109, and KOD1002 on PDA/2+YE, 6 days; i) *M. humilis* PMI1414- on PDA/2+YE, 6 days.

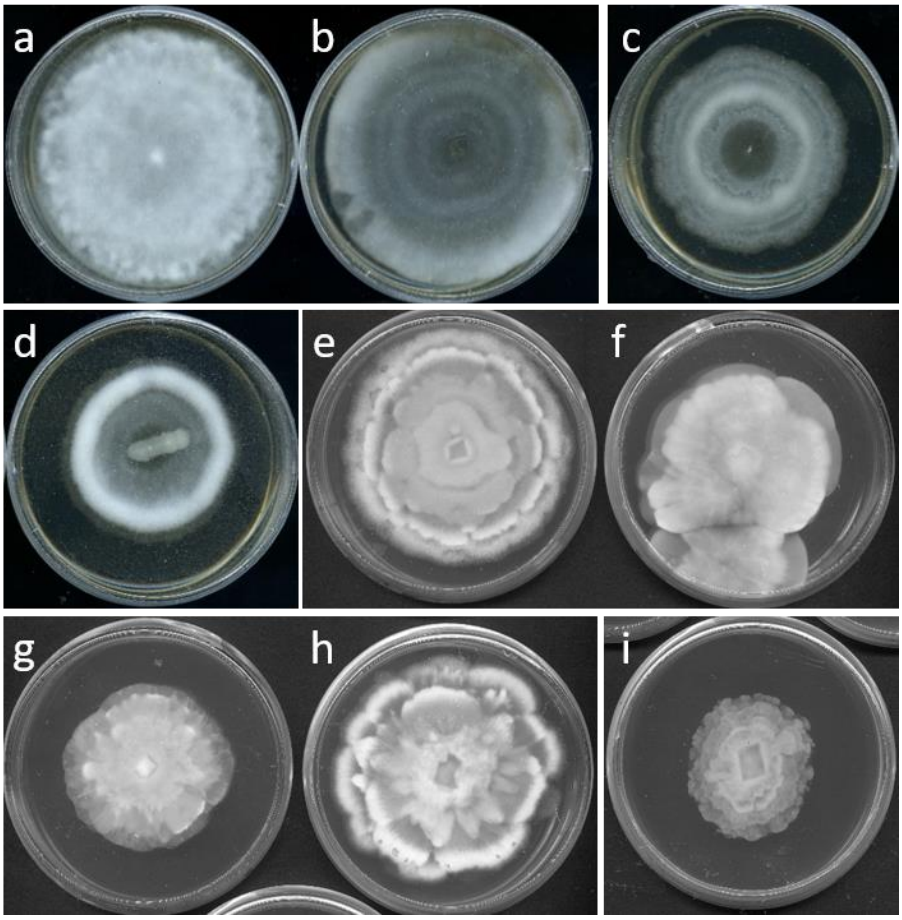


Figure 2.2 – Common Mortierellaceae spore forms

Mortierella elongata NVP64 a) sporangiospores and bent sporangiophores on agar surface; b) intercalary chlamydospore and septate, evacuated hyphae; c) branched sporangiophore bearing sporangiospores; and d) sexual zygospore from heterothallic mating with *M. elongata* NVP5. Scale bars – a, 100 µm; b, 10 µm; c, 200 µm; d, 20 µm.

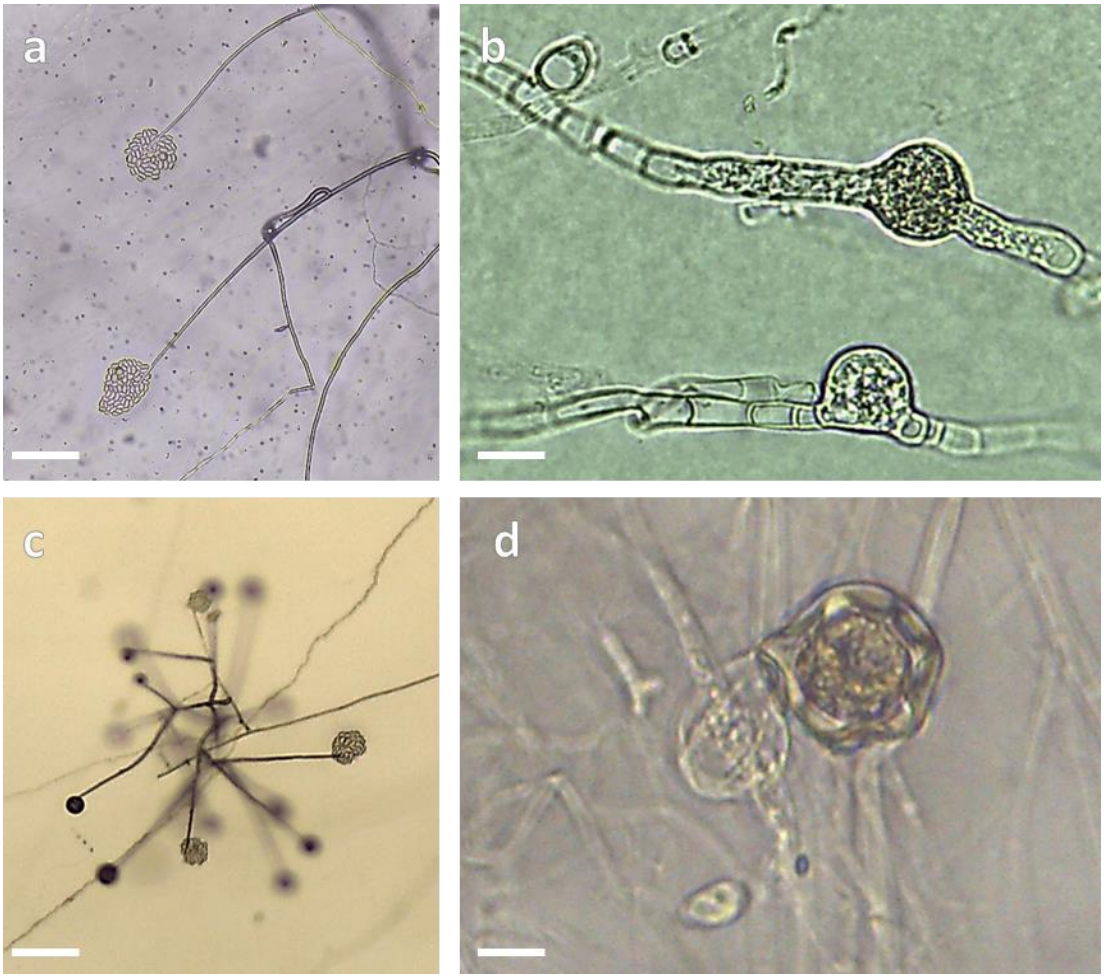


Figure 2.3 – Media-dependent macromorphology
Mortierella sp. aff ambigua JL86, 10 days, on three media a) PDA/2+YE, b) MEA+YE, c) CZA.

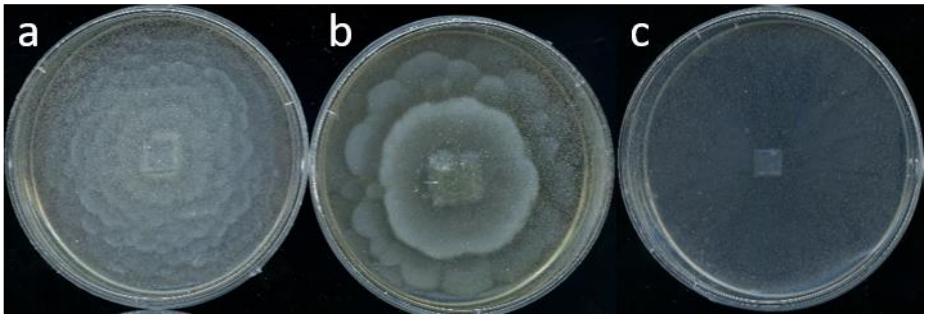


Figure 2.4 – Maximum likelihood analysis of LCG dataset

Maximum likelihood analysis of a concatenated matrix of 109,439 nucleotide characters of protein-coding sequences from 434 genes. Clade colors indicate monophyletic groupings, lines and clade names denote previously defined clades for the purpose of discussion. Node numbers indicate bootstrap support.

Figure 2.5 – Unconstrained Maximum Likelihood analysis of the concatenated MGP dataset

Taxa are named according to the initial ITS-based species identification and current taxonomy. Clade colors indicate monophyletic groupings according to the proposed taxonomy, lines and names denote previously defined clades.

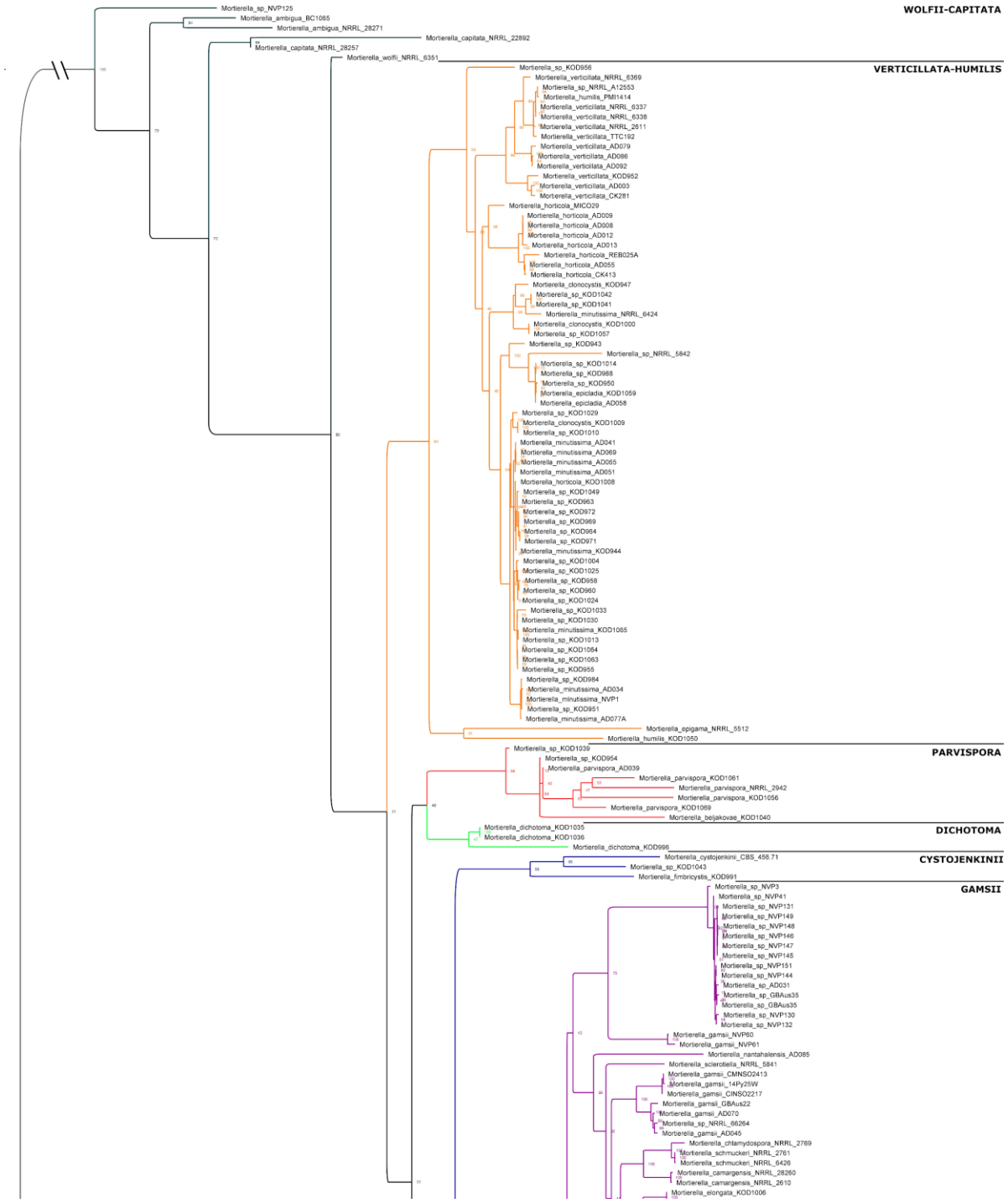


Figure 2.5 (cont'd)

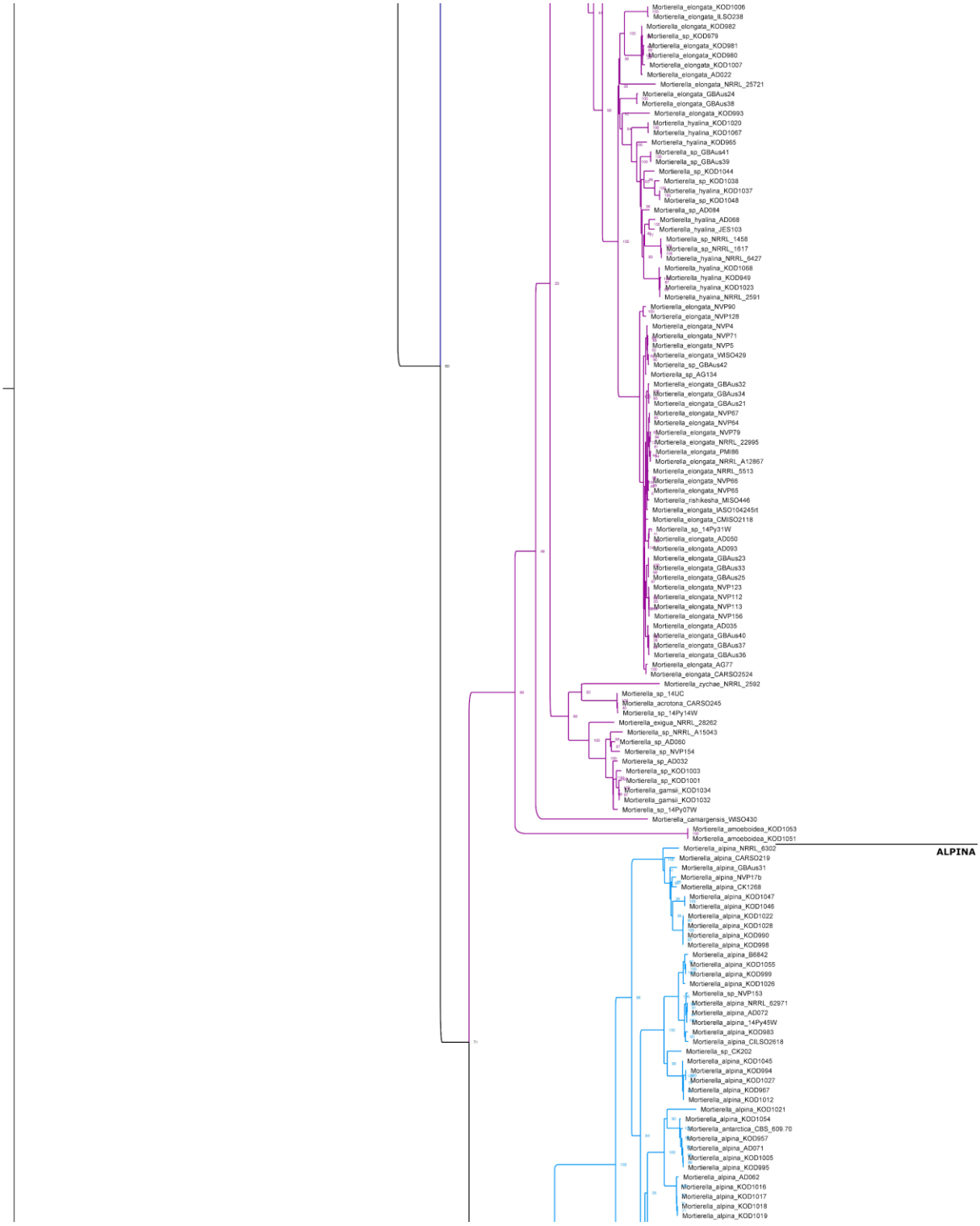


Figure 2.5 (cont'd)

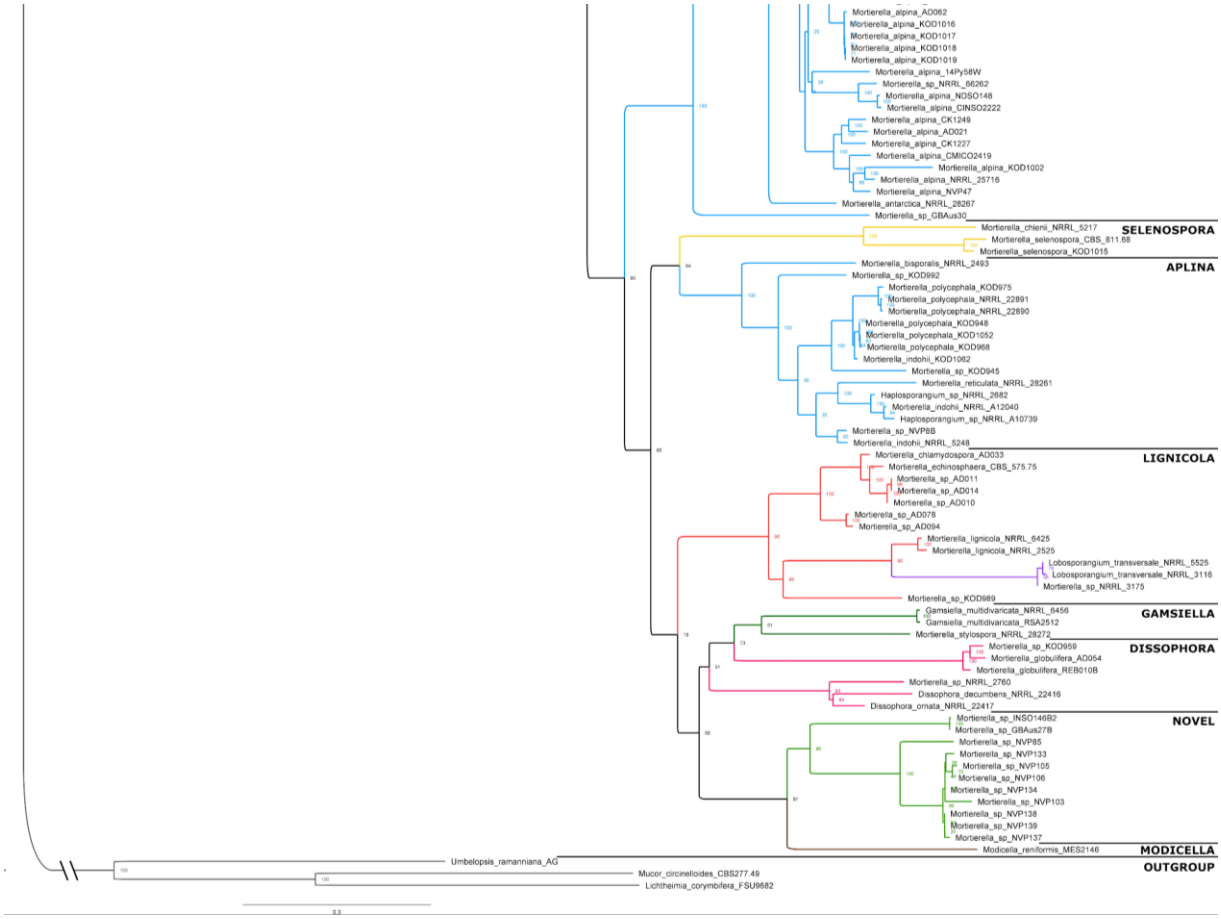


Figure 2.6 – MrBayes muilt-gene Mortierellaceae phylogeny

A Bayesian analysis of the concatenated MGP dataset using a series of partial constraints defined by major nodes in the LCG phylogeny. Clade colors indicate groupings according to the Constrained RAxML MGP phylogeny.

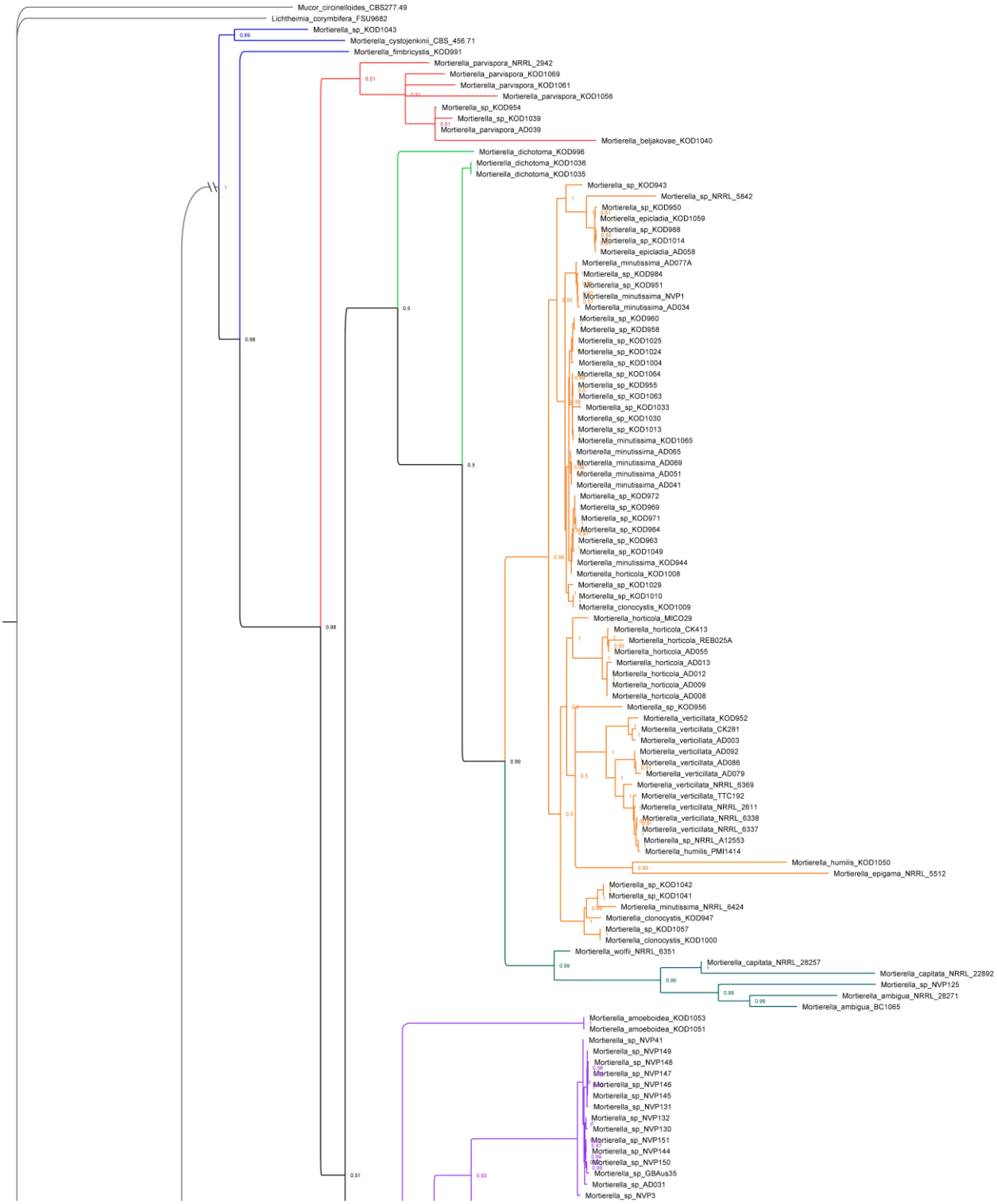


Figure 2.6 (cont'd)

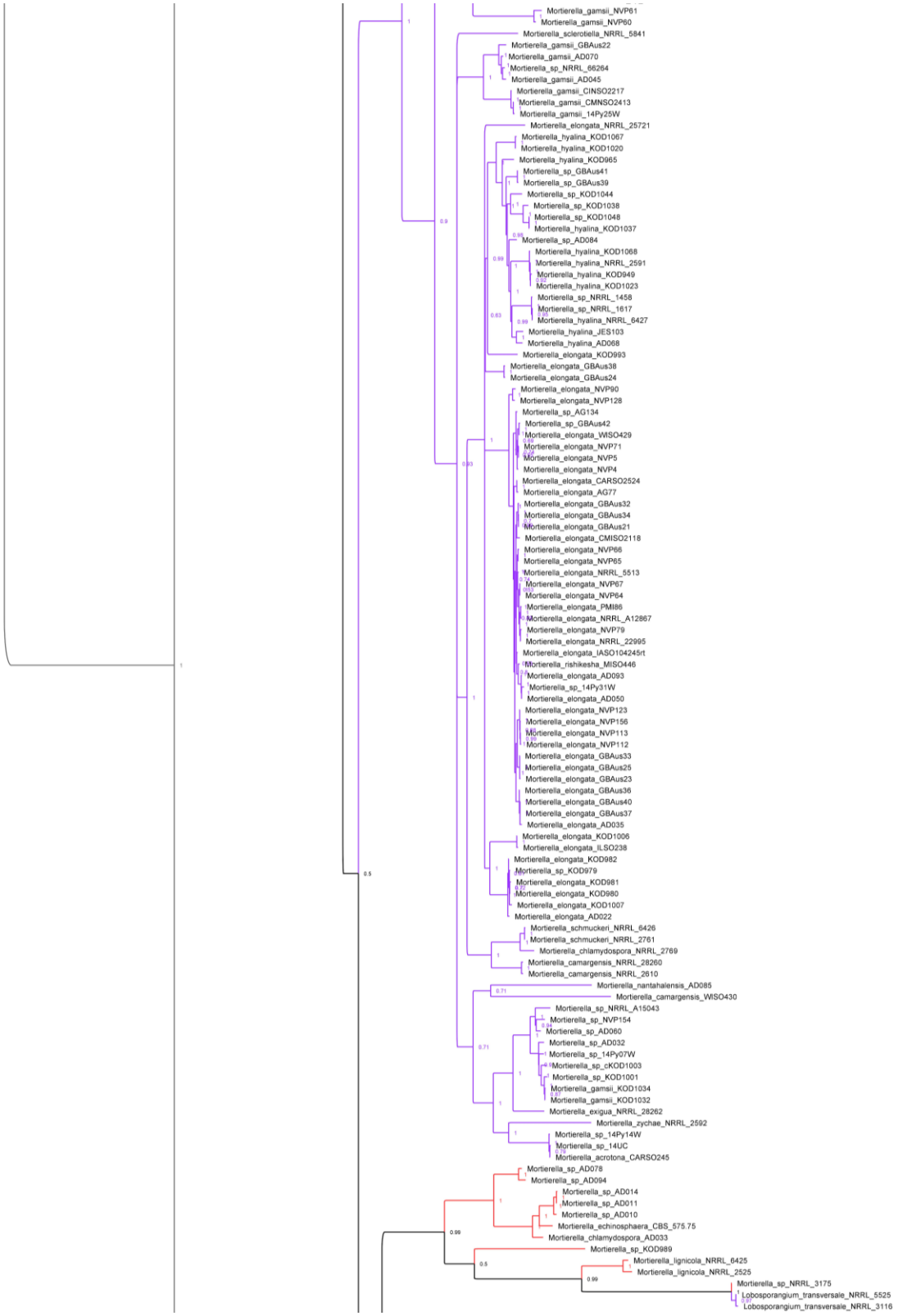


Figure 2.6 (cont'd)

Figure 2.7 – Constrained Maximum Likelihood analysis of the MGP dataset

Maximum Likelihood analysis of the concatenated 6-gene MGP dataset composed of 8181 nucleotide characters and constrained by the LCG phylogeny. Taxa are named according to the initial ITS-based species identification and proposed genus-level taxonomy. Clade colors indicate monophyletic groupings according to the proposed taxonomy, lines and names denote previously defined clades for the purpose of discussion.

Figure 2.7 (cont'd)

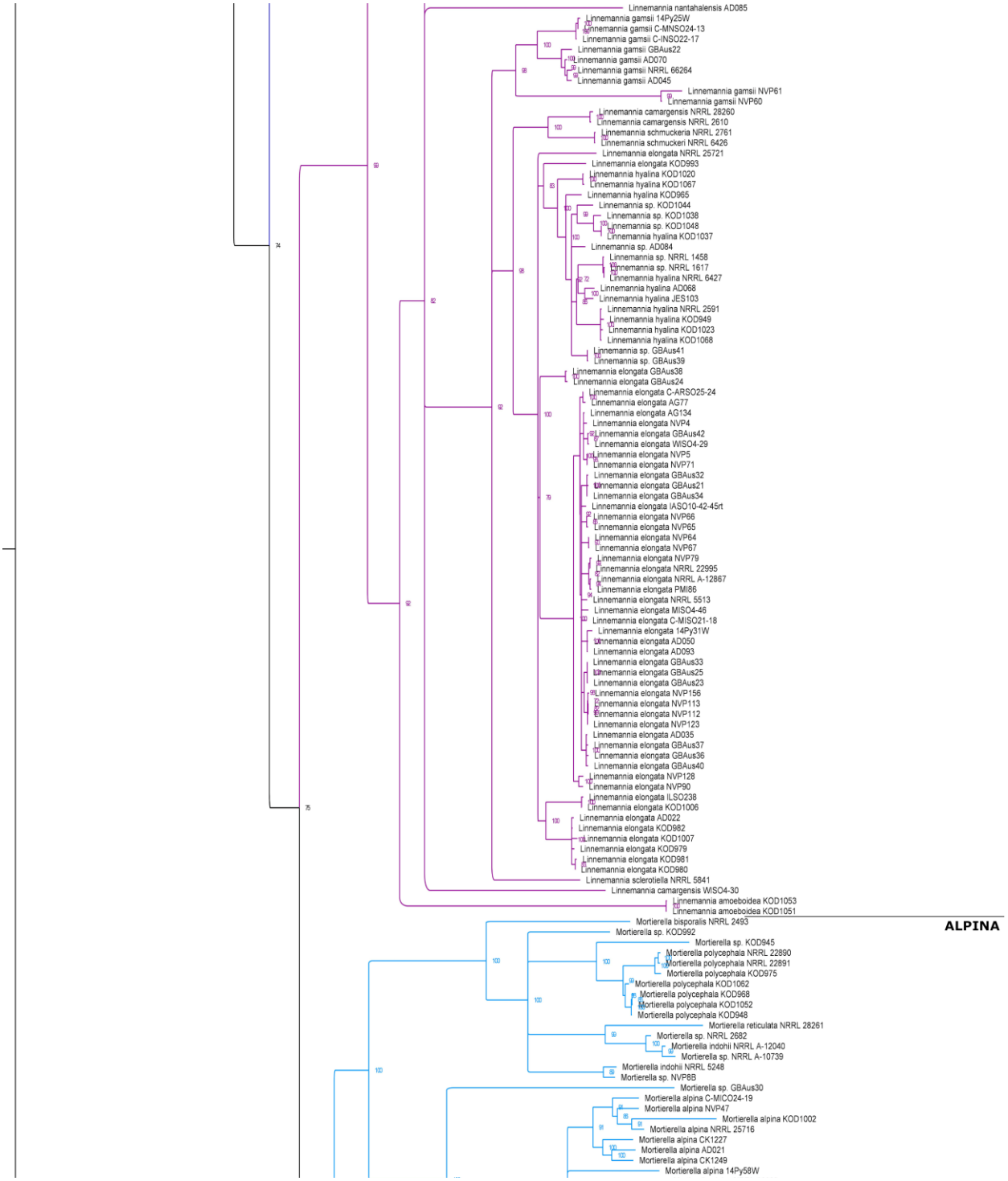


Figure 2.7 (cont'd)

Table 2.1 – Mortierellaceae species not included in this study

A summary of the described species not included in this study, the estimated placement under the proposed taxonomy, the basis for the estimation, and the reference for the original species definition.

Current Name	Closest included species by ITS	Justification	Predicted Genus	Species Description
Aquamortierella elegans				Embree & Indoh, Bulletin of the Torrey Botanical Club 94: 464 (1967)
Echinochlamydo-sporium variabile		NCBI BLAST (EU688962)		X.Z. Jiang, H.Y. Yu, M.C. Xiang, X.Y. Liu & X.Z. Liu, Fungal Diversity 46: 46 (2011)
Modicella malleola	*Modicella reniformis*	Wagner *et al*. (2013)	*Modicella*	Harkn., Proceedings of the California Academy of Sciences 1 (8): 280 (1899)
Mortierella alliaceae	*Sepiachlamydospori um fimbricystis*	Wagner *et al*. (2013)	*Sepiachlamy-dosporium*	Linnem., ZentBl. Bakt. ParasitKde, Abt. 2: 225 (1953)
Mortierella angusta = *Mortierella polycephala var. angusta* = *Mortierella simplex*	*Dissophora ornata*	Wagner *et al*. (2013) NCBI BLAST (MH858055.1) at 86%	*Dissophora*	Linnem., Mucorales, eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe: 172 (1969) Linnem., Mucor.-Gatt. Mortierella Coem.: 29 (1941)
Mortierella apiculata				Marchal, Bull. Soc. R. Bot. Belg. 30(no. 2): 135 (1891)
Mortierella arachnoides				Therry & Thierry, Revue mycol., Toulouse 4(no. 15): 161 (1882)
Mortierella arcuata				E. Wolf, Zentbl. Bakt. ParasitKde, Abt. II 107: 530 (1954)

Table 2.1 (cont'd)

Mortierella armillariicola	*Linnemannia acrotona*	Wagner *et al*. (2013)	*Linnemannia*	W. Gams, Persoonia 9 (1): 128 (1976)
Mortierella baccata				E. Wolf, Zentbl. Bakt. ParasitKde, Abt. II 107: 530 (1954)
Mortierella bainieri	*Linnemannia exigua*	Wagner *et al*. (2013)	*Linnemannia*	Costantin, Bulletin de la Société Mycologique de France 4: 152 (1889)
Mortierella basiparvispora	*Entomortierella parvispora*	Wagner *et al*. (2013)	*Entomortierella*	W. Gams & Grinb., Persoonia 9 (1): 130 (1976)
Mortierella biramosa = Mortierella wuyishanensis	*Linnemannia nantahalensis*	Wagner *et al*. (2013)	*Linnemannia*	Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 110 (1875) F.J. Chen, Mycosystema 5: 57 (1992)
Mortierella calciphila	*Entomortierella beljakovae*	NCBI BLAST (KT964845.1) at 84%	*Entomortierella*	Li et al., Fungal Diversity 78: 201 (2016)
Mortierella cephalosporina				Chalab., Mikrobiol. Zh. 27: 31 (1965)
Mortierella claussenii	*Linnemannia camargensis*	Wagner *et al*. (2013)	*Linnemannia*	Linnem., Archiv für Mikrobiologie 30: 265 (1958)
Mortierella cotigans	*Linnemannia exigua*	Wagner *et al*. (2013)	*Linnemannia*	Degawa, Mycologia 90: 1040 (1998)
Mortierella decipens = Haplosporangium decipiens	*Mortierella bisporalis*	Wagner *et al*. (2013)	*Mortierella*	Björl., Botaniska Notiser 1936: 126 (1936) Thaxt., Botanical Gazette Crawfordsville 58: 363 (1914)
Mortierella delamerensis				W.Gams in GBIF Secretariat (2019)
Mortierella diffluens				Sorokin (1873)

Table 2.1 (cont'd)

Mortierella echinula	-	NCBI BLAST (MH860124.1)		Linnem., Zentbl. Bakt. ParasitKde, Abt. II 107: 229 (1953)
Mortierella elasson	*Dissophora decumbens*	NCBI BLAST (HQ630368.1) at 99%	*Dissophora*	Sideris & G.E. Paxton, Mycologia 21(4): 176 (1929)
Mortierella elongatula	*Sepiachlamydo-sporium fimbricystis*	Wagner *et al.* (2013) NCBI BLAST (NR_111582.1) at 96%	*Sepiachlamy-dosporium*	W. Gams & Domsch, Persoonia 9(1): 119 (1976)
Mortierella fatshederae	*Linnemannia exigua*	Wagner *et al*. (2013)	*Linnemannia*	Hyang B. Lee, K. Voigt & T.T.T. Nguyen, in Hyde et al., Fungal Diversity 80: 255 (2016)
Mortierella ficariae				Therry & Thierry, Revue mycol., Toulouse 4(no. 15): 161 (1882)
Mortierella fimbriata				S.H. Ou, Sinensia, Shanghai 1: 442 (1940)
Mortierella fluviae	*Linnemannia exigua*	NCBI BLAST (KX227756.1) at 99%	*Linnemannia*	Hyang B. Lee, K. Voigt & T.T.T. Nguyen, Fungal Diversity 80: 255 (2016)
Mortierella formicae	*Entomortierella beljakovae*	NCBI BLAST (KY793000.1) at 84%	*Entomortierella*	Siedlecki, in Hyde et al., Fungal Diversity 87: 222 (2017)
Mortierella formicicola	*Entomortierella beljakovae*	Wagner *et al*. (2013)	*Entomortierella*	D.S. Clark & W. Gams (?)
Mortierella formosana	*Linnemannia gamsii*	NCBI BLAST (KP744428.1) at 86%	*Linnemannia*	Ariyawansa et al., Fungal Diversity 75: 254 (2015)
Mortierella formosensis				C.Y.Chien in GBIF Secretariat (2019)
Mortierella fusispora				Tiegh., Annls Sci. Nat., Bot., sér. 6 4(4): 385 (1878) [1876]

Table 2.1 (cont'd)

Mortierella gemmifera	*Entomortierella lignicola*	Wagner *et al*. (2013)	*Entomortierella*	M. Ellis, Transactions of the British Mycological Society 24: 95 (1940)
Mortierella globalpina	*Thaxteriella minutissima*	Wagner *et al*. (2013)	*Thaxteriella*	W. Gams & Veenb.-Rijks, Persoonia 9 (1): 113 (1976)
Mortierella hepiali				Q.T. Chen & B. Liu, in Chen, Wang & Liu, Journal of Shanxi University, Natural Science 4: 70 (1986)
Mortierella heterospora				W.Gams in GBIF Secretariat (2019)
Mortierella histoplasmatoides	*Linnemannia hyalina*	Wagner *et al*. (2013)	*Linnemannia*	W. Gams, Boletus 15: 35 (1991)
Mortierella humicola				Oudem., Archives Néerlandaises 7: 276 (1902)
Mortierella humilissima				Pišpek, Acta bot. Inst. bot., Zagreb 4: 99 (1929)
Mortierella hypsicladia	*Mortierella reticulata*	NCBI BLAST (MH802523.1) at 93%	*Mortierella*	Degawa & W. Gams, Stud. Mycol. 50(2): 567 (2004)
Mortierella insignis				Linnem., Mucor.-Gatt. Mortierella Coem.: 34 (1941)
Mortierella jenkinii = Mortierella bainieri var. jenkinii	*Entomortierella parvispora*	Wagner *et al*. (2013)	*Entomortierella*	(A.L. Sm.) Naumov, Opredelitel Mukorovykh (Mucorales): 97 (1935)
Mortierella kuhlmanii	*Entomortierella echinosphaera*	Wagner *et al*. (2013)	*Entomortierella*	W. Gams, Persoonia 9 (1): 122 (1976)

Table 2.1 (cont'd)

Mortierella longigemmata	*Linnemannia hyalina*	NCBI BLAST (JX976055.1) at 94%	*Linnemannia*	Linnem., Mucorales, eine Beschreibung aller Gattungen und Arten dieser Pilzgruppe: 199 (1969)
Mortierella macrocystis = Mortierella microspora var. macrocystis	*Sepiachlamydo-sporium cystojenkinii*	Wagner *et al*. (2013)	*Sepiachlamy-dosporium*	W. Gams, Nova Hedwigia 3: 69 (1961)
Mortierella macrocystopsis	*Sepiachlamyd-osporium cystojenkinii*	Wagner *et al*. (2013)	*Sepiachlamy-dosporium*	W. Gams & Carreiro, Studies in Mycology 31: 85 (1989)
Mortierella mairei				Vuill., Bull. Soc. mycol. Fr. 34(1-2): 46 (1918)
Mortierella mehrotraensis				Baijal, Sydowia 21: 269 (1968) [1967]
Mortierella microspora				E. Wolf, Zentbl. Bakt. ParasitKde,
				Abt. II 107: 528 (1954)
Mortierella microzygospora	-	Wagner *et al*. (2013) NCBI BLAST (MH862681.1)		Degawa, Mycologia 90: 1041 (1998)
Mortierella mundensis				Linnem., Mucor.-Gatt. Mortierella Coem.: 48 (1941)
Mortierella niveovelutina				Cif. & Ashford, Porto Rico J. Publ. Health Trop. Med. 5(2): 142 (1929)
Mortierella oliogospora	*Mortierella polycephala*	Wagner *et al*. (2013)	*Mortierella*	Björl., Botaniska Notiser 1936: 121 (1936)
Mortierella ovalispora				Chalab., Mikrobiol. Zh. 27: 28 (1965)
Mortierella paraensis	*Entomortierella beljakovae*	Wagner *et al*. (2013)	*Entomortierella*	Pfenning & W. Gams, Mycotaxon 46: 287 (1993)

Table 2.1 (cont'd)

Mortierella parazychae				Degawa, in Degawa & Tokumasu, Mycologia 90(6): 1041 (1998)
Mortierella pilulifera				Tiegh., Annls Sci. Nat., Bot., sér. 6 1: 105 (1875)
Mortierella pisiformis	*Entomortierella echinosphaera*	NCBI BLAST (KP744416.1) at 87%	*Entomortierella*	H.M. Ho, S.F. Wei & K. Voigt, in Ariyawansa et al., Fungal Diversity 75: 252 (2015)
Mortierella plectoconfusa				E. Wolf, Zentbl. Bakt. ParasitKde, Abt. II 107: 527 (1954)
Mortierella pseudozygospora	-	Wagner *et al*. (2013) NCBI BLAST (JX975880.1)		W. Gams & Carreiro, Studies in Mycology 31: 87 (1989)
Mortierella pulchella	*Sepiachlamydo-sporium fimbricystis*	Wagner *et al*. (2013)	*Sepiachlamy-dosporium*	W. Gams & Domsch, Persoonia 9(1): 119 (1976)
Mortierella pusilla *= Mortierella nodosa* *= Mortierella stricta*				Oudem., Arch. néerl. Sci., Sér. 2 7: 277 (1902) E. Wolf, Zentbl. Bakt. ParasitKde, Abt. II 107: 531 (1954)
Mortierella pygmaea				Chalab., Mikrobiol. Zh. 27: 30 (1965)
Mortierella repens				A.L. Sm., J. Bot., Lond. 36: 180 (1898)
Mortierella rhizogena				Dasz., Bull. Soc. bot. Genève, 2 sér. 4: 310 (1912)
Mortierella rostafinskii	-	Wagner *et al*. (2013) NCBI BLAST (JX975885.1)		Bref., Untersuchungen aus dem Gesammtgebiete der Mykologie 4: 81 (1881)

Table 2.1 (cont'd)

Mortierella sarnyensis	*Linnemannia nantahalensis*	Wagner *et al*. (2013)	*Linnemannia*	Milko, Novosti Sistematiki Nizshikh Rastenii 10: 87 (1973)
Mortierella signyensis	*Linnemannia schmuckeri*	NCBI BLAST (JQ693160.1) at 98%	*Linnemannia*	K. Voigt, P.M. Kirk & Bridge, in Bridge & Hughes, Index Fungorum 7: 1 (2012)
Mortierella simplex (= Mortierella angusta)	*Dissophora ornata*	Wagner *et al*. (2013) NCBI BLAST (JX975870.1) at 86%	*Dissophora*	Tiegh. & G. Le Monn., Annales des Sciences Naturelles Botanique 17: 350 (1873)
Mortierella sossauensis	*Necromyco-mortierella dichotoma*	Wagner *et al*. (2013)	*Necromyco-mortierella*	E. Wolf, Zentralblatt für Bakteriologie und Parasitenkunde, Abteilung 2 107: 533 (1954)
Mortierella strangulata	-	Wagner *et al*. (2013) NCBI BLAST (JX975997.1)		Tiegh., Annales des Sciences Naturelles Botanique sér. 6, 1: 102 (1875)
Mortierella striospora				K.B. Deshp. & Mantri, Mycopath. Mycol. appl. 20: 223 (1963)
Mortierella subtilissima				Oudem., in Oudemans & Koning, Arch. néerl. Sci., Sér. 2 7: 277 (1902)
Mortierella sugadairana	-	NCBI BLAST (MF510830.1)		Y. Takash., Degawa & K. Narisawa, Mycoscience 59(3): 201 (2018)
Mortierella thereuopodae	*Linnemannia hyalina*	NCBI BLAST (AB862879.1) at 83%	*Linnemannia*	Linnem., Mucorales (Lehre): 199 (1969)

Table 2.1 (cont'd)

Mortierella tirolensis				Linnem., Mucorales (Lehre): 192 (1969)
Mortierella traversoana				Peyronel [as 'traversiana'], I germi astmosferici dei fungi con micelio, Diss. (Padova): 17 (1913)
Mortierella tsukubaensis				Ts. Watan., in Watanabe, Watanabe, Fukatsu & Kurane, Mycol. Res. 105(4): 506 (2001)
Mortierella tuberosa				Tiegh., Annls Sci. Nat., Bot., sér. 6 1: 106 (1875)
Mortierella verrucosa	*Linnemannia hyalina*	NCBI BLAST (MH878485.1) at 98%	*Linnemannia*	Linnem., Zentbl. Bakt. ParasitKde, Abt. II 107: 227 (1953)
Mortierella zonata	*Linnemannia hyalina*	Wagner *et al*. (2013)	*Linnemannia*	Linnem., Flora (Regensburg) 130: 210 (1936)

Table 2.2 – Isolate metadata

The substrate, geographic origin, collector, collection year, vouchered by, and synonymous isolate identification numbers known for each isolate used in this study.

Isolate ID	Preliminary Identification	Status	Isolated From	Geographic Origin	Year	Collected By	Vouchered By	Alternate IDs
NRRL_22416	*Dissophora decumbens*		plant, ground-up Quercus (Oak) and Acer (Maple) leaves	USA: Rhode Island		M. Carreiro	NRRL	CBS 592.88
NRRL_22417	*Dissophora ornata*		soil, in mountain forest under Weinmannia, Clusia etc., alt. 3100 m.	Colombia: Cordillera Central, Cauca en Huila, Parque Nacional del Puracé		T. van der Hammen & R. Jaramillo	NRRL	CBS 348.77, IMI 287528
NRRL_2682	*Haplosporangium sp.*		Dog dung	USA: Palo Verde, California			NRRL	NRRL A-7647
NRRL_3175	*Haplosporangium sp.*		Greenhouse soil				NRRL	NRRL A-13808
NRRL_A-10739	*Haplosporangium sp.*		Pack rat dung	USA: Vidal Junction, California			NRRL	
NRRL_A-10996	*Haplosporangium sp.*							
FSU9682	*Lichtheimia corymbifera*		Soil	Afghanistan		J.J. Curtis	CBS or NRRL	CBS 429.75, ATCC 46771, NRRL 2981

Table 2.2 (cont'd)

NRRL_3116	*Lobosporangium transversale*	ISOTYPE	Soil beneath Purshia tridentata	USA: Virginia City, Nevada			NRRL	ATCC 16960, CBS 357.67, IMI 130776, NRRL A-12901, VKM F-1384
NRRL_5525	*Lobosporangium transversale*		Soil	USA: Texas			NRRL	ATCC 18036
MES-2146	*Modicella reniformis*			Argentina		Matthew Smith		
C-ARSO24-5	*Mortierella acrotona*		Soil	USA: Arkansas		Martin Chilvers	Martin Chilvers	
14Py14W	*Mortierella alpina*		Soil	USA: Illinois		Martin Chilvers	Martin Chilvers	
AD021	*Mortierella alpina*		Rhizosphere of Pinus sp.	USA: Bryce Canyon, UT	2015	Alessandro Desirò	Gregory Bonito	
AD062	*Mortierella alpina*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
AD071	*Mortierella alpina*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
AD072	*Mortierella alpina*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
B6842	*Mortierella alpina*		human leg lesion	USA: Minnesota	2011	Andrii Gryganskyi & Greg Bonito		

Table 2.2 (cont'd)

C-ARSO21-9	*Mortierella alpina*		Soil	USA: Arkansas		Martin Chilvers	Martin Chilvers	
C-ILSO26-18	*Mortierella alpina*		Soil	USA: Illinois		Martin Chilvers	Martin Chilvers	
C-INSO22-22	*Mortierella alpina*		Soil	USA: Indiana		Martin Chilvers	Martin Chilvers	
CK1227	*Mortierella alpina*		biological soil crust	USA: Utah	2014	C. Kuske	Andrea Porras-Alfaro	
CK1249	*Mortierella alpina*		biological soil crust	USA: Utah	2014	C. Kuske	Andrea Porras-Alfaro	
CK1268	*Mortierella alpina*		biological soil crust	USA: Utah	2014	C. Kuske	Andrea Porras-Alfaro	
CK202	*Mortierella alpina*		biological soil crust	USA: Utah	2014	C. Kuske	Andrea Porras-Alfaro	
C-MICO24-19	*Mortierella alpina*		Soil	USA: Michigan		Martin Chilvers	Martin Chilvers	
GBAus31	*Mortierella alpina*		Soil	Australia		Gregory Bonito	Gregory Bonito	
KOD1002	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2155-1
KOD1005	*Mortierella alpina*		Boot Mud	USA: Illinois	2013	Andrew Miller	Andrew Miller	2178-1
KOD1012	*Mortierella alpina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2203-2
KOD1016	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2242-2
KOD1017	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2242-3
KOD1018	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2244-1

Table 2.2 (cont'd)

KOD1019	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2244-3
KOD1021	*Mortierella alpina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2253-2
KOD1022	*Mortierella alpina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2253-3
KOD1026	*Mortierella alpina*		Bat	USA: Illinois	2013	Andrew Miller	Andrew Miller	2305-1
KOD1027	*Mortierella alpina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2313-3
KOD1028	*Mortierella alpina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2327-3
KOD1045	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2624-1
KOD1046	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2642-2
KOD1047	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2645-1
KOD1054	*Mortierella alpina*		Leaf Litter	USA: Illinois	2012	Andrew Miller	Andrew Miller	1064-11
KOD1055	*Mortierella alpina*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1210-11
KOD957	*Mortierella alpina*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1650-1
KOD958	*Mortierella alpina*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1690-1
KOD967	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	1908-1
KOD983	*Mortierella alpina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	1979-2
KOD990	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2052-2
KOD994	*Mortierella alpina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2093-2

Table 2.2 (cont'd)

KOD995	*Mortierella alpina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2093-3
KOD998	*Mortierella alpina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2136-1
KOD999	*Mortierella alpina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2147-2
NDSO1-48	*Mortierella alpina*		Soil	USA: North Dakota		Martin Chilvers	Martin Chilvers	
NRRL_62971	*Mortierella alpina*		Cropfield Soil, CR700	USA: Kilbourne, Illinois		D.T. Wicklow	NRRL	ENDO 3847
NRRL_6302	*Mortierella alpina*					G. Linnemann	NRRL	CBS 250.53
NRRL_66262	*Mortierella alpina*			USA: San Nicolas Island, California			NRRL	NRRL A-10995
NRRL_A-15043	*Mortierella alpina*		Fescue hay	USA: Kentucky			NRRL	
NVP17b	*Mortierella alpina*		Truffle Fruiting Body	Italy	2015	Natalie Vandepol	Gregory Bonito	
NVP47	*Mortierella alpina*		Soil	USA: Traverse City, Michigan	2015	Natalie Vandepol	Gregory Bonito	
BC1065	*Mortierella ambigua*		fungivorous millipedes	USA: Ouachita Mountains, Arkansas	2011	Andrii Gryganskyi		
NRRL_28271	*Mortierella ambigua*					U. Schulz, Bayer	NRRL	CBS 450.88
KOD1051	*Mortierella amoeboidea*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1031-12
KOD1053	*Mortierella amoeboidea*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1059-11
KOD1030	*Mortierella antarctica*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2344-1
NRRL_28267	*Mortierella antarctica*	ISOTYPE	Soil, rock crevice near glacier	Antarctica: Hallett Station	1966	O.L. Lange	NRRL	CBS 609.70

Table 2.2 (cont'd)

NVP157	*Mortierella antarctica*		Soil	Antarctica: Hallett Station	1966	G. Linnemann & O.L. Lange	CBS	
NRRL_25716	*Mortierella aplina*		*Aspergillus flavus* sclerotium buried in soil	USA: Tifton, Georgia	1996	DT Wicklow	NRRL	
KOD1040	*Mortierella beljakovae*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2448-3
NRRL_2493	*Mortierella bisporalis*		Rotted *Populus* log with dung pellets mixed with litter	USA: Gull Lake, Michigan			NRRL	
NRRL_A-12553	*Mortierella bisporalis*		Soil under sugar maple tree	USA: Massachusetts			NRRL	
NRRL_2610	*Mortierella camargensis*						NRRL	NRRL A-7266
NRRL_28260	*Mortierella camargensis*	ISOTYPE	Sandy soil	France: Bois de Rièges, Camargue	1951	J. Nicot	NRRL	CBS 221.58
WISO4-30	*Mortierella camargensis*		Soil	USA: Wisconsin		Martin Chilvers	Martin Chilvers	
NRRL_22892	*Mortierella capitata*	ISOTYPE of *M. vesiculosa*	forest soil	India: Rishikesh			NRRL	NRRL A-12039, CBS 648.68
NRRL_28257	*Mortierella capitata*		Forest soil	India		B.S. Mehrotra	NRRL	CBS 648.68, NRRL A-12039
NRRL_5217	*Mortierella chienii*	TYPE of *M. umbellata*	Forest soil	USA: Athens, Georgia	1970	C.-Y. Chien	NRRL	ATCC 22481, CBS 124.71, IMI 158112, NRRL A-18233

Table 2.2 (cont'd)

AD033	*Mortierella chlamydospora*		Soil	USA: East Lansing, MI	2015	Alessandro Desirò, Shelby Hughey	Gregory Bonito	
NRRL_2769	*Mortierella chlamydospora*						NRRL	ATCC 42541
NRRL_2760	*Mortierella claussenii*	SYNTYPE	Soil under Castanea sativa, pH 4.7, alt. 300 m.	Switzerland: Ticino, Cavigliano, Centovalli		G. Linnemann	NRRL	CBS 294.59, NRRL A-16564, NRRL A-9140
KOD1000	*Mortierella clonocystis*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2148-1
KOD947	*Mortierella clonocystis*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1040-1
CBS456.71	*Mortierella cystojenkinii*	TYPE	Soil	Netherlands: Wageningen		J.W. Veenbaas-Rijks	CBS	
KOD1035	*Mortierella dichotoma*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2379-1
KOD1036	*Mortierella dichotoma*		Bat	USA: Illinois	2013	Andrew Miller	Andrew Miller	2395-1
KOD996	*Mortierella dichotoma*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2104-1
CBS575.75	*Mortierella echinosphaera*	HOLOTYPE	Begonia	Netherlands: Aalsmeer		A.J. van der Plaats-Niterink	CBS	IMI 242503, CBS H-7365 (holotype); CBS H-7366 (isotype)
AD022	*Mortierella elongata*		Rhizosphere of Pine	USA: Bryce Canyon, UT	2015	Alessandro Desirò	Gregory Bonito	
AD035	*Mortierella elongata*		Soil	USA: Seattle, WA	2015	Alessandro Desirò, Shelby Hughey	Gregory Bonito	

Table 2.2 (cont'd)

AD050	*Mortierella elongata*		Rhizosphere of poplar	USA: Kellogg Biological Station, KBS, Michigan	2015	Alessandro Desirò, Gian Maria Niccolò Benucci	Gregory Bonito	
AD093	*Mortierella elongata*		Soil	USA: Coatesville, PA	2015	Alessandro Desirò, Andrii Griganski, Zhen Hao	Gregory Bonito	
AG77	*Mortierella elongata*		Soil	USA: Duke Forest, Korstian Div., North Carolina			Gregory Bonito	
C-ARSO25-24	*Mortierella elongata*		Soil	USA: Arkansas		Martin Chilvers	Martin Chilvers	
C-MISO21-18	*Mortierella elongata*		Soil	USA: Michigan		Martin Chilvers	Martin Chilvers	
GBAus21	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus23	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus24	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus25	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus32	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus33	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus34	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus36	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus37	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus38	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	

Table 2.2 (cont'd)

GBAus40	*Mortierella elongata*		Soil	Australia		Gregory Bonito	Gregory Bonito	
IASO10-42-45rt	*Mortierella elongata*		Soil	USA: Iowa		Martin Chilvers	Martin Chilvers	
ILSO2-38	*Mortierella elongata*		Soil	USA: Illinois		Martin Chilvers	Martin Chilvers	
INSO1-46B2	*Mortierella elongata*		Soil	USA: Indiana		Martin Chilvers	Martin Chilvers	
KOD1006	*Mortierella elongata*		Boot Mud	USA: Illinois	2013	Andrew Miller	Andrew Miller	2179-1
KOD1007	*Mortierella elongata*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2184-3
KOD980	*Mortierella elongata*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	1976-3
KOD981	*Mortierella elongata*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	1976-4
KOD982	*Mortierella elongata*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	1979-1
KOD984	*Mortierella elongata*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	1979-3
KOD993	*Mortierella elongata*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2089-1
NRRL_5513	*Mortierella elongata*		Soil	USA: Monroe, Georgia		C.-Y. Chien	NRRL	ATCC 42661, CBS 121.71
NVP112	*Mortierella elongata*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP113	*Mortierella elongata*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP123	*Mortierella elongata*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP128	*Mortierella elongata*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	

Table 2.2 (cont'd)

NVP156	*Mortierella elongata*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP4	*Mortierella elongata*		Soil	USA: Hart, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NVP5	*Mortierella elongata*		Soil	USA: Hart, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NVP64	*Mortierella elongata*		Soil	USA: Jackson, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NVP65	*Mortierella elongata*		Soil	USA: Jackson, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NVP66	*Mortierella elongata*		Soil	USA: Grand Junction, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NVP67	*Mortierella elongata*		Soil	USA: Grand Junction, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NVP71	*Mortierella elongata*		Soil	USA: Brighton, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NVP79	*Mortierella elongata*		Soil	USA: Jackson, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NVP90	*Mortierella elongata*		Soil	USA: Michigan	2015	Natalie Vandepol	Gregory Bonito	
WISO4-29	*Mortierella elongata*		Soil	USA: Wisconsin		Martin Chilvers	Martin Chilvers	
AD058	*Mortierella epicladia*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
KOD1059	*Mortierella epicladia*		Leaf Litter	USA: Illinois	2012	Andrew Miller	Andrew Miller	1274-13
NRRL_5512	*Mortierella epigama*	ISOTYPE	Composted refuse	Germany		K.H. Domsch	NRRL	ATCC 24027, CBS 489.70

Table 2.2 (cont'd)

NRRL_28262	*Mortierella exigua*		Wheat field soil	Germany: Kiel-Kitzeberg		W. Gams	NRRL	CBS 870.68
KOD991	*Mortierella fimbricystis*		Procyon Latrine	USA: Illinois	2013	Andrew Miller	Andrew Miller	2063-1
14Py25W	*Mortierella gamsii*		Soil	USA: Illinois		Martin Chilvers	Martin Chilvers	
AD045	*Mortierella gamsii*		Rhizosphere/Roots of Spruce, Pine and Oak	USA: Lake Lansing, East Lansing, MI	2015	Alessandro Desirò	Gregory Bonito	
AD070	*Mortierella gamsii*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
C-INSO22-17	*Mortierella gamsii*		Soil	USA: Indiana		Martin Chilvers	Martin Chilvers	
C-MNSO24-13	*Mortierella gamsii*		Soil	USA: Minnesota		Martin Chilvers	Martin Chilvers	
GBAus22	*Mortierella gamsii*		Soil	Australia		Gregory Bonito	Gregory Bonito	
KOD1032	*Mortierella gamsii*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2345-2
KOD1034	*Mortierella gamsii*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2348-3
NVP60	*Mortierella gamsii*		Soil	USA: Cassopolis, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NVP61	*Mortierella gamsii*		Soil	USA: Cassopolis, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NRRL_66264	*Mortierella geracilis*		Pyrenomycete on wood	USA: Massachusetts			NRRL	NRRL A-12637
AD054	*Mortierella globulifera*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	

Table 2.2 (cont'd)

REB-010B	*Mortierella globulifera*		Soil	USA: Loblolly Pine Plantation, Duke Forest, North Carolina	2013	C. Kuske	Gregory Bonito	
AD008	*Mortierella horticola*		Rhizosphere of Allisonia sp.	New Zealand: Kelly Creek, South Island	2015	Alessandro Desirò	Gregory Bonito	
AD009	*Mortierella horticola*		Rhizosphere of Allisonia sp.	New Zealand: Kelly Creek, South Island	2015	Alessandro Desirò	Gregory Bonito	
AD012	*Mortierella horticola*		Rhizosphere of Allisonia sp.	New Zealand: Kelly Creek, South Island	2015	Alessandro Desirò	Gregory Bonito	
AD013	*Mortierella horticola*		Rhizosphere of Allisonia sp.	New Zealand: Kelly Creek, South Island	2015	Alessandro Desirò	Gregory Bonito	
AD055	*Mortierella horticola*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
CK413	*Mortierella horticola*		biological soil crust	USA: Utah	2014	C. Kuske	Andrea Porras-Alfaro	
MICO2-9	*Mortierella horticola*		Soil	USA: Michigan		Martin Chilvers	Martin Chilvers	
REB-025A	*Mortierella horticola*		Soil	USA: Loblolly Pine Plantation, Duke Forest, North Carolina	2013	C. Kuske	Gregory Bonito	
KOD1050	*Mortierella humilis*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1023-11
PMI1414	*Mortierella humilis*		Soil	USA: Massachusetts	2009	Brantlee Sprakes-Richter	Gregory Bonito	

Table 2.2 (cont'd)

AD068	*Mortierella hyalina*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
JES103	*Mortierella hyalina*							
KOD1020	*Mortierella hyalina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2246-1
KOD1023	*Mortierella hyalina*		Woody Debris	USA: Illinois	2013	Andrew Miller	Andrew Miller	2262-1
KOD1037	*Mortierella hyalina*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2429-1
KOD1067	*Mortierella hyalina*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1643-11
KOD1068	*Mortierella hyalina*		Woody Debris	USA: Illinois	2012	Andrew Miller	Andrew Miller	1670-12
KOD949	*Mortierella hyalina*		Procyon Latrine	USA: Illinois	2012	Andrew Miller	Andrew Miller	1145-2
KOD965	*Mortierella hyalina*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	1898-2
NRRL_2591	*Mortierella hyalina*		Sewage				NRRL	NRRL A-7162
NRRL_6427	*Mortierella hyalina*		Hypoxylon deustum	USA: Schoharie County, New York	1969	C.T. Rogerson	NRRL	ATCC 42665, CBS 100563, C.T.R. 69-229, NRRL A-17771
NRRL_A-12040	*Mortierella hyalina*		soil	India			NRRL	CBS 650.68
NRRL_5248	*Mortierella indohii*		Dung	USA: Athens, Georgia	1971	C.-Y. Chien	NRRL	CBS 460.75, IMI 242505

Table 2.2 (cont'd)

NRRL_2525	*Mortierella lignicola*						NRRL	NRRL A-16362
NRRL_6425	*Mortierella lignicola*						NRRL	NRRL A-16560, ATCC 42664
AD034	*Mortierella minutissima*		Soil	USA: East Lansing, MI	2015	Alessandro Desirò & Shelby Hughey	Gregory Bonito	
AD041	*Mortierella minutissima*		Rhizosphere/Roots of Spruce, Pine and Oak	USA: Lake Lansing, East Lansing, MI	2015	Alessandro Desirò	Gregory Bonito	
AD051	*Mortierella minutissima*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
AD065	*Mortierella minutissima*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
AD069	*Mortierella minutissima*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
AD077A	*Mortierella minutissima*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
KOD944	*Mortierella minutissima*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1029-1
KOD959	*Mortierella minutissima*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1695-1
NRRL_6424	*Mortierella minutissima*			Germany		G. Linnemann	NRRL	CBS 226.35, NRRL A-16546

Table 2.2 (cont'd)

NVP1	*Mortierella minutissima*		Soil	USA: Cincinnati, Ohio	2015	Natalie Vandepol	Gregory Bonito	
NRRL_6456	*Mortierella multidivaricata*	ISOTYPE	decaying stump	Russia: Sokolniki Park, Moskva			NRRL	CBS 227.78, IMI 236322, RSA 2152
RSA2512	*Mortierella multidivaricata*							
AD085	*Mortierella nantahalensis*		Soil	USA: Coatesville, PA	2015	Alessandro Desirò, Andrii Griganski, & Zhen Hao	Gregory Bonito	
NRRL_5842	*Mortierella nantahalensis*	ISOTYPE	Soil	USA: Joyce Kilmer Memorial Forest, Nantahala National Forest, North Carolina	1970	C.-Y. Chien	NRRL	ATCC 22480, CBS 610.70, IMI 158113, NRRL 5216, NRRL A-18051
AD039	*Mortierella parvispora*		Rhizosphere/Roots of Spruce, Pine and Oak	USA: Lake Lansing, East Lansing, MI	2015	Alessandro Desirò	Gregory Bonito	
KOD1056	*Mortierella parvispora*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1240-11
KOD1061	*Mortierella parvispora*		Bat	USA: Illinois	2012	Andrew Miller	Andrew Miller	1422-11
KOD1062	*Mortierella parvispora*		Bat	USA: Illinois	2012	Andrew Miller	Andrew Miller	1455-11
KOD1069	*Mortierella parvispora*		Boot Mud	USA: Illinois	2012	Andrew Miller	Andrew Miller	1777-12
NRRL_2942	*Mortierella parvispora*						NRRL	NRRL A-10895

Table 2.2 (cont'd)

KOD1052	*Mortierella polycephala*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1033-12
KOD948	*Mortierella polycephala*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1044-1
KOD968	*Mortierella polycephala*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	1913-1
KOD975	*Mortierella polycephala*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	1948-3
NRRL_22890	*Mortierella polycephala*						NRRL	NRRL A-18178
NRRL_22891	*Mortierella polycephala*						NRRL	NRRL A-18179
NRRL_28261	*Mortierella reticulata*			UK		M. Turner	NRRL	CBS 859.68
MISO4-46	*Mortierella rishikesha*		Soil	USA: Michigan		Martin Chilvers	Martin Chilvers	
NRRL_2761	*Mortierella schmuckeri*	SYNTYPE	Soil under Opuntia sp., pH 6.7	Mexico: Queretaro		G. Linnemann	NRRL	ATCC 42658, CBS 295.59, NRRL A-16570, NRRL A-9141, NRRL 6426

Table 2.2 (cont'd)

NRRL_6426	*Mortierella schmuckeri*	SYNTYPE	Soil under Opuntia sp., pH 6.7	Mexico: Queretaro		G. Linnemann	NRRL	ATCC 42658, CBS 295.59, NRRL 2761, NRRL A-16570, NRRL A-9141
NRRL_5841	*Mortierella sclerotiella*	ISOTYPE	Mouse dung	Ukraine			NRRL	ATCC 18732, CBS 529.68, IMI 133978, VKM F-1099
CBS811.68	*Mortierella selenospora*	TYPE	mushroom compost with *Entomophthora coronata* & *Aphanocladium album*	Netherlands: Horst	1968	Proefstation v.d. Champignoncultuur	CBS	
KOD1015	*Mortierella selenospora*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2229-1
14Py07W	*Mortierella sp.*		Soil	USA: Illinois		Martin Chilvers	Martin Chilvers	
14Py31W	*Mortierella sp.*		Soil	USA: Illinois		Martin Chilvers	Martin Chilvers	
14Py45W	*Mortierella sp.*		Soil	USA: Illinois		Martin Chilvers	Martin Chilvers	
14UC	*Mortierella sp.*							
AD010	*Mortierella sp.*		Rhizosphere of Allisonia sp.	New Zealand: Kelly Creek, South Island	2015	Alessandro Desirò	Gregory Bonito	

Table 2.2 (cont'd)

AD011	*Mortierella sp.*		Rhizosphere of Allisonia sp.	New Zealand: Kelly Creek, South Island	2015	Alessandro Desirò	Gregory Bonito	
AD014	*Mortierella sp.*		Rhizosphere of Allisonia sp.	New Zealand: Kelly Creek, South Island	2015	Alessandro Desirò	Gregory Bonito	
AD031	*Mortierella sp.*		Soil	USA: Belleville-Woods, MI	2015	Alessandro Desirò, Shelby Hughey	Gregory Bonito	
AD032	*Mortierella sp.*		Soil	USA: Chandler Crossing, MI	2015	Alessandro Desirò, Shelby Hughey	Gregory Bonito	
AD060	*Mortierella sp.*		Rhizosphere/Roots of Spruce and Pine	USA: Sleepy Hollow State Park, Laingsburg, MI	2015	Alessandro Desirò	Gregory Bonito	
AD078	*Mortierella sp.*		Soil	USA: Coatesville, PA	2015	Alessandro Desirò, Andrii Griganski, Zhen Hao	Gregory Bonito	
AD084	*Mortierella sp.*		Soil	USA: Coatesville, PA	2015	Alessandro Desirò, Andrii Griganski, Zhen Hao	Gregory Bonito	
AD094	*Mortierella sp.*		Soil	USA: Coatesville, PA	2015	Alessandro Desirò, Andrii Griganski, Zhen Hao	Gregory Bonito	
GBAus27B	*Mortierella sp.*	TYPE	Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus30	*Mortierella sp.*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus35	*Mortierella sp.*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus39	*Mortierella sp.*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus41	*Mortierella sp.*		Soil	Australia		Gregory Bonito	Gregory Bonito	

Table 2.2 (cont'd)

GBAus42	*Mortierella sp.*		Soil	Australia		Gregory Bonito	Gregory Bonito	
GBAus43	*Mortierella sp.*		Soil	Australia		Gregory Bonito	Gregory Bonito	
KOD1001	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2148-2
KOD1003	*Mortierella sp.*		Woody Debris	USA: Illinois	2013	Andrew Miller	Andrew Miller	2159-1
KOD1004	*Mortierella sp.*		Leaf Litter	USA: Illinois	2013	Andrew Miller	Andrew Miller	2166-1
KOD1008	*Mortierella sp.*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2188-1
KOD1009	*Mortierella sp.*		Bat	USA: Illinois	2013	Andrew Miller	Andrew Miller	2192-1
KOD1010	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2200-1
KOD1013	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2215-1
KOD1014	*Mortierella sp.*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2217-1
KOD1024	*Mortierella sp.*		Boot Mud	USA: Illinois	2013	Andrew Miller	Andrew Miller	2272-1
KOD1025	*Mortierella sp.*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2283-2
KOD1029	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2339-1
KOD1033	*Mortierella sp.*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2348-1
KOD1038	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2429-3
KOD1039	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2430-1
KOD1041	*Mortierella sp.*		Woody Debris	USA: Illinois	2013	Andrew Miller	Andrew Miller	2474-1

Table 2.2 (cont'd)

KOD1042	*Mortierella sp.*		Woody Debris	USA: Illinois	2013	Andrew Miller	Andrew Miller	2474-2
KOD1043	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2511-1
KOD1044	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2548-1
KOD1048	*Mortierella sp.*		Bat	USA: Illinois	2012	Andrew Miller	Andrew Miller	1012-11
KOD1049	*Mortierella sp.*		Bat	USA: Illinois	2012	Andrew Miller	Andrew Miller	1012-13
KOD1057	*Mortierella sp.*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1252-11
KOD1063	*Mortierella sp.*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1518-11
KOD1064	*Mortierella sp.*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1518-13
KOD1065	*Mortierella sp.*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1562-12
KOD943	*Mortierella sp.*		Bat	USA: Illinois	2012	Andrew Miller	Andrew Miller	1014-2
KOD945	*Mortierella sp.*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1033-2
KOD950	*Mortierella sp.*		Leaf Litter	USA: Illinois	2012	Andrew Miller	Andrew Miller	1274-2
KOD951	*Mortierella sp.*		Leaf Litter	USA: Illinois	2012	Andrew Miller	Andrew Miller	1274-3
KOD954	*Mortierella sp.*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1479-3
KOD955	*Mortierella sp.*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1550-1
KOD956	*Mortierella sp.*		Cave wall	USA: Illinois	2012	Andrew Miller	Andrew Miller	1611-3
KOD960	*Mortierella sp.*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1695-2

Table 2.2 (cont'd)

KOD963	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	1894-2
KOD964	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	1897-1
KOD969	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	1922-3
KOD971	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	1929-1
KOD972	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	1930-1
KOD979	*Mortierella sp.*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	1976-1
KOD988	*Mortierella sp.*		Cave wall	USA: Illinois	2013	Andrew Miller	Andrew Miller	2035-1
KOD989	*Mortierella sp.*		Soil	USA: Illinois	2013	Andrew Miller	Andrew Miller	2039-3
KOD992	*Mortierella sp.*		Woody Debris	USA: Illinois	2013	Andrew Miller	Andrew Miller	2066-3
NRRL_1458	*Mortierella sp.*		Plant leaf				NRRL	ATCC 56653,
								Blakeslee C1066
NRRL_1617	*Mortierella sp.*						NRRL	
NRRL_22995	*Mortierella sp.*		Sclerotium of Aspergillus flavus buried in cornfield soil	USA: IRVSF, Kilbourne, Illinois	1994	DT Wicklow	NRRL	
NRRL_25721	*Mortierella sp.*		Aspergillus flavus sclerotium buried in field soil	USA: IRVSF, Kilbourne, Illinois	1996	DT Wicklow	NRRL	
NRRL_A-12867	*Mortierella sp.*		Soil	USA: Austin, Texas			NRRL	
NVP103	*Mortierella sp.*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	

Table 2.2 (cont'd)

NVP105	*Mortierella sp.*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP106	*Mortierella sp.*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP125	*Mortierella sp.*		Soil	Fiji	2016	Natalie Vandepol	Gregory Bonito	
NVP130	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP131	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP132	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP133	*Mortierella sp.*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP134	*Mortierella sp.*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP137	*Mortierella sp.*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP138	*Mortierella sp.*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP139	*Mortierella sp.*		Soil	Uganda	2016	Natalie Vandepol	Gregory Bonito	
NVP144	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP145	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP146	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP147	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP148	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP149	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	

Table 2.2 (cont'd)

NVP150	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP151	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP153	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP154	*Mortierella sp.*		Soil	Australia	2016	Natalie Vandepol	Gregory Bonito	
NVP3	*Mortierella sp.*		Soil	USA: Cincinnati, Ohio	2015	Natalie Vandepol	Gregory Bonito	
NVP41	*Mortierella sp.*		Soil	USA: Detroit, Michigan	2015	Natalie Vandepol	Gregory Bonito	
NVP85	*Mortierella sp.*		Soil	USA: Hamilton, Ohio	2015	Natalie Vandepol	Gregory Bonito	
NVP8B	*Mortierella sp.*		Soil	USA: Los Gatos, California	2015	Natalie Vandepol	Gregory Bonito	
PMI86	*Mortierella sp.*		Populus deltoides roots	USA: North Carolina	2011	Khalid Hameed & Gregory Bonito	Gregory Bonito	
14Py58W	*Mortierella sp.*		Soil	USA: Illinois		Martin Chilvers	Martin Chilvers	
NRRL_28272	*Mortierella stylospora*	ISOTYPE	Soil, sandy loam	Australia: Victoria			NRRL	CBS 211.32, IMI 038599
AD003	*Mortierella verticillata*		Rhizosphere of Allisonia sp.	New Zealand: Kelly Creek, South Island	2015	Alessandro Desirò	Gregory Bonito	
AD079	*Mortierella verticillata*		Soil	USA: Coatesville, PA	2015	Alessandro Desirò, Andrii Griganski, Zhen Hao	Gregory Bonito	
AD086	*Mortierella verticillata*		Soil	USA: Coatesville, PA	2015	Alessandro Desirò, Andrii Griganski, Zhen Hao	Gregory Bonito	

Table 2.2 (cont'd)

AD092	*Mortierella verticillata*		Soil	USA: Coatesville, PA	2015	Alessandro Desirò, Andrii Griganski, Zhen Hao	Gregory Bonito	
CK281	*Mortierella verticillata*		Soil	USA: Loblolly Pine Plantation, Duke Forest, North Carolina	2014	C. Kuske	Andrea Porras-Alfaro	
KOD952	*Mortierella verticillata*		Soil	USA: Illinois	2012	Andrew Miller	Andrew Miller	1476-3
NRRL_2611	*Mortierella verticillata*						NRRL	NRRL A-7267
NRRL_6337	*Mortierella verticillata*		Sandy forest soil	England: Freshfield, Lancashire		S.T. Williams	NRRL	CBS 131.66, NRRL A-16547
NRRL_6338	*Mortierella verticillata*		Sandy forest soil	England: Freshfield, Lancashire		S.T. Williams	NRRL	CBS 130.66, NRRL A-16548
NRRL_6369	*Mortierella verticillata*		Soil	Canada: Great Bear Lake, Northwest Territories	1969	E.E. Butler	NRRL	CBS 100561, NRRL A-6111
TTC192	*Mortierella verticillata*		Soil	USA: Loblolly Pine Plantation, Duke Forest, North Carolina	2013	C. Kuske	Gregory Bonito	
NRRL_6351	*Mortierella wolfii*		Lung from cow dying of mycotic pneumonia	New Zealand			NRRL	NRRL A-18027
NRRL_66265	*Mortierella wolfii*					B.S. Mehrotra	NRRL	NRRL A-12631

Table 2.2 (cont'd)

NRRL_2592	*Mortierella zychae*		Dead wood	USA: Brownfield Wood, Urbana, Illinois			NRRL	NRRL A-7087
CBS277.49	*Mucor circinelloides*					A.F. Blakeslee	CBS	ATCC 1216b, MUCL 15438, NRRL 3631
AG13-4	*Umbelopsis sp.*		Soil	USA: North Carolina			Gregory Bonito	
AG#	*Umbelopsis ramanniana*					Andrii Gryganskyi		

Table 2.3 – LCG assembly statistics

The LCG assembly statistics, BUSCO analysis on the fungi_odb9 dataset which contained 290 single-copy marker genes, protein ortholog detection, number of markers used out of 434 total, and assembly deposition/accession details. Sample identifications were adjusted to correct misidentified samples or outdated taxonomy. “REF” indicates reference *de novo* genomes that were used to guide LCG sequence analysis, which are best accessed by BioSample number.

Sample Name	Contig							BUSCO Marker Percentage				Assembly			
	#	Mbp	Min	Max	Med-ian	L50	N50	Full	Sin-gle	Dup-licate	Frag-ment	Cov-erage	Mark-ers Used	BioSample	Assembly Accession
Dissophora ornata NRRL_22417	4002	38.4	500	123321	3612	467	23711	94.5	82.4	12.1	4.8	14.9	408	SAMN05720457	JAAAUD000000000
Gamsiella multidivaricata NRRL_6456	3523	36.0	500	118702	4952	469	23035	91.4	79.7	11.7	6.6	11.7	404	SAMN05720499	JAAAIT000000000
Lobos-porangium transversale NRRL_3116	6585	35.4	500	70116	2722	895	10948	81.4	75.2	6.2	12.1	15.1	417	SAMN05720431	Superceded by REF genome
Lobos-porangium transversale NRRL_5525	6418	35.6	500	128939	2737	864	11602	79.7	71.4	8.3	12.8	16.3	388	SAMN06281768	JAAAHT000000000
Modicella reniformis MES-2146	12478	43.6	500	267961	2062	2102	5999	81.7	75.5	6.2	14.5	17.2	368	SAMN05720516	JAAAHW000000000
Mortierella alpina AD071	1601	35.1	499	336245	5760	164	64418	96.8	83.4	13.4	2.1	13.2	416	SAMN05720461	JAAAHX000000000
Mortierella alpina AD072	2684	37.3	500	174171	6707	352	30823	95.2	82.8	12.4	3.4	11.1	411	SAMN05720462	JAAAUT000000000
Mortierella alpina CK1249	4026	36.3	500	92935	4968	605	18393	94.4	83.4	11	4.5	8.5	403	SAMN05720518	JAAAHY000000000
Mortierella alpina GBAus31	2501	37.2	500	216994	5988	311	36335	96.8	83.4	13.4	1.7	12.2	416	SAMN05720773	JAAAHZ000000000

Table 2.3 (cont'd)

Mortierella alpina NRRL_66262	1181	37.7	500	458088	5288	114	101414	98	81.4	16.6	0.7	34	415	SAMN10361219	JAABKD000000000
Mortierella alpina NVP157	2186	35.5	500	272146	4268	226	47442	96.5	83.1	13.4	2.1	11	409	SAMN05720774	JAAAIB000000000
Mortierella ambigua BC1065	2284	37.9	498	238780	3660	243	48912	97.9	96.2	1.7	1	25.7	415	SAMN09074672	JAAAJB000000000
Mortierella ambigua BC1291	2279	37.6	500	252519	4804	262	44814	97.3	95.9	1.4	2.4	22.6	414	SAMN09074671	JAAAUS000000000
Mortierella ambigua NRRL_28271	2034	35.1	500	259283	5340	227	47393	96.9	94.5	2.4	2.4	12.7	411	SAMN05720519	JAAAIA000000000
Mortierella beljakovae KOD1040	4906	37.8	500	114383	3110	598	18574	93.7	83.4	10.3	3.8	13.1	407	SAMN05720775	JAAAVF000000000
Mortierella camargensis NRRL_2610	2797	44.9	491	271031	7635	377	36446	96.2	83.1	13.1	2.1	15.6	414	SAMN05727885	JAAAUF000000000
Mortierella chlamydospora AD033	3653	39.0	499	149264	5051	479	24358	94.5	82.4	12.1	3.4	16.9	408	SAMN05720793	JAAAIC000000000
Mortierella chlamydospora NRRL_2769	5304	38.2	500	76672	4037	810	14167	93.4	83.4	10	4.8	10.7	400	SAMN05720521	JAAAID000000000
Mortierella clonocystis AM1000	2928	41.4	500	208146	6033	376	33105	94.8	83.4	11.4	3.4	13.8	408	SAMN05720794	JAAAIE000000000
Mortierella clonocystis KOD947	3048	39.5	500	157240	5381	392	31100	96.5	85.5	11	2.8	13.3	410	SAMN05720795	JAAAIF000000000
Mortierella cystojenkinii CBS456.71	3044	42.9	291	200391	4581	346	37152	95.2	83.8	11.4	3.8	14.6	411	SAMN05720522	JAAAIG000000000
Mortierella elongata GBAus34	3514	46.9	501	111933	7016	494	28149	95.9	83.8	12.1	2.8	12.6	415	SAMN05720796	JAAAUV000000000

Table 2.3 (cont'd)

Mortierella elongata GBAus40	4256	46.8	500	122649	6079	611	22018	94.8	84.8	10	3.8	10.8	405	SAMN05720440	JAAAIH000000000
Mortierella elongata NVP5	5336	46.0	500	123362	5213	884	15830	92.1	83.1	9	5.9	10.1	404	SAMN05720527	JAAAII000000000
Mortierella elongata NVP71	6092	45.6	500	89460	4818	1061	13229	91	83.4	7.6	6.2	10.8	391	SAMN05720528	JAAAUW000000000
Mortierella epicladia AD058	3610	40.8	500	181942	6369	543	22197	92.7	85.5	7.2	5.5	11.6	406	SAMN05720441	JAAAIJ000000000
Mortierella epicladia KOD1059	2579	38.1	500	152774	7478	363	31490	91.4	79.7	11.7	6.2	12.1	410	SAMN05720442	JAAAIK000000000
Mortierella epigama NRRL_5512	3903	32.9	500	81695	5327	649	15510	94.4	87.2	7.2	3.1	12.6	408	SAMN05720443	JAAAVE000000000
Mortierella exigua NRRL_28262	5084	48.1	500	123319	4776	732	19879	94.5	84.8	9.7	3.8	9.5	398	SAMN05720535	JAAAIL000000000
Mortierella gamsii AD045	3359	49.3	500	305885	6658	430	33479	95.5	81	14.5	2.4	12.2	412	SAMN05720529	JAAAIM000000000
Mortierella gamsii NVP60	6418	49.5	500	166065	4282	967	15258	93.8	85.9	7.9	4.1	10.3	400	SAMN05720530	JAAAIN000000000
Mortierella globulifera AD054	4204	38.2	500	114911	4447	560	19603	93.5	80.7	12.8	4.1	10	400	SAMN05720444	JAAAIO000000000
Mortierella globulifera REB-010B	2632	39.2	500	200293	5893	303	37013	95.9	83.1	12.8	3.1	15.5	410	SAMN05720531	JAAAIP000000000
Mortierella horticola AD009	3587	40.0	500	118901	5989	510	23161	92.4	83.8	8.6	6.9	10.2	395	SAMN05720532	JAAAIQ000000000
Mortierella horticola CK413	2108	40.4	500	256907	5327	222	56369	96.9	85.5	11.4	1.4	14.9	409	SAMN05720445	JAAAUX000000000

Table 2.3 (cont'd)

Mortierella humilis KOD1050	2079	29.2	554	102834	10992	551	16383	79	72.8	6.2	3.8	12.5	354	SAMN05720536	JAAAIR000000000
Mortierella hyalina NRRL_2591	1564	46.1	500	535183	2879	102	128808	97.3	82.1	15.2	1	38.5	416	SAMN10361082	JAAAXW000000000
Mortierella lignicola NRRL_2525	4029	35.3	501	100968	4138	530	19168	92.8	82.8	10	5.2	16.3	403	SAMN05720451	JAAAUG000000000
Mortierella minutissima AD069	3215	39.4	500	134544	4516	389	30349	95.6	86.6	9	3.1	14.5	416	SAMN05720476	JAAAIS000000000
Mortierella minutissima NVP1	3345	39.0	477	132954	4234	410	29770	96.2	85.9	10.3	2.1	14.2	412	SAMN05720446	JAAAUY000000000
Mortierella polycephala KOD948	2595	32.6	500	148289	4233	303	31731	95.1	83.4	11.7	2.4	12.3	408	SAMN05720452	JAAAJA000000000
Mortierella schmuckeri NRRL_6426	3515	46.1	500	124421	7322	542	25996	96.6	85.2	11.4	2.1	11.3	405	SAMN05720483	JAAAUQ000000000
Mortierella selenospora KOD1015	8632	40.5	500	66104	2780	1396	8341	87.9	81.7	6.2	11.7	9.5	389	SAMN05720454	JAABOA000000000
Mortierella sp. 14UC	2793	45.2	500	223587	6512	341	39533	95.5	84.1	11.4	2.4	12.4	411	SAMN05720455	JAAAUP000000000
Mortierella sp. AD010	4436	38.6	500	105019	4067	581	18859	94.8	83.1	11.7	4.5	14.4	407	SAMN05720791	JAAAUR000000000
Mortierella sp. AD011	4450	38.6	500	120180	4019	592	19053	94.8	84.5	10.3	4.5	13.5	411	SAMN05720798	JAAAVD000000000
Mortierella sp. AD031	3076	44.8	500	176793	5604	364	37102	95.5	85.5	10	2.8	12.5	416	SAMN05720799	JAAAUL000000000
Mortierella sp. AD032	4189	49.3	500	108262	5304	568	26892	95.2	86.9	8.3	3.1	10.4	411	SAMN05720491	JAAAIU000000000
Mortierella sp. AD094	5229	43.8	500	149436	3238	609	20836	93.4	81.7	11.7	5.2	13.5	414	SAMN05720438	JAAAUZ000000000
Mortierella sp. AM989	4302	40.2	498	192044	2533	404	28934	94.1	80.3	13.8	3.8	17.8	406	SAMN05720439	JAAAUM000000000

Table 2.3 (cont'd)

Mortierella sp. GBAus30	1938	37.0	497	204409	6156	222	51412	95.9	79.3	16.6	2.4	18.6	409	SAMN05720448	JAAAIV000000000
Mortierella sp. GBAus35	3099	44.8	500	165087	5608	382	35220	95.2	85.5	9.7	3.4	11.3	411	SAMN05720449	JAAAUN000000000
Mortierella sp. GBAus39	4038	50.9	499	190274	4665	455	32930	95.8	85.5	10.3	2.8	10.6	414	SAMN05720493	JAAAUO000000000
Mortierella sp. GBAus43	4063	39.2	500	149293	2139	343	33168	96.9	85.2	11.7	1.7	12.6	404	SAMN05720494	JAAAIW000000000
Mortierella sp. KOD1030	3767	38.8	500	159479	3974	457	25173	94.5	85.2	9.3	4.1	13.2	411	SAMN05720520	JAAAUU000000000
Mortierella sp. NRRL_3175	3995	36.1	500	91252	4133	515	20648	93.8	85.2	8.6	4.8	16.8	406	SAMN05727888	JAAAUH000000000
Mortierella sp. NRRL_A-10739	1697	33.5	500	216595	5245	182	56943	96.2	82.4	13.8	2.8	17.6	410	SAMN05727889	JAAAUI000000000
Mortierella sp. NRRL_A-10996	1626	33.5	500	304264	5338	170	59832	95.9	81.4	14.5	3.1	17.1	412	SAMN05727887	JAAAUJ000000000
Mortierella sp. NRRL_A-12553	11106	39.0	500	105785	1297	1162	8506	81.7	74.1	7.6	15.2	13.5	382	SAMN05727890	JAAAUE000000000
Mortierella sp. NVP41	2509	45.1	500	353893	4783	266	51498	97.2	84.8	12.4	1.4	14.3	419	SAMN05720450	JAAAIX000000000
Mortierella sp. NVP85	5178	42.8	490	199404	2911	548	21933	95.2	83.8	11.4	3.8	13.2	405	SAMN05720496	JAAAVA000000000
Mortierella verticillata AD079	3481	39.7	500	131992	5436	475	24790	94.8	84.1	10.7	3.8	14.9	407	SAMN07687489	JAAAVB000000000
Mortierella verticillata NRRL 2611	3118	39.9	500	185175	5614	402	29432	96.9	85.5	11.4	2.4	11.9	410	SAMN05720458	JAAAUK000000000
Mortierella verticillata TTC192	3609	42.1	500	260596	5928	509	24172	93.4	83.1	10.3	5.5	13.1	408	SAMN07687234	JAAAVC000000000
Mortierella wolfii NRRL 66265	1088	34.1	500	432786	7590	110	90211	98.3	96.2	2.1	1.4	16.8	406	SAMN05720778	JAAAIZ000000000

Table 2.3 (cont'd)

Mortierella wolfii NRRL_6351	1180	34.2	493	534714	5458	113	93672	98.3	96.2	2.1	1.4	14.7	403	SAMN05720777	JAAAIY000000000
Mortierella zychae NRRL_2592	1260	45.7	500	611785	5298	120	120966	97.3	82.1	15.2	1.4	33.5	412	SAMN10361244	JAAAHU000000000
Mortierella zychae PUST_F9C	2068	44.8	1002	257400	10265	282	46863	93.8	79.7	14.1	4.8	17.4	410	SAMN11510820	JAAAHV000000000
REF: *Lobo-sporangium transversale* NRRL_3116	138	42.8	1957	1854208	182234	22	672590	92	80.3	11.7	3.8	-	417	SAMN05421885	N/A
REF: *Mortierella alpina* B6842	838	39.3	1000	745939	9779	82	144249	96.2	78.3	17.9	1.4	-	416	SAMN02370960	N/A
REF: *Mortierella elongata* AG-77	473	50.0	1003	1529192	5302	31	518384	96.9	81	15.9	2.1	-	418	SAMN02745706	N/A
REF: *Mortierella humilis* PMI1414	523	36.2	1152	456461	49981	99	118088	87.6	75.2	12.4	1.7	-	376	SAMN06266088	N/A
REF: *Mortierella* sp. GBAus27b	140	45.0	1029	2979878	118251	15	820600	97.9	83.8	14.1	0.3	-	413	SAMN06310397	N/A
REF: *Mortierella verticillata* NRRL 6337	56	41.9	5293	5074928	17244	6	2912254	96.2	83.1	13.1	1.4	-	417	SAMN00699802	N/A

Table 2.4 – Primer sets produced by the MGP locus selection pipeline

All primer sets produced by the MGP locus selection pipeline. Status indicates which tests the primer set has passed: *in silico* = simulated PCR in IPCRESS; *in vitro* = amplification & sequencing of each independent primer set using genomic DNA from a panel of isolates; *in vivo* = multiplex PCR and sequencing to generate mutli-gene phylogenetic data. Failure at each stage came in the form of 1) non-specific *in silico* "amplification", 2) off target *in vitro* amplification or failure to amplify across the panel of isolates, and 3) *in vivo* MGP sequence data analysis revealing selective pressure or potential gene duplication of that locus.

Primer Set Status	ExonID in *M. elongata* AG77 (gene_exon)	Size (bp)	Putative Gene Identification	Forward Primer				Reverse Primer			
				Sequence (5' to 3')	Len (bp)	GC %	Tm °C	Sequence (5' to 3')	Len (bp)	GC %	Tm °C
Used	**11759_6**	1481	RPB1 largest subunit	TCACGWCCTCCCATGGCGT	19	63	55.4	AAGGAGGGTCGTCTTCGTGG	20	60	55.9
	1870_4_1	1693	Xanthine dehydrogenase	GTGGTCAGGAGCAGTTCTACC	21	57	56.3	ATGCGCTCRGGWGTGGCAG	19	63-68	55.4-57.6
	2451_3	1932	Calcium-translocating P-type ATPase	CTGGAACTGCAAGAACTTGC	20	50	51.8	GGYGTSAGTATCTACGAGGA	20	50-55	51.8-53.8
	3770_1	1320	EF-1alpha	CTTGCCACCCTTGCCATCG	19	63	55.4	AACGTCGTCGTTATCGGACAC	21	52	54.4
	5512_3	1606	Glycosyltransferase Family 21 protein	TTCTTCTCRGTCACAGTCTTGAC	23	43-48	53.5-55.3	TTGAGAGAGAGGTCMGSAGCA	21	52-57	54.4-56.3
	4955_7	747	Hypothetical protein, DNA replication licensing factor, MCM5 component	ATACGGATRATRGCYTCCAGCTG	23	43-57	53.5-58.8	GAAGCTTCGTGGAGATATCAACG	23	48	55.3
Passed *in silico*	**10927_4**	2000	Class V Myosin motor head	TCTCGAACGAWCCTGCATCCT	21	52	54.4	AACAAGGCHCGCAAGGAGCT	20	55-60	53.8-55.9
Passed *in vitro*	**2175_2**	1400	CTP synthase	TGAACGAYGGTGGWGAGGT	19	53-58	51.1-53.2	GCCAWCTCGACAATCTCCA	19	53	51.1
	370_5	980	Acyl-CoA oxidase	ATCAACTACCCCATGGTCCA	20	50	51.8	GCTCAGWCTGRGCCTTRTCC	20	55-65	53.8-57.9
Failed *in vivo*	**4121_2**	950	Hypothetical protein, Amino acid transporters	ACAAAGATCAASGWGCAGTTGCC	23	48	55.3	TGGGTGGVTGGATCGGWGTC	20	60-65	55.9-57.9

Table 2.4 (cont'd)

	5401_4	1400	onanonoxo-7-onima-8-eninoihtemlysoneda	CSAGACGCCAGCATCCACT	19	63-65	55.4	GTSAACAGCCCYATCATGTC	20	50-55	51.8-53.8
	5491_1	944	delta-12 fatty acid desaturase	GCATGGTAGAAYGGCATCTG	20	50-55	51.8-53.8	CCCGAGTTCACSATCAAGGA	20	55	53.8
	615_2	1560	chitin synthase	TTGGCCATGTTGTGSCGCTTGG	22	59	58.6	CGGCATGGGDTACTAYTTCAACG	23	48-57	55.3-58.8
Passed *in silico* Failed *in vitro*	10616_4	1005		TYTCGTTGATRTCGGGCTTGTTG	23	43-52	53.5-57.1	CCTTCAGYAACCAGGACAAGTG	22	50-55	54.8-56.7
	2714_3-_2	1615		GACATRCCGACYTGCTGGAC	20	55-65	53.8-57.9	CCAAAAGATTGTCAAGAAGCACGAC	25	44	56
	4725_7	2020		TAGTAGTADGGRCGGTCAAAGAC	23	43-52	53.5-57.1	CTGGTACTTCCCTCTSTGGCA	21	57	56.3
	5489_5	1160		CCTCACAGAGCAGSACCAGA	20	60	55.9	AGCGMGASTGGGAGATTGAC	20	55-60	53.8-55.9
	5925_8_3	1275		CTTGKTGATGCGCTTGATACC	21	48-52	52.4-54.4	GAGATCAAGMGRTTCGAGGA	20	45-55	49.7-53.8
	615_3-_2	730-820		AGTTGATCCAGCGACGACGCT	21	57	56	CCCGGWAACCGWGGAAAGC	19	63	55.4
	662_2	800		GTCTTGACACCRCAYTGGAAYTT	23	43-52	53.5-57.1	ACCTCCAAGGAYCTSACCAT	20	50-55	51.8-53.8
	7496_4	2000		ATCTCGGGKGTCTCGAACGA	20	55-60	53.8-55.9	CTCGAGGGAAAAGTGGTGGA	20	55	53.8
	959_4	1317		AGAAYCACGGCATCAACTGGT	21	48-52	52.4-54.4	ACGACTTYTGRGCAGCGTT	19	47-58	48.9-53.2
Passed *in silico* Un-tested *in vitro*	10278_1	661		TGGTARACATCRGCRTGCTC	20	45-60	49.7-55.9	TCGCCAARTCCTCMAAGGTCGT	22	50-55	54.8-58.6
	1296_2	2000-2100		GGATGGCTGTTYTGRTGHGCCTG	23	52-65	57.1-62.4	ATCTGGGACACDGAGCTYGCCTG	23	57-65	58.8-62.4
	1326_1	1100		GACTGRATRCMCTTGTAGAA	20	35-50	45.6-51.8	TGCTGCCCMGGAATGTTCAA	20	50-55	51.8-53.8
	1672_3	725		AACATMTACTCGCTCAAYTGGTGG	24	42-50	54-57.4	ACTTGSCGCTTGTAYTCSTCGT	22	50-55	54.8-56.7
	1777_5	1243		TGGGMTCKGCTATYAAGAACAAGG	24	42-54	54-59.1	AGGTACTCCATCGAGAACTCGC	22	55	56.7

Table 2.4 (cont'd)

	180_1	1900		GAAGCATCTTYTTG GCMGCRATCAT	25	40-52	54.4-59.3	TCCATCTCGGACYT GACCACAAC	23	52-57	57.1-58.8
	1830_4	763		GACTGGACYAAGAA GGGAGAGG	22	55-59	56.7-58.6	TGACWGCRTCCATC TTGTCGCG	22	55-59	56.7-58.6
	1870_4_2	1511		AAGGCCCARTGGTT CCGCC	19	63-68	55.4-57.6	GTCTGGCCCTCAAA GACCTTG	21	57	56.3
	2281_4	1071-1100		TCATCCTCRTCCAT MGACTC	20	45-60	49.7-55.9	TCRTTCTTGACBATT CGCAC	20	40-50	47.7-51.8
	2290_4	1170		TACCCWTACCTYCA GAAGCG	20	50-55	51.8-53.8	ACTCATCTTCTTCTT CTCGCG	21	48	52.4
	2463_6-_4	1480		TCCTCMARCCTCYT CCACTC	20	50-65	51.8-57.9	GAACAYASCATGGA GATGCCC	21	52-57	54.4-56.3
	2486_4	2045		GCRTTGTTCATRTT GGGGAC	20	45-55	49.7-53.8	GATATYCTGACCAA RCGCGA	20	45-55	49.7-53.8
Passed *in silico*	2561_2	1200		TGGGGCTCYATCTT TGGWTTCTTG	24	46-50	55.7-57.4	ACCATGTCGACGGM CTGVGAGCA	23	57-65	58.8-62.4
Un-tested *in vitro*	2575_2	1700		TCDTCAATKCCMGA CCARTACTT	23	35-52	49.9-57.1	GCVCCVTTCTGCAT GAAYATG	21	43-57	50.5-56.3
	313_2	890		CGTCGTCGTACTTG TAGAA	19	47	48.9	GGMGTCGTCTTTAT CAAGTTC	21	43-48	50-52
	313_3	840		TCCTGCRACYTCTC CDGCRACCAT	24	50-63	57.4-62.5	TGGCSCACGACCAY GARATCAT	22	50-59	54.8-58.6
	3998_2	2009		AGCGAAGARGARGA RGAGTC	20	45-60	49.7-55.9	GAGCTGGTATCRAT CTGGAC	20	50-55	51.8-53.8
	4269_2	1063		CTGCTCGTCCCGTT ATYTCCGT	21	55-59	56.7-58.6	GTCTTGGGGGCCTT CTTGGCA	21	62	58.3
	4352_3	1600		AAGCAGCCKCGMG TCATCTC	20	55-65	53.8-57.9	KGCAAARTCVTCGG GGTC	18	50-67	48-54.9
	4352_3_2	1500		AAGCAGCCKCGMG TCATCTC	20	55-65	53.8-57.9	AGVACCTCDGCACG CTC	17	59-71	49.5-54.3
	4690_5	2244		CCTTGGAGATGCAC ATRAAGC	21	48-52	52.4-54.4	TTTCGATTGTWCGC ACCAAGGA	22	45	53
	5431_1	1141		ACATYCACTCKCGC ACCTTCTC	22	50-59	54.8-58.6	ACCTCGGCCTKGAA GACCTC	20	60-65	55.9-57.9

Table 2.4 (cont'd)

Passed *in silico* Un-tested *in vitro*	5535_5	665		AACTTCTCDGGRCGSGTCGA	20	55-65	53.8-57.9	TACTTCYTKGGHGAYGCCA	19	42-63	46.8-55.4
	5557_5-_6	1860		CAGTTYCTSTACCGVCCCTT	20	50-60	51.8-55.9	CCTTGTGSGGCTCGTGCA	18	67	54.9
	5910_1	812		CCAAGGTCGGYCAGAAYGCC	20	60-70	55.9-60	GAAGATRCCKCCRATCCAGG	20	50-65	51.8-57.9
	5925_8_1	2008-2047		TTGACGATRCGGTAGATGGG	20	50-55	51.8-53.8	TCAAGGTSATGCGMAAGGTC	20	50-55	51.8-53.8
	5925_8_2	1630		GCCTCYTGCTGRCGACTCTTG	20	57-67	56.3-60.2	CCTAYGTCAAGAACGGWCCTCA	22	50-55	54.8-56.7
	6157_7-_5	2000-2200		GAYGAYTTGCCAGCWCC	17	53-65	47.1-51.9	TGGATCCARAACGCSAC	17	53-59	47.1-49.5
	648_4	1898		CTTCAGACGGCGRCGGTTG	19	63-68	55.4-57.6	TCGACAAGTACAACGAGGAGTGC	23	52	57.1
	7496_4-_3	2300		GTCGAAAGATTTGTTCTGCTG	21	43	50.5	ACCAACCCWATCATGGAAGC	20	50	51.8
	8603_2-_3	400-670		TATGGTCTKTAYTTGATGGTCGA	23	35-43	49.9-53.5	AAGTAGTTKGADCCWGMCATCC	22	41-55	51.1-56.7
	957_2	1358		AGATTCGCTCSGTYATTGGTGC	22	50-55	54.8-56.7	CCTCGATATCACCRACCATGTAGA	24	46-50	55.7-57.4
	9823_5-_6	1550-1650		ATGGCHTACAACGATCT	17	41-47	42.2-44.6	GAGYTCCARATTCCAGTT	18	39-50	43.5-48
Failed *in silico*	10146_1-_2			TCAAGTACGGTTGGGAYTT	19	42-47	46.8-48.9	GCRGGGATCTTGACYTTG	18	50-61	48-52.6
	10482_2	900		ARTCAATGCTGTCGCAGGT	19	47-53	48.9-51.1	TCCAAGAACGAGATCCAGTC	20	50	51.8
	10927_4-_3	1600		GATTTGTTCTGCTGATGCGCCA	22	50	54.8	TCCTTCATCGGTGTCCTCGA	20	55	53.8
	12903_1	2000		GCCTCCTCCAAGTARTTGTC	20	50-55	51.3-53.8	ATCGAGAAGCGMACCTGGC	19	58-63	53.2-55.4
	1327_1	1200		ATCARCTCGGTCATGWAAGG	20	45-50	49.7-51.8	ATCTGCTTYTGCTGCCCMGG	20	55-65	53.8-57.9

Table 2.4 (cont'd)

Failed *in silico*	13292_2	730		GTCTTGGCCTTGCTCATCA	19	53	51.1	CACCGCCACATCYTGTC	17	59-65	49.5-51.9
	1359_4	650		ACATCRCCRAACATGACGGC	20	50-60	51.8-55.9	CTTTGARCGYATCCTCTGGAGA	22	45-55	53-56.7
	1412_4	700		CTTCTCCTTCTTRGMCTTCTT	21	38-48	48.5-52.4	AGCTCATYAAGAACAAGARCGA	22	36-45	49.2-53
	14290_2-_1	1300-1670		CTTGATCTTCTTGCGSGCAAT	21	48	52.4	GCCACCACCTGCCCCTC	17	76	56.7
	1655_4	1600		TCWCCRGTCGARGCCATCTC	20	55-65	53.8-57.9	AGTCKGTYGGHGAGGTCATG	20	50-65	51.8-57.9
	2258_3	2200		CCATRTCCTTGGASTCRTCGTGC	23	52-61	57.1-60.6	GTCTCGTTCGGCCCCAAGCA	20	65	57.9
	2550_2	1543		CCTTGWACTTCTCGGCCTCG	20	60	55.9	ACCTACTCBTGCGTTGCCGTCT	22	55-59	56.7-58.6
	3484_2	1261		AACGASCAGGGTMACCGTAYCA	22	50-59	54.8-58.6	TCATGGSACGCTCWCCCTCGTA	22	59	58.6
	376_2	1400		TCGCTYGCCTACGGWGGYATG	21	57-67	56.3-60.2	TAGTTDGTRGGYTCGTCCAG	20	45-60	49.7-55.9
	671_2	1100		GATACCDCCCAAGTTCTG	18	50-56	48-50.3	CTTCTGCTGCCCHGGYATGT	20	55-65	53.8-57.9
	730_2	600		TBCGCCTKTTGATCTCBCAC	2	45-60	49.7-55.9	ACAATRGTGAACATGCGCTC	20	45-50	47.9-51.8
	819_3	1827		ATCTTGTTCTGCTCACGRGCCT	21	50-55	54.8-56.7	GGGYATGCCCCATCGTGGT	19	63-68	55.4-57.6
	834_4	600		TGTYTGCCTGGMTGCTTCT	19	47-58	48.9-53.2	GAARTCGTCAAAGTGCCAG	19	47-53	48.9-51.1

Table 2.5 – Raw sequences per locus

The total number of sequences recovered, the total number of isolates represented, and the ratio between the number of sequences and isolates, full and partial length, for each locus

	5512		**370**		**1870**		**2175**		**5491**		**2451**		**4121**	
	Full	Part	Full	Part	Full	Part	Full	Part	Full	Part	Full	Part	Full	Part
Sequences	214	39	238	61	295	70	353	201	334	63	170	81	370	119
Samples	214	32	205	57	283	38	289	119	301	53	169	55	269	50
Ratio	1	1.22	1.16	1.07	1.04	1.84	1.22	1.69	1.11	1.19	1.01	1.47	1.38	2.38
	4955		**5401**		**EF1a**		**RPB1**		**615**		**10927**		**ITS**	
	Full	Part	Full	Part	Full	Part	Full	Part	Full	Part	Full	Part	Full	Part
Sequences	299	44	253	77	298	698	335	110	305	92	175	89	307	42
Samples	282	40	246	54	287	110	307	78	253	60	171	61	303	28
Ratio	1.06	1.1	1.03	1.43	1.04	6.35	1.09	1.41	1.21	1.53	1.02	1.46	1.01	1.5

Table 2.6 – Rejected Strains

The strain number, preliminary identification, ITS-based identification, number of full-length sequences for each locus, and reason the strain was excluded from the final MGP analyses.

Reason	Strain ID	Preliminary ID	ITS ID	5512	1870	2451	4955	EF1a	RPB1	ITS
Erratic placement(s)	NRRL_5247	*Mortierella elongata*	*Mortierella elongata*		1			1	1	1
	NRRL_28263	*Mortierella rostafinskii*			1		1	1	1	
	NRRL_2665	*Mortierella sp.*	*Mortierella sp. clade5*		1		1	1	1	1
	NRRL_A-16826	*Mortierella sp.*	*wolfii, clade 5*		1		1	1	1	1
	NRRL_A-13231	*Umbelopsis vinacea*	*Mortierella sp. clade6 & Umbelopsis*		1		1	1		2
	NRRL_28259	*Umbelopsis vinacea*	*Umbelopsis vinacea*		1		1	1		1
Sequence Duplication	NRRL_22986	*Umbelopsis vinacea*		1	5		5	5	7	1
	NVP39	*Mortierella minutissima*	*Mortierella sp. clade7*				2		1	1
	NRRL_28270	*Mortierella echinula*		1	2		2	2	2	
	NRRL_28640	*Mortierella wolfii*			3		3	1	2	
Only one MGP locus	AD030	*Mortierella sp.*					1			
	NVP93	*Mortierella minutissima*	*Mortierella minutissima*					1		1
	NRRL_A-17819	*Mortierella rostafinskii*	*Mortierella rostafinskii*					1		1
	AD036	*Mortierella chlamydospora*	*Mortierella chlamydospora*					1		1
	KOD1061	*Mortierella sp.*	*Mortierella parvispora*						1	1
No MGP loci	C-SDSO22-35	*Mortierella elongata*	*Mortierella elongata*							1
	KSSO1-41	*Mortierella gamsii*								
	KSSO2-49	*Mortierella elongata*								
	C-MISO26-28	*Mortierella alpina*								2
	C-MNSO23-21	*Mortierella alpina*								
	AG14-9		*Umbelopsis*							1
	AG18-7		*Mortierella sp. clade7*							1
	AG24-3		*Umbelopsis*							1
	AG69		*Umbelopsis*							partial

Table 2.6 (cont'd)

No MGP loci	AG6-9		*Umbelopsis*							1
	AG12		*Umbelopsis*							1

Table 2.7 – MGP Sequences by Isolate

Values indicate the Genbank reference number for the sequence included in the final dataset. Numbers in parentheses indicate the initial degree of sequence duplication for that sample at that locus. “0.5” indicates at least one partial sequence was detected but could not be included due to insufficient length. Asterisks indicate sequences obtained from a low coverage or de novo genome sequence, rather than PCR amplification.

Isolate ID	Preliminary Identification	Updated Identification	In LCG	Locus 1870	Locus 2451	Locus 4955	Locus 5512	Locus EF1a	Locus RPB1
14Py07W	*Mortierella sp.*	*Linnemannia sp*		MN878503		MN878963	MN879069	MN878222	MN744146
14Py14W	*Mortierella alpina*	*Linnemannia sp*		MN878497		MN878964	MN879070	MN878223	MN744147
14Py25W	*Mortierella gamsii*	*Linnemannia gamsii*		MN878490		MN878859	MN879036	MN878114	MN744033
14Py31W	*Mortierella sp.*	*Linnemannia elongata*		MN878434	MN883114	MN878965	MN879071	MN878224	MN744148
14Py45W	*Mortierella sp.*	*Mortierella alpina*		MN878549	MN878713	MN878751	MN879072	MN878017	MN743921
14Py58W		*Mortierella alpina*		MN878518	MN878701	MN878752	MN879073	MN878018	MN743922
14UC	*Mortierella sp.*	*Linnemannia sp*	Y	MN878286*	0.5*	MN878747*	0.5*	MN878175*	MN743903*
AD003	*Mortierella verticillata*	*Podila verticillata*		MN878332	MN883115	MN879004	MN879037	MN878269	MN744194
AD008	*Mortierella horticola*	*Podila horticola*		MN878350	MN883116	MN878870	MN879074	MN878125	MN744044
AD009	*Mortierella horticola*	*Podila horticola*	Y	MN878349	MN878651	MN878871	MN879075	MN878126	MN744045
AD010	*Mortierella sp.*	*Entomortierella sp*	Y	MN878453	0.5*	MN878954 (2)		MN878212	MN744134 (2)
AD011	*Mortierella sp.*	*Entomortierella sp*	Y	0.5*	0.5*	MN878955		MN879018	MN744135
AD012	*Mortierella horticola*	*Podila horticola*		MN878348	0.5	MN878872	MN879076	MN878127	MN744046
AD013	*Mortierella horticola*	*Podila horticola*		MN878351	MN878650	MN878873	MN879077	MN878128	MN744047
AD014	*Mortierella sp.*	*Entomortierella sp*		0.5	0.5	MN878956		MN878213	MN744136
AD021	*Mortierella alpina*	*Mortierella alpina*		MN878516	MN878706	MN878753	MN879078	MN878019	MN743923
AD022	*Mortierella elongata*	*Linnemannia elongata*		MN878462	MN878629	MN878810	MN879079	MN878067	MN743983

Table 2.7 (cont'd)

AD031	*Mortierella sp.*	*Linnemannia sp nov*	Y	MN878406	MN883117	MN878966		MN878225	MN744149
AD032	*Mortierella sp.*	*Linnemannia sp*	Y	MN878504	0.5*	MN878967	MN879080	MN878226	MN744150
AD033	*Mortierella chlamydospora*	*Entomortierella chlamydospora*	Y					MN878059*	MN879017*
AD034	*Mortierella minutissima*	*Podila minutissima*		MN878360	MN878664	MN878892	MN879081	MN878150	MN744069
AD035	*Mortierella elongata*	*Linnemannia elongata*		MN878456	MN878605	MN878811	MN879082	MN878068	MN743984
AD039	*Mortierella parvispora*	*Entomortierella parvispora*				MN878903		MN878163	MN744080
AD041	*Mortierella minutissima*	*Podila minutissima*		MN878383		MN878893	MN879083	MN878151	MN744070
AD045	*Mortierella gamsii*	*Linnemannia gamsii*	Y	MN878495	MN878632	MN878860	0.5*	MN878115	MN744034
AD050	*Mortierella elongata*	*Linnemannia elongata*		MN878432	MN878598	MN878812	MN879084	MN878069	MN743985
AD051	*Mortierella minutissima*	*Podila minutissima*		MN878384		MN878894	MN879085	MN878152	MN744071
AD054	*Mortierella globulifera*	*Dissophora globulifera*	Y	MN878297	MN878682	MN878868	MN879086	MN878124	MN744042
AD055	*Mortierella horticola*	*Podila horticola*		0.5	MN878653	MN878874	MN879087	MN878129	MN744048
AD058	*Mortierella epicladia*	*Podila epicladia*	Y	MN878358	MN878659	MN878854	MN879088	MN878110	MN744028
AD060	*Mortierella sp.*	*Linnemannia sp*		MN878501	0.5	MN878968	MN879089	MN878227	MN744151
AD062	*Mortierella alpina*	*Mortierella alpina*		MN878526	MN878729	MN878754	MN879090	MN878020	MN743924
AD065	*Mortierella minutissima*	*Podila minutissima*		MN878382	0.5	MN878895	MN879091	MN878153	MN744072
AD068	*Mortierella hyalina*	*Linnemannia hyalina*		MN878488	MN878625	MN878880	MN879092	MN878135	MN744054
AD069	*Mortierella minutissima*	*Podila minutissima*	Y	MN878288*	MN878564*	MN878745*	MN879093*	MN878154*	MN743905*

Table 2.7 (cont'd)

AD070	*Mortierella gamsii*	*Linnemannia gamsii*		MN878496	0.5	MN878861	MN879038	MN878116	MN744035
AD071	*Mortierella alpina*	*Mortierella alpina*	Y	MN878523	MN878735	MN878755	MN879094	MN878021	MN743925
AD072	*Mortierella alpina*	*Mortierella alpina*	Y	MN878289*	0.5*	MN878744*	0.5*	MN878022*	MN743906*
AD077A	*Mortierella minutissima*	*Podila minutissima*		MN878364	MN878666	MN878896	MN879095	MN878155	MN744073
AD078	*Mortierella sp.*	*Entomortierella sp*			MN878678			MN878214	MN744137
AD079	*Mortierella verticillata*	*Podila verticillata*	Y			MN878741*		MN878270*	MN743902*
AD084	*Mortierella sp.*	*Linnemannia hyalina*		MN878489	MN878626	MN878969	MN879096	MN878228	MN744152
AD085	*Mortierella nantahalensis*	*Linnemannia nantahalensis*		MN878327	MN878578	MN878902	MN879097	MN878162	MN744079
AD086	*Mortierella verticillata*	*Podila verticillata*		MN878334	MN878642	MN879005	MN879039	MN878271	MN744195
AD092	*Mortierella verticillata*	*Podila verticillata*		MN878335	MN878641	MN879006	MN879040	MN878272	MN744196
AD093	*Mortierella elongata*	*Linnemannia elongata*		MN878431	MN878602	MN878813	MN879098	MN878070	MN743986
AD094	*Mortierella sp.*	*Entomortierella sp*	Y	0.5*	MN878679	MN878918		MN878176	MN744096
AG#	*Umbelopsis ramanniana*	*Umbelopsis ramanniana*			MN883129*			MN879019*	MN743913*
AG13-4	*Umbelopsis*	*Linnemannia elongata*		MN878425				MN878229	MN744153
AG77	*Mortierella elongata*	*Linnemannia elongata*	Y	MN878282*	MN878561*	MN878737*	MN879099*	MN878071*	MN743897*
B6842	*Mortierella alpina*	*Mortierella alpina*	Y	MN878283*	MN878562*	MN878738*	MN879100*	MN878023*	MN743898*
BC1065	*Mortierella ambigua*	*Actinomortierella ambigua*				MN878740*			MN743900*
C-ARSO21-9	*Mortierella alpina*	*Mortierella alpina*		MN878531	MN878699	MN878756	MN879101	0.5	MN743926
C-ARSO24-5	*Mortierella acrotona*	*Linnemannia acrotona*		MN878498		MN878750	MN879102	MN878016	MN743920

Table 2.7 (cont'd)

C-ARSO25-24	*Mortierella elongata*	*Linnemannia elongata*		MN878437	MN878604	MN878814	MN879103	MN878072	MN743987
C-ILSO26-18	*Mortierella alpina*	*Mortierella alpina*		MN878546	MN878714	MN878757	MN879105	MN878024	MN743927
C-INSO22-17	*Mortierella gamsii*	*Linnemannia gamsii*		MN878491		MN878862	MN879041	MN878117	MN744036
C-INSO22-22	*Mortierella alpina*	*Mortierella alpina*		MN878511	MN878709	MN878758	MN879106	MN878025	MN743928
C-MICO24-19	*Mortierella alpina*	*Mortierella alpina*		MN878515	MN878704	MN878761		MN878027	MN743931
C-MISO21-18	*Mortierella elongata*	*Linnemannia elongata*		MN878442	MN878592	MN878815	MN879111	MN878073	MN743988
C-MNSO24-13	*Mortierella gamsii*	*Linnemannia gamsii*		MN878492		MN878863	MN879042	MN878118	MN744037
CBS277.49	*Mucor circinelloides*	*Mucor circinelloides*	Y		MN883128*			MN879021*	MN743912*
CBS456.71	*Mortierella cystojenkinii*	*Gryganskiella cystojenkinii*	Y	0.5			MN879043	MN878062	MN743978
CBS575.75	*Mortierella echinosphaera*	*Entomortierella echinospaera*				MN878809		MN878066	MN743982
CBS811.68	*Mortierella selenospora*	*Lunasporangiospora selenospora*		MN878316	MN878567	MN878917	0.5		0.5
CK1227	*Mortierella alpina*	*Mortierella alpina*		MN878517	MN878707	MN878759	MN879107	0.5	MN743929
CK1249	*Mortierella alpina*	*Mortierella alpina*	Y	MN878290*	MN883118*	0.5*	0.5*	MN878026*	MN743908*
CK1268	*Mortierella alpina*	*Mortierella alpina*		MN878535	MN878690	MN878760	MN879108	0.5	MN743930
CK202	*Mortierella alpina*	*Mortierella sp*		MN878551	MN878719	MN878919	MN879109	MN878177	MN744097
CK281	*Mortierella verticillata*	*Podila verticillata*		MN878333	MN878640	MN879007	MN879044	MN878273	MN744197

Table 2.7 (cont'd)

CK413	*Mortierella horticola*	*Podila horticola*	Y	MN878293*	0.5*	MN878743*	MN879110*	0.5*	MN743907*
FSU9682	*Lichtheimia corymbifera*	*Lichtheimia corymbifera*						MN879020*	MN743911*
GBAus21	*Mortierella elongata*	*Linnemannia elongata*		MN878445	MN878593	MN878816	MN879112	0.5	MN743989
GBAus22	*Mortierella gamsii*	*Linnemannia gamsii*		MN878493	MN878579	MN878864	MN879045	MN878119	MN744038
GBAus23	*Mortierella elongata*	*Linnemannia elongata*		MN878452		MN878817	MN879113	MN878074	MN743990
GBAus24	*Mortierella elongata*	*Linnemannia elongata*	Y	MN878469	MN878627	MN878818	MN879114	MN878075	MN743991
GBAus25	*Mortierella elongata*	*Linnemannia elongata*		MN878450	MN878610	MN878819	MN879115	MN878076	MN743992
GBAus27B	*Mortierella sp.*	*Benniella sp nov 1*	Y	MN878303	MN878569	MN878998			MN744182
GBAus30	*Mortierella sp.*	*Mortierella sp*	Y	MN878320	MN883119	MN878999	MN879046	MN878258	MN744183
GBAus31	*Mortierella alpina*	*Mortierella alpina*	Y	MN878534	MN878696	MN878762	MN879116	MN878028	MN743932
GBAus32	*Mortierella elongata*	*Linnemannia elongata*		MN878443	MN878603	MN878820	MN879117	MN878077	MN743993
GBAus33	*Mortierella elongata*	*Linnemannia elongata*		MN878451	MN878609	MN878821	MN879118	MN878078	MN743994
GBAus34	*Mortierella elongata*	*Linnemannia elongata*	Y	MN878444	MN878633	MN878822	MN879119	MN878079	MN743995
GBAus35	*Mortierella sp.*	*Linnemannia sp nov*	Y	MN878408	MN878634	MN878970		MN878230	MN744154
GBAus36	*Mortierella elongata*	*Linnemannia elongata*		MN878457	MN878607	MN878823	MN879120	MN878080	MN743996
GBAus37	*Mortierella elongata*	*Linnemannia elongata*		MN878455	MN878606	MN878824	MN879121	MN878081	MN743997
GBAus38	*Mortierella elongata*	*Linnemannia elongata*		MN878470	MN878628	MN878825	MN879122	MN878082	MN743998
GBAus39	*Mortierella sp.*	*Linnemannia hyalina*	Y	MN878482	MN878619	MN878971	MN879123	MN878231	MN744155
GBAus40	*Mortierella elongata*	*Linnemannia elongata*	Y	MN878454	MN878608	MN878826	MN879124	MN878083	MN743999

Table 2.7 (cont'd)

GBAus41	*Mortierella sp.*	*Linnemannia hyalina*		MN878483	MN878618	MN878972	MN879125	MN878232	MN744156
GBAus42	*Mortierella sp.*	*Linnemannia elongata*		MN878423	MN878588	MN878973	MN879126	MN878233	MN744157
IASO10-42-45rt	*Mortierella elongata*	*Linnemannia elongata*		MN878447	MN878597	MN878827	MN879127	0.5	MN744000
ILSO2-38	*Mortierella elongata*	*Linnemannia elongata*		MN878460	MN878582	MN878828	MN879128	MN878084	MN744001
INSO1-46B2	*Mortierella elongata*	*Benniella sp nov 1*		MN878304	0.5	MN878829			MN744002
JES103	*Mortierella hyalina*	*Linnemannia hyalina*	Y	MN878284*	MN878563*	MN878739*	MN879129*	MN878136*	MN743899*
KOD1000	*Mortierella clonocystis*	*Podila clonocystis*	Y	MN878347	MN878668	MN878923	MN879130	MN878181	MN744102
KOD1001	*Mortierella sp.*	*Linnemannia sp*		MN878506	0.5	MN878974	MN879131	MN878234	MN744158
KOD1002	*Mortierella alpina*	*Mortierella alpina*			MN878702	MN878763		MN878029	MN743933
KOD1003	*Mortierella sp.*	*Linnemannia sp*		MN878505		MN878975	MN879132	MN878235	MN744159
KOD1004	*Mortierella sp.*	*Podila minutissima*		MN878387		MN878924	MN879133	MN878182	MN744103
KOD1005	*Mortierella alpina*	*Mortierella alpina*		MN878521	MN878733	MN878764	MN879134	MN878030	MN743934
KOD1006	*Mortierella elongata*	*Linnemannia elongata*		MN878461	MN878583	MN878830	MN879135	MN878085	MN744003
KOD1007	*Mortierella elongata*	*Linnemannia elongata*		MN878468	0.5	MN878831	MN879136	MN878086	MN744004
KOD1008	*Mortierella sp.*	*Podila horticola*		MN878388		MN878875	MN879137	MN878130	MN744049
KOD1009	*Mortierella sp.*	*Podila clonocystis*		MN878376	MN878672	MN878805	MN879138	MN878060	MN743976
KOD1010	*Mortierella sp.*	*Podila minutissima*		MN878377	MN878673	MN878925	MN879139	MN878183	MN744104
KOD1012	*Mortierella alpina*	*Mortierella alpina*		MN878554	MN878722	MN878765	MN879140	MN878031	MN743935
KOD1013	*Mortierella sp.*	*Podila minutissima*		MN878370		MN878926	MN879141	MN878184	MN744105
KOD1014	*Mortierella sp.*	*Podila epicladia*		MN878355	MN878658	MN878927	MN879142	MN878185	MN744106
KOD1015	*Mortierella selenospora*	*Lunasporangiospora selenospora*	Y	MN878317	MN878566	0 (2)		MN878174	MN744095* (2)

Table 2.7 (cont'd)

KOD1016	*Mortierella alpina*	*Mortierella alpina*		MN878527	MN878726	MN878766	MN879143	MN878032	MN743936
KOD1017	*Mortierella alpina*	*Mortierella alpina*		MN878528	MN878727	MN878767	0.5	MN878033	MN743937
KOD1018	*Mortierella alpina*	*Mortierella alpina*		MN878529	MN878728	MN878768	MN879144	0.5	MN743938
KOD1019	*Mortierella alpina*	*Mortierella alpina*		MN878530	MN878730	MN878769	MN879145	MN878034	MN743939
KOD1020	*Mortierella hyalina*	*Linnemannia hyalina*		MN878471	MN878613	MN878881	MN879146	MN878137	MN744055
KOD1021	*Mortierella alpina*	*Mortierella alpina*		MN878519	MN878725	MN878770 (2)	MN879147	MN878035	MN743940
KOD1022	*Mortierella alpina*	*Mortierella alpina*		MN878542	MN878695	MN878771	MN879148	0.5	MN743941
KOD1023	*Mortierella hyalina*	*Linnemannia hyalina*		MN878480	MN878622	MN878882	MN879149	MN878138	MN744056
KOD1024	*Mortierella sp.*	*Podila minutissima*		MN878393		MN878928	MN879150	MN878186	MN744107
KOD1025	*Mortierella sp.*	*Podila minutissima*		MN878390		0.5	MN879151	MN879022	MN744108
KOD1026	*Mortierella alpina*	*Mortierella alpina*		MN878544	MN878718	MN878772	MN879152	MN878036	MN743942
KOD1027	*Mortierella alpina*	*Mortierella alpina*		MN878555	MN878721	MN878773	MN879153	0.5	MN743943
KOD1028	*Mortierella alpina*	*Mortierella alpina*		MN878540	MN878694	MN878774	MN879154	0.5	MN743944
KOD1029	*Mortierella sp.*	*Podila minutissima*		MN878366	MN878671	MN878929	MN879155	MN878187	MN744109
KOD1030	*Mortierella antarctica*	*Podila minutissima*	Y	MN878371	MN878667	MN878930	MN879156	MN878188	MN744110
KOD1032	*Mortierella gamsii*	*Linnemannia sp*		MN878507	0.5	MN878865	MN879157	MN878120	MN744039
KOD1033	*Mortierella sp.*	*Podila minutissima*		MN878354		MN878931	MN879158	MN878189	MN744111
KOD1034	*Mortierella gamsii*	*Linnemannia sp*		MN878508		MN878866	MN879159	MN878121	MN744040
KOD1035	*Mortierella dichotoma*	*Necromycomortierella dichotoma*		MN878365		MN878807		MN878063	MN743979

Table 2.7 (cont'd)

KOD1036	*Mortierella dichotoma*	*Necromycomortierella dichotoma*		0 (2)		0 (2)		MN878064	MN743980
KOD1037	*Mortierella hyalina*	*Linnemannia hyalina*		MN878485	MN878614	MN878883	MN879160	MN878139	MN744057
KOD1038	*Mortierella sp.*	*Linnemannia hyalina*		MN878484	MN878616	MN878976	MN879161	MN878236	MN744160
KOD1039	*Mortierella sp.*	*Entomortierella parvispora*		MN878367		MN878920	0.5	MN878178	MN744099
KOD1040	*Mortierella beljakovae*	*Entomortierella beljakovae*	Y	MN878300	0.5*	0.5*		MN878053	MN743968
KOD1041	*Mortierella sp.*	*Podila clonocystis*		MN878343	MN878655	MN878932	MN879162	MN878190	MN744112
KOD1042	*Mortierella sp.*	*Podila clonocystis*		MN878344	MN878656	MN878933	MN879163	MN878191	MN744113
KOD1043	*Mortierella sp.*	*Gryganskiella sp*		0 (2)		MN878921	0.5	MN878179	MN744100 (2)
KOD1044	*Mortierella sp.*	*Linnemannia hyalina*		MN878487	MN878617	MN878977	MN879164	MN878237	MN744161
KOD1045	*Mortierella alpina*	*Mortierella alpina*		MN878556	MN878723	MN878775	MN879165	MN878037	MN743945
KOD1046	*Mortierella alpina*	*Mortierella alpina*		MN878538	MN878697	MN878776	MN879166	MN878038	MN743946
KOD1047	*Mortierella alpina*	*Mortierella alpina*		MN878539	MN878692	MN878777	MN879167	0.5	MN743947
KOD1048	*Mortierella sp.*	*Linnemannia hyalina*		MN878486	MN878615	MN878978	0.5	MN878238	MN744162
KOD1049	*Mortierella sp.*	*Podila minutissima*		MN878386		MN878934	MN879168	MN878192	MN744114
KOD1050	*Mortierella humilis*	*Podila verticillata*	Y	MN878323	0.5*	MN878878	MN879169	MN878133	MN744052
KOD1051	*Mortierella amoeboidea*	*Linnemannia amoedoidea*		MN878324	0.5	MN878795		MN878050	MN743965
KOD1052	*Mortierella polycephala*	*Mortierella polycephala*		MN878319	0.5	MN878906	MN879047	MN878168	MN744084
KOD1053	*Mortierella amoeboidea*	*Linnemannia amoedoidea*		MN878325	MN878577	MN878796		MN878051	MN743966
KOD1054	*Mortierella alpina*	*Mortierella alpina*		MN878520	MN878732	MN878778	MN879170	MN878039	MN743948

Table 2.7 (cont'd)

KOD1055	*Mortierella alpina*	*Mortierella alpina*		MN878545	MN878716	MN878779	MN879171	0.5	MN743949
KOD1056	*Mortierella parvispora*	*Entomortierella parvispora*		MN878302		MN878904		MN878165	MN744081
KOD1057	*Mortierella sp.*	*Podila clonocystis*		MN878346	0.5	MN878935	MN879172	MN878193	MN744115
KOD1059	*Mortierella epicladia*	*Podila epicladia*	Y	MN878356	MN878660	MN878855	MN879173	MN878111	MN744029
KOD1061	*Mortierella parvispora*	*Entomortierella parvispora*						MN878164	MN879015
KOD1062	*Mortierella parvispora*	*Mortierella polycephala*				MN878890	MN879048	0.5	MN744064
KOD1063	*Mortierella sp.*	*Podila minutissima*		MN878374		MN878936	MN879174	MN878194	MN744116
KOD1064	*Mortierella sp.*	*Podila minutissima*		MN878372	0.5	MN878937	MN879175	MN878195	MN744117
KOD1065	*Mortierella sp.*	*Podila minutissima*		MN878373		MN878897	MN879176	MN878156	MN744074
KOD1067	*Mortierella hyalina*	*Linnemannia hyalina*		MN878472	MN883120	MN878884	MN879177	MN878140	MN744058
KOD1068	*Mortierella hyalina*	*Linnemannia hyalina*		MN878477	MN878623	MN878885	MN879178	MN878141	MN744059
KOD1069	*Mortierella parvispora*	*Entomortierella parvispora*				MN878905		MN878166	MN744082
KOD943	*Mortierella sp.*	*Podila epicladia*		MN878353	MN878663	MN878938	MN879179	MN878196	MN744118
KOD944	*Mortierella minutissima*	*Podila minutissima*		MN878385		MN878898	MN879180	MN878157	MN744075
KOD945	*Mortierella sp.*	*Mortierella sp*		MN878391	MN883121	MN878959	MN879049	MN878217	MN744141
KOD947	*Mortierella clonocystis*	*Podila clonocystis*	Y	MN878345	MN878654	MN878806	MN879181	MN878061	MN743977
KOD948	*Mortierella polycephala*	*Mortierella polycephala*	Y	MN878318	MN878687	MN878907	MN879050	MN878169	MN744085
KOD949	*Mortierella hyalina*	*Linnemannia hyalina*		MN878479	MN883122	MN878886	MN879068	MN878142	MN744060
KOD950	*Mortierella sp.*	*Podila epicladia*		MN878357	MN878662	MN878939	MN879182	MN878197	MN744119
KOD951	*Mortierella sp.*	*Podila minutissima*		MN878361	MN878665	MN878940	MN879183	MN878198	MN744120

Table 2.7 (cont'd)

KOD952	*Mortierella verticillata*	*Podila verticillata*		MN878331	MN883123	MN879008	MN879051	MN878274	MN744198
KOD954	*Mortierella sp.*	*Entomortierella parvispora*				MN878922		MN878180	MN744101
KOD955	*Mortierella sp.*	*Podila minutissima*		MN878375		MN878953	MN879184	MN878211	MN744133
KOD956	*Mortierella sp.*	*Podila sp*		MN878329	MN878649	MN878941	MN879185	MN878199	MN744121
KOD957	*Mortierella alpina*	*Mortierella alpina*		MN878524	MN878731	MN878780	MN879186	MN878040	MN743950
KOD958	*Mortierella alpina*	*Podila minutissima*		MN878392		MN878942	MN879187	MN878200	MN744122
KOD959	*Mortierella minutissima*	*Dissophora globulifera*		MN878298	MN878681 (2)	MN878958		MN878215	MN744139
KOD960	*Mortierella sp.*	*Podila minutissima*		MN878394		MN878943	MN879188	MN878201	MN744123
KOD963	*Mortierella sp.*	*Podila minutissima*		MN878389		MN878952	MN879189	MN878210	MN744132
KOD964	*Mortierella sp.*	*Podila minutissima*		MN878378		MN878944	MN879190	MN878202	MN744124
KOD965	*Mortierella hyalina*	*Linnemannia hyalina*		MN878481	0.5	MN878887	MN879191	MN878143	MN744061
KOD967	*Mortierella alpina*	*Mortierella alpina*		MN878553	MN878724	MN878781	MN879192	MN878041	MN743951
KOD968	*Mortierella polycephala*	*Mortierella polycephala*		0.5	MN878686	MN878908	MN879052	MN878170	MN744086
KOD969	*Mortierella sp.*	*Podila minutissima*		MN878380		MN878945	MN879193	MN878203	MN744125
KOD971	*Mortierella sp.*	*Podila minutissima*		MN878381		MN878946	MN879194	MN878204	MN744126
KOD972	*Mortierella sp.*	*Podila minutissima*		MN878379		MN878947	MN879195	MN878205	MN744127
KOD975	*Mortierella polycephala*	*Mortierella polycephala*		0 (2)	MN878688	MN878909 (4)	MN879053	MN879023	MN744087 (2)
KOD979	*Mortierella sp.*	*Linnemannia elongata*		MN878467	MN878630	MN878979	MN879196	MN878239	MN744163
KOD980	*Mortierella elongata*	*Linnemannia elongata*		MN878463	0.5	MN878832	MN879197	MN878087	MN744005
KOD981	*Mortierella elongata*	*Linnemannia elongata*		MN878465	MN878635	MN878833	MN879198	MN878088	MN744006
KOD982	*Mortierella elongata*	*Linnemannia elongata*		MN878466	MN878631	MN878834	MN879199	MN878089	MN744007

Table 2.7 (cont'd)

KOD983	*Mortierella alpina*	*Mortierella alpina*		MN878547	MN878711	MN878782	MN879200	MN878042	MN743952
KOD984	*Mortierella elongata*	*Podila minutissima*		MN878362	MN878669	MN878948	MN879201	MN878206	MN744128
KOD988	*Mortierella sp.*	*Podila epicladia*		MN878359	MN878661	MN878949	MN879202	MN878207	MN744129
KOD989	*Mortierella sp.*	*Entomortierella sp*	Y	MN878314	MN878677	MN878957	0.5	MN879024	MN744138
KOD990	*Mortierella alpina*	*Mortierella alpina*		MN878537	MN878693	MN878783	MN879203	MN879025	MN743953
KOD991	*Mortierella fimbricystis*	*Gryganskiella fimbricystis*		MN878301		MN878858	MN879204	MN879026	MN744032
KOD992	*Mortierella sp.*	*Mortierella sp*		MN878533	0.5	MN878960	MN879054	MN878218	MN744142
KOD993	*Mortierella elongata*	*Linnemannia elongata*		MN878419	MN878581	MN878835	MN879205	MN878090	MN744008
KOD994	*Mortierella alpina*	*Mortierella alpina*		MN878552	MN878720	MN878793	MN879206	MN878049	MN743963
KOD995	*Mortierella alpina*	*Mortierella alpina*		MN878522	MN878734	MN878784	MN879207	MN878043	MN743954
KOD996	*Mortierella dichotoma*	*Necromycomortierella dichotoma*		MN878464	0.5	MN878808	MN879055	MN878065	MN743981 (2)
KOD998	*Mortierella alpina*	*Mortierella alpina*		MN878541	MN878691	MN878785	MN879208		MN743955
KOD999	*Mortierella alpina*	*Mortierella alpina*		MN878543	MN878717	MN878786	MN879209	MN878044	MN743956
MES-2146	*Modicella reniformis*	*Modicella reniformis*	Y	MN878299	MN878568	0.5	0.5*	MN878015	MN743919
MICO2-9	*Mortierella horticola*	*Podila horticola*		MN878352	0.5	MN878876	MN879210	MN878131	MN744050
MISO4-46	*Mortierella rishikesha*	*Linnemannia elongata*		MN878446	MN878595	MN878913	MN879211	MN878171	MN744091
NDSO1-48	*Mortierella alpina*	*Mortierella alpina*		MN878512	MN878710	MN878787	MN879212	MN878045	MN743957
NRRL 1458	*Mortierella sp.*	*Linnemannia hyalina*		MN878475	MN878621	MN878997	MN879213	MN878257	MN744181
NRRL 1617	*Mortierella sp.*	*Linnemannia hyalina*		MN878473	MN883124	MN878996	MN879214	MN878256	MN744180

Table 2.7 (cont'd)

NRRL 22416	*Dissophora decumbens*	*Dissophora decumbens*		MN878559		0.5		MN879027	MN743914
NRRL 22417	*Dissophora ornata*	*Dissophora ornata*	Y	MN878558	0.5*	0.5*		MN878010	MN743915
NRRL 22890	*Mortierella polycephala*	*Mortierella polycephala*		0.5	0.5	MN878911	MN879056	MN879028	MN744089
NRRL 22891	*Mortierella polycephala*	*Mortierella polycephala*		MN883132	MN878689	MN878910 (2)	MN879057	MN879029	MN744088
NRRL 22892	*Mortierella capitata*	*Actinomortierella capitata*		MN883130 (2)	MN878560	MN878736		MN879030	MN743896
NRRL 22995	*Mortierella sp.*	*Linnemannia elongata*		MN878436	MN878591	MN878853	MN879215	MN878109	MN744027
NRRL 2493	*Mortierella bisporalis*	*Mortierella bisporalis*		MN878557	MN878685		MN879058	MN878054	MN743969
NRRL 2525	*Mortierella lignicola*	*Entomortierella lignicola*	Y	0.5*	MN878676	0.5*		MN878149	MN744068
NRRL 25716	*Mortierella aplina*	*Mortierella alpina*		MN878514	MN878703	MN878792		MN878048	MN743962
NRRL 25721	*Mortierella sp.*	*Linnemannia elongata*		MN878459		MN878852	MN879216	MN878108	MN744026
NRRL 2591	*Mortierella hyalina*	*Linnemannia hyalina*		MN878478	MN878624	MN878889	MN879217	MN878145	MN744063
NRRL 2592	*Mortierella zychae*	*Linnemannia zychae*		MN878395		MN879014		MN878281	MN744204
NRRL 2610	*Mortierella camargensis*	*Linnemannia camargensis*	Y	MN878414	MN878638	MN878799	MN879218	MN878056	MN743971
NRRL 2611	*Mortierella verticillata*	*Podila verticillata*	Y	MN878340	MN878646	MN879011	MN879059	MN878278	MN744201
NRRL 2682	*Haplosporangium sp.*	*Mortierella sp*		MN883131	0.5	MN878749	MN879060	MN878012	MN743917
NRRL 2760	*Mortierella claussenii*	*Dissophora sp*				MN878804		MN879031	MN744093
NRRL 2761	*Mortierella schmuckeri*	*Linnemannia schmuckeri*		MN878411	MN878637	MN878915	MN879219	MN879032	MN743975

Table 2.7 (cont'd)

NRRL 2769	*Mortierella chlamydospora*	*Entomortierella chlamydospora*	Y	MN878413	0.5*	MN878803*		MN879033	0.5*
NRRL 28257	*Mortierella capitata*	*Actinomortierella capitata*		MN878368 (2)		MN878801* (2)	0.5	MN879034	MN743973
NRRL 28260	*Mortierella camargensis*	*Linnemannia camargensis*		MN878415	MN878639	MN878800	MN879220	MN878057	MN743972
NRRL 28261	*Mortierella reticulata*	*Mortierella reticulata*		MN878458		MN878912	0.5	0 (4)	MN744090
NRRL 28262	*Mortierella exigua*	*Linnemannia exigua*	Y	MN878499	0.5*	MN878857	MN879221	MN878113	MN744031
NRRL 28267	*Mortierella antarctica*	*Mortierella antarctica*		MN878509		MN878797		MN878052	MN743967
NRRL 28271	*Mortierella ambigua*	*Actinomortierella ambigua*	Y	0.5*		MN878794	MN879222	MN879035	MN743964
NRRL 28272	*Mortierella stylospora*	*Gamsiella stylospora*		MN878322	MN878683	MN879003		MN878268	0.5
NRRL 2942	*Mortierella parvispora*	*Entomortierella parvispora*		MN878416		0.5		MN878167	MN744083
NRRL 3116	*Lobosporangium transversale*	*Lobosporangium transversale*	Y	MN878295		0.5*	0.5*	MN878014 (2)	0 (2)
NRRL 3175	*Haplosporangium sp.*	*Entomortierella sp*	Y	0.5*	MN878675	0.5*	0.5*		MN744098
NRRL 5217	*Mortierella chienii*	*Lunasporangiospora chienii*	Y	MN878315	MN878565	MN878802		MN878058	MN743974
NRRL 5248	*Mortierella indohii*	*Mortierella indohii*		MN878474		0.5	MN879061	MN878146	MN744065
NRRL 5512	*Mortierella epigama*	*Podila epigama*	Y	MN878321		MN878856	0.5*	MN878112	MN744030
NRRL 5513	*Mortierella elongata*	*Linnemannia elongata*		MN878448	MN878600	MN878850	MN879223	MN878106	MN744024
NRRL 5525	*Lobosporangium transversale*	*Lobosporangium transversale*	Y	MN878294		0.5*	0.5*	MN878013 (2)	MN879016 (3)
NRRL 5841	*Mortierella sclerotiella*	*Linnemannia sclerotiella*		MN878417		MN878916	MN879067	MN878173	MN744094

Table 2.7 (cont'd)

NRRL 5842	*Mortierella nantahalensis*	*Podila sp*		MN878525	0.5	MN878950	MN879224	MN878208	MN744130 (3)
NRRL 62971	*Mortierella alpina*	*Mortierella alpina*		MN878550	MN878715	MN878791	MN879225	0.5	MN743961
NRRL 6302	*Mortierella alpina*	*Mortierella alpina*		MN878532	MN878700	MN878790	0.5	MN878047	MN743960
NRRL 6337	*Mortierella verticillata*	*Podila verticillata*	Y	MN878342	MN878647	MN879012	MN883111	MN878279	MN744202
NRRL 6338	*Mortierella verticillata*	*Podila verticillata*		MN878341	MN878648	MN879010	MN883112	MN878277	MN744200
NRRL 6351	*Mortierella wolfii*	*Actinomortierella wolfii*	Y	MN878369		MN879013	0.5*	MN878280	MN744203
NRRL 6369	*Mortierella verticillata*	*Podila verticillata*		MN878336	MN878643	MN879009	MN883113	MN878276	MN744199
NRRL 6424	*Mortierella minutissima*	*Podila minutissima*		MN878337	MN878657	MN878900	MN879226	MN878159	MN744077
NRRL 6425	*Mortierella lignicola*	*Entomortierella lignicola*			MN878674	0.5		MN878148	MN744067 (3)
NRRL 6426	*Mortierella schmuckeri*	*Linnemannia schmuckeri*	Y	MN878412	MN878636	MN878914	MN879227	MN878172	MN744092
NRRL 6427	*Mortierella hyalina*	*Linnemannia hyalina*		MN878476	MN878620	MN878888	MN879228	MN878144	MN744062
NRRL 6456	*Mortierella multidivaricata*	*Gamsiella multidivaricata*	Y	0.5*	MN878684	MN878901	0.5*	MN878161	MN744078
NRRL 66262	*Mortierella alpina*	*Mortierella alpina*		MN878510	MN878708	MN878962	MN879229	MN878221	MN744145
NRRL 66264	*Mortierella geracilis*	*Linnemannia gamsii*		MN878494	MN878580	MN878994		MN878254	MN744178
NRRL A-10739	*Haplosporangium sp.*	*Mortierella sp*	Y	MN878330	0.5*	MN878748	MN879062	MN878011	MN743916
NRRL A-12040	*Mortierella hyalina*	*Mortierella sp*				MN878891	MN879063	MN878147	MN744066
NRRL A-12553	*Mortierella bisporalis*	*Podila verticillata*	Y	MN878338	MN878645	MN878951	MN879064	MN878209	MN744131

Table 2.7 (cont'd)

NRRL A-12867	*Mortierella sp.*	*Linnemannia elongata*		MN878433	MN878601	MN878851	MN879230	MN878107	MN744025
NRRL A-15043	*Mortierella alpina*	*Linnemannia sp*		MN878502		MN878995	MN879231	MN878255	MN744179
NVP1	*Mortierella minutissima*	*Podila minutissima*	Y	MN878363	MN878670	MN878899	MN879232	MN878158	MN744076
NVP103	*Mortierella sp.*	*Benniella sp nov 3*		MN878310		MN879000		MN878259	MN744184
NVP105	*Mortierella sp.*	*Benniella sp nov 3*		MN878308				MN878260	MN744185
NVP106	*Mortierella sp.*	*Benniella sp nov 3*		MN878307				MN878261	MN744186
NVP112	*Mortierella elongata*	*Linnemannia elongata*		MN878439	MN878611	0.5	MN879233	MN878091	MN744009
NVP113	*Mortierella elongata*	*Linnemannia elongata*		MN878440	MN883125	MN878836	MN879234	MN878092	MN744010
NVP123	*Mortierella elongata*	*Linnemannia elongata*		MN878438	MN878612	MN878837	MN879235	MN878093	MN744011
NVP125	*Mortierella sp.*	*Actinomortierella sp*						MN878216	MN744140
NVP128	*Mortierella elongata*	*Linnemannia elongata*		MN878420	MN878584	MN878838	MN879236	MN878094	MN744012
NVP130	*Mortierella sp.*	*Linnemannia sp nov*		MN878404	0.5	MN878980		MN878240	MN744164
NVP131	*Mortierella sp.*	*Linnemannia sp nov*		MN878398	MN878571	MN878981		MN878241	MN744165
NVP132	*Mortierella sp.*	*Linnemannia sp nov*		MN878405	MN878574	MN878982		MN878242	MN744166
NVP133	*Mortierella sp.*	*Benniella sp nov 3*		MN878309		MN879001		MN878262	MN744187
NVP134	*Mortierella sp.*	*Benniella sp nov 3*		MN878306		0.5		0.5	MN744188
NVP137	*Mortierella sp.*	*Benniella sp nov 3*		MN878311				MN878263	MN744189
NVP138	*Mortierella sp.*	*Benniella sp nov 3*		MN878312				MN878264	MN744190
NVP139	*Mortierella sp.*	*Benniella sp nov 3*		MN878313				MN878265	MN744191
NVP144	*Mortierella sp.*	*Linnemannia sp nov*		MN878407	MN878575	MN878983		MN878243	MN744167
NVP145	*Mortierella sp.*	*Linnemannia sp nov*		MN878399	MN878572	MN878984		MN878244	MN744168
NVP146	*Mortierella sp.*	*Linnemannia sp nov*		MN878400	0.5	MN878985		MN878245	MN744169
NVP147	*Mortierella sp.*	*Linnemannia sp nov*		MN878401	MN883126	MN878986		MN878246	MN744170
NVP148	*Mortierella sp.*	*Linnemannia sp nov*		MN878402		MN878987		MN878247	MN744171

Table 2.7 (cont'd)

NVP149	*Mortierella sp.*	*Linnemannia sp nov*		MN878397	0.5	MN878988		MN878248	MN744172
NVP150	*Mortierella sp.*	*Linnemannia sp nov*		MN878409	0.5	MN878989		MN878249	MN744173
NVP151	*Mortierella sp.*	*Linnemannia sp nov*		MN878410	MN878576	MN878990		MN878250	MN744174
NVP153	*Mortierella sp.*	*Mortierella alpina*		MN878548	MN878712	MN878961	MN879237	MN878219	MN744143
NVP154	*Mortierella sp.*	*Linnemannia sp*		MN878500		MN878991	MN879238	MN878251	MN744175
NVP156	*Mortierella elongata*	*Linnemannia elongata*		MN878441	0.5	MN878839	MN879239	MN878095	MN744013
NVP157	*Mortierella alpina*	*Mortierella alpina*	Y	MN878287*		MN878746*	MN879104*		MN743904*
NVP17b	*Mortierella alpina*	*Mortierella alpina*		MN878536	MN878698	MN878788	MN879240	0.5	MN743958
NVP3	*Mortierella sp.*	*Linnemannia sp nov*		MN878396	0.5	MN879002		MN878266	MN744192
NVP4	*Mortierella elongata*	*Linnemannia elongata*		MN878427	MN878586	MN878840	MN879241	MN878096	MN744014
NVP41	*Mortierella sp.*	*Linnemannia sp nov*	Y	MN878403	MN878573	MN878992		MN878252	MN744176
NVP47	*Mortierella alpina*	*Mortierella alpina*		MN878513	MN878705	MN878789		MN878046	MN743959
NVP5	*Mortierella elongata*	*Linnemannia elongata*	Y	MN878422	MN878587	MN878841	MN879242	MN878097	MN744015
NVP60	*Mortierella gamsii*	*Linnemannia gamsii*	Y	MN878292*		MN878742*	MN879243*	MN878122*	MN743910*
NVP61	*Mortierella gamsii*	*Linnemannia gamsii*		MN878418	MN878570	MN878867		MN878123	MN744041
NVP64	*Mortierella elongata*	*Linnemannia elongata*		MN878428	MN878599	MN878842	MN879244	MN878098	MN744016
NVP65	*Mortierella elongata*	*Linnemannia elongata*		MN878449	MN878594	MN878843	MN879245	MN878099	MN744017
NVP66	*Mortierella elongata*	*Linnemannia elongata*				MN878844		MN878100	MN744018
NVP67	*Mortierella elongata*	*Linnemannia elongata*		MN878429	0.5	MN878845	MN879246	MN878101	MN744019
NVP71	*Mortierella elongata*	*Linnemannia elongata*	Y	MN878426	MN878585	MN878846	MN879247	MN878102	MN744020

Table 2.7 (cont'd)

NVP79	*Mortierella elongata*	*Linnemannia elongata*		MN878435	MN878590	MN878847	MN879248	MN878103	MN744021
NVP85	*Mortierella sp.*	*Benniella sp nov 2*	Y	MN878305		0.5		MN878267	MN744193
NVP8B	*Mortierella sp.*	*Mortierella sp*					MN879065	MN878220	MN744144
NVP90	*Mortierella elongata*	*Linnemannia elongata*		MN878421	MN883127	MN878848	MN879249	MN878104	MN744022
PMI1414	*Mortierella humilis*	*Podila verticillata*	Y	MN878339	MN878644	MN878879	MN879066	MN878134	MN744053
PMI86	*Mortierella sp.*	*Linnemannia elongata*	Y	MN878430	MN878596	MN878993	MN879250	MN878253	MN744177
REB-010B	*Mortierella globulifera*	*Dissophora globulifera*	Y	MN878296	MN878680	MN878869	MN879251	0 (2)	MN744043
REB-025A	*Mortierella horticola*	*Podila horticola*		MN878328	MN878652	MN878877	MN879252	MN878132	MN744051
RSA2512	*Mortierella multidivaricata*	*Gamsiella multidivaricata*	Y	MN878285*				MN878160* (2*)	MN743901*
TTC192	*Mortierella verticillata*	*Podila verticillata*	Y	MN878291*				MN878275*	MN743909*
WISO4-29	*Mortierella elongata*	*Linnemannia elongata*		MN878424	MN878589	MN878849	MN879253	MN878105	MN744023
WISO4-30	*Mortierella camargensis*	*Linnemannia camargensis*		MN878326	0.5	MN878798		MN878055	MN743970

Table 2.8 – Primer mismatch

The total number of isolates belonging to each of the ITS-based clades defined by Wagner et al. (2013), the number of those detected in each locus, and the number of usable isolates included in the phylogenetic analyses. The larger the discrepancy between total and detected isolates, the poorer the performance of the primer set on this lineage of Mortierellomycotina.

Clade	Samp. Num	1870				2451				4955				5512				EF1a				RPB1			
		Found		Useable		Found		Useable		Found		Useable		Found		Useable		Found		Useable		Found		Useable	
		#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
1	16	13	81.3	11	68.8	8	50.0	5	31.3	12	75.0	11	68.8	5	31.3	4	25.0	13	81.3	13	81.3	16	100	15	93.8
2	64	61	95.3	60	93.8	38	59.4	32	50.0	59	92.2	56	87.5	53	82.8	50	78.1	64	100	63	98.4	64	100	64	100
3	13	10	76.9	8	61.5	7	53.8	5	38.5	12	92.3	9	69.2	6	46.2	6	46.2	13	100	13	100	13	100	13	100
4	22	22	100	21	95.5	17	77.3	12	54.5	21	95.5	18	81.8	7	31.8	5	22.7	21	95.5	21	95.5	22	100	21	95.5
5	7	7	100	6	85.7	0	0.0	0	0.0	6	85.7	4	57.1	6	85.7	2	28.6	7	100	7	100	6	85.7	6	85.7
6	63	59	93.7	58	92.1	56	88.9	51	81.0	62	98.4	61	96.8	58	92.1	55	87.3	61	96.8	49	77.8	63	100	63	100
7	110	106	96.4	103	93.6	93	84.5	77	70.0	106	96.4	104	94.5	95	86.4	92	83.6	109	99.1	107	97.3	110	100	110	100
NA	2	2	100	2	100	2	100	1	50.0	2	100	2	100	0	0.0	0	0.0	2	100	2	100	2	100	2	100

Table 2.9 – Locus sequence variability at the species level

Summary statistics from pairwise blastn analyses of the locus sequences as submitted to Genbank. Empty cells indicate that the locus was not recovered for any strains in that species. '*n*' = the number of non-self pairwise blastn analyses conducted, where '0' indicates that only one sequence was available to represent the species and therefore no intraspecific analyses could be conducted. "Min% - Max%" = the range of percent sequence variability, where '0' means the sequences were identical. Values in bold indicate a meaningful difference between the maximum observed intraspecific variation and the minimum observed interspecific variation.

	1870				**2451**			
	Intraspecific		**Interspecific**		**Intraspecific**		**Interspecific**	
Species	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%
Actinomortierella ambigua								
Actinomortierella capitata	0	-	174	1.3 - 18.0	0	-	85	19.2 - 21.5
Actinomortierella wolfii	0	-	85	0 - 10.9				
Benniella erionia	1	0	182	11.6 - 14.0	0	-	81	19.1 - 22.0
Benniella sp nov1	0	-	77	6.8 - 15.2				
Benniella sp nov2	55	**0 - 0.7**	719	**6.8 - 14.4**				
Dissophora decumbens	0	-	84	12.7 - 13.3				
Dissophora globulifera	5	**1.3 - 1.5**	274	**14.8 - 16.6**	5	**1.6 - 2.0**	273	**15.8 - 20.2**
Dissophora ornata	0	-	85	12.1 - 13.0				
Entomortierella beljakovae	0	-	83	13.8 - 17.3				
Entomortierella chlamydospora	0	-	88	0.1 - 8.5				
Entomortierella echinospaera								
Entomortierella lignicola					1	0.7	179	0 - 21.9
Entomortierella parvispora	0	-	271	0.2 - 14.1				
Gamsiella multidivaricata	0	-	89	13.0 - 14.1	0	-	89	15.0 - 18.0
Gamsiella stylospora	0	-	90	11.9 - 13.1	0	-	81	16.8 - 20.3
Gryganskiella cystojenkinii								
Gryganskiella fimbricystis	0	-	83	13.0 - 14.0				
Linnemannia acrotona	0	-	88	0.1 - 8.4				
Linnemannia amoedoidea	1	0	185	11.0 - 12.0	0	-	84	16.9 - 21.8
Linnemannia camargensis	1	0	281	3.1 - 9.8	1	0	183	4.8 - 20.2
Linnemannia elongata	2351	0 - 4.3	2485	0 - 7.6	1681	0 - 14.7	2459	3.1 - 24.6
Linnemannia exigua	0	-	87	3.0 - 7.5				
Linnemannia gamsii	63	0 - 5.2	812	4.1 - 8.5	5	**0.6 - 1.7**	370	**6.5 - 21.6**
Linnemannia hyalina	341	0 - 3.4	1525	0 - 8.2	287	0 - 5.0	1476	3.1 - 23.4
Linnemannia nantahalensis	0	-	87	8.0 - 9.5	0	-	88	11.7 - 20.8
Linnemannia schmuckeri	1	0	185	0.1 - 8.4	1	0	185	4.8 - 20.3
Linnemannia sclerotiella	0	-	86	4.5 - 8.0				
Linnemannia sp nov	209	**0 - 1.0**	1263	**6.4 - 8.3**	71	**0 - 1.4**	805	**14.6 - 21.4**
Linnemannia zychae	0	-	87	6.9 - 9.6				

Table 2.9 (cont'd)

Lobosporangium transversale	1	**0**	180	**16.6 - 17.7**				
Lunasporangiospora chienii	0	-	82	9.1 - 13.7	0	-	82	13.8 - 23.1
Lunasporangiospora selenospora	1	2.3	182	9.1 - 13.1	1	2.5	179	13.8 - 22.7
Modicella reniformis	0	-	80	14.0 - 16.6	0	-	80	20.2 - 23.9
Mortierella alpina	2161	0 - 12.1	2470	0.3 - 14.8	2132	0 - 11.3	2501	2.2 - 22.7
Mortierella antarctica	0	-	83	6.2 - 12.0				
Mortierella bisporalis	0	-	89	9.8 - 11.9	0	-	83	11.1 - 21.1
Mortierella indohii	0	-	85	0 - 7.8				
Mortierella polycephala	5	**0.1 - 4.0**	272	**11.8 - 13.4**	11	0 - 3.5	367	0.1 - 20.9
Mortierella reticulata	0	-	85	0 - 7.6				
Necromycomortierella dichotoma	0	-	173	0.1 - 10.3				
Podila clonocystis	29	0 - 5.4	549	0 - 11.1	19	0 - 8.6	458	0 - 23.0
Podila epicladia	29	0 - 3.3	548	2.8 - 11.1	29	0 - 4.4	550	3.9 - 20.7
Podila epigama	0	-	86	12.2 - 13.3				
Podila horticola	55	0 - 7.7	721	0 - 13.5	19	**0 - 2.4**	460	**6.8 - 21.4**
Podila minutissima	928	0 - 7.3	2122	0 - 11.8	89	0 - 8.3	885	0 - 22.4
Podila verticillata	109	0 - 12.2	1059	0.4 - 13.9	89	**0 - 5.0**	884	**8.3 - 21.6**
Lichtheimia corymbifera								
Mucor circinelloides					0	-	75	30.4 - 34.9
Umbelopsis ramanniana					0	-	81	30.3 - 32.9

Table 2.9 (cont'd)

	4955				5512			
	Intraspecific		Interspecific		Intraspecific		Interspecific	
Species	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%
Actinomortierella ambigua	1	9.1	172	10.3 - 20.4	0	-	91	22.9 - 24.8
Actinomortierella capitata	1	0	177	10.3 - 21.1				
Actinomortierella wolfii	0	-	86	5.9 - 9.6				
Benniella erionia	1	0	174	14.1 - 16.2				
Benniella sp nov1	-	-	-	-				
Benniella sp nov2	0	-	182	0.4 - 10.3				
Dissophora decumbens	-	-	-	-				
Dissophora globulifera	5	**2.1 - 2.4**	271	**14.0 - 15.5**	1	2.2	183	17.6 - 19.1
Dissophora ornata	-	-	-	-				
Entomortierella beljakovae	-	-	-	-				
Entomortierella chlamydospora	0	-	85	0 - 10.1				
Entomortierella echinospaera	0	-	80	1.6 - 16.6				
Entomortierella lignicola	-	-	-	-				
Entomortierella parvispora	19	0.6 - 11.1	450	11.0 - 17.1				
Gamsiella multidivaricata	0	-	84	11.7 - 14.3				
Gamsiella stylospora	0	-	83	14.3 - 15.8				
Gryganskiella cystojenkinii	-	-	-	-	0	-	82	18.5 - 20.8
Gryganskiella fimbricystis	0	-	84	5.6 - 9.6	0	-	86	19.0 - 20.3
Linnemannia acrotona	0	-	84	0 - 8.5	0	-	87	0.1 - 17.8
Linnemannia amoedoidea	1	0	183	13.6 - 15.7				
Linnemannia camargensis	5	0 - 11.0	276	3.8 - 12.1	1	0	186	4.6 - 17.5
Linnemannia elongata	2255	0 - 4.8	2481	0 - 10.2	2255	0 - 6.0	2485	2.8 - 17.2
Linnemannia exigua	0	-	85	4.2 - 10.9	0	-	89	3.7 - 17.2
Linnemannia gamsii	71	0 - 6.3	804	4.5 - 11.3	29	0 - 8.7	552	6.0 - 19.7
Linnemannia hyalina	341	0 - 3.0	1523	0 - 10.2	305	0 - 4.1	1463	2.8 - 17.2
Linnemannia nantahalensis	0	-	85	7.2 - 10.6	0	-	88	10.6 - 17.9
Linnemannia schmuckeri	1	0	182	0 - 10.1	1	0	186	4.6 - 16.9
Linnemannia sclerotiella	0	-	84	5.3 - 8.3	0	-	86	7.3 - 15.9
Linnemannia sp nov	209	0 - 0.8	1262	0.4 - 11.9				
Linnemannia zychae	0	-	92	3.7 - 11.3				
Lobosporangium transversale								
Lunasporangiospora chienii	0	-	81	17.6 - 19.5				
Lunasporangiospora selenospora	0	-	84	15.4 - 17.1				
Modicella reniformis								

Table 2.9 (cont'd)

Mortierella alpina	2158	0 - 10.4	2476	2.2 - 14.5	1559	0 - 9.8	2384	3.3 - 18.3
Mortierella antarctica	0	-	86	6.5 - 13.4				
Mortierella bisporalis					0	-	84	10 - 20.1
Mortierella indohii					0	-	83	1.7 - 20.1
Mortierella polycephala	41	0 - 2.4	632	0 - 16.6	41	0 - 2.3	637	0 - 19.7
Mortierella reticulata	0	-	84	7.0 - 16.8				
Necromycomortierella dichotoma	0	-	175	0 - 13.9	0	-	87	15.5 - 17.5
Podila clonocystis	29	0 - 4.6	545	0 - 15.0	29	0 - 4.1	551	0 - 18.2
Podila epicladia	29	0 - 3.5	547	2.2 - 15.2	29	0 - 3.1	554	0.1 - 17.9
Podila epigama	0	-	83	15.2 - 17.6				
Podila horticola	71	0 - 3.8	802	0 - 14.5	71	0 - 6.7	808	0.2 - 19.5
Podila minutissima	869	0 - 4.5	2081	0 - 14.9	929	0 - 4.2	2126	0 - 18.0
Podila verticillata	129	0 - 13.9	1038	4.1 - 15.0	109	0 - 15.8	961	5.7 - 21.1
Lichtheimia corymbifera								
Mucor circinelloides								
Umbelopsis ramanniana								

Table 2.9 (cont'd)

	ef1a				rpb1			
	Intraspecific		Interspecific		Intraspecific		Interspecific	
Species	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%
Actinomortierella ambigua	0	-	82	1.7 - 4.8	0	-	174	10 - 20.1
Actinomortierella capitata	0	-	173	0.3 - 7.4	1	**0**	176	**7.6 - 16.6**
Actinomortierella wolfii	0	-	87	0.1 - 3.3	0	-	77	12.4 - 16.1
Benniella erionia					1	0.2	175	11.0 - 16.0
Benniella sp nov1	0	-	83	2.5 - 7.8	0	-	70	7.0 - 18.0
Benniella sp nov2	41	0 - 2.9	630	2.5 - 7.2	55	0 - 3.3	711	7.0 - 16.1
Dissophora decumbens	0	-	80	6.6 - 7.6	0	-	80	12.8 - 14.6
Dissophora globulifera	1	3.0	177	5.4 - 6.3	5	**1.8 - 2.2**	266	**13.2 - 16.3**
Dissophora ornata	0	-	84	2.9 - 3.8	0	-	80	12.6 - 14.3
Entomortierella beljakovae	0	-	86	3.8 - 5.1	0	-	81	0.1 - 14.4
Entomortierella chlamydospora	0	-	173	1.3 - 5.3	0	-	170	0 - 15.6
Entomortierella echinospaera	0	-	79	1.7 - 5.4	0	-	78	2.1 - 15.8
Entomortierella lignicola	1	1.3	173	3.5 - 5.5	1	0.7	169	11.8 - 17.7
Entomortierella parvispora	41	0.1 - 2.9	630	1.7 - 5.3	41	0 - 9.1	627	0.1 - 15.4
Gamsiella multidivaricata	1	0.7	179	3.7 - 5.3	1	0	172	13.9 - 16.1
Gamsiella stylospora	0	-	85	3.4 - 4.3				
Gryganskiella cystojenkinii	0	-	84	4.2 - 5.1	0	-	82	12.6 - 13.8
Gryganskiella fimbricystis	0	-	85	1.4 - 3.4	0	-	83	13.1 - 15.2
Linnemannia acrotona	0	-	90	0.2 - 2.7	0	-	88	0 - 8.9
Linnemannia amoedoidea	1	0	178	3.4 - 4.2	1	0.2 - 0.2	186	11.1 - 12.8
Linnemannia camargensis	2	1.4 - 4.2	275	2.5 - 4.7	3	0 - 11.6	281	3.8 - 11.7
Linnemannia elongata	1968	0 - 2.0	2768	1.2 - 3.2	2449	0 - 5.2	2488	2.9 - 10.3
Linnemannia exigua	0	-	88	1.4 - 3.2	0	-	88	3.9 - 9.8
Linnemannia gamsii	71	0.2 - 1.7	803	1.5 - 3.3	71	0 - 6.1	808	4.8 - 9.6
Linnemannia hyalina	323	0 - 2.7	1546	1.6 - 3.9	341	0 - 3.5	1528	2.9 - 9.8
Linnemannia nantahalensis	0	-	89	1.9 - 2.9	0	-	92	7.9 - 9.3
Linnemannia schmuckeri	0	**-**	186	**2.5 - 4.0**	1	0	185	0 - 9.5
Linnemannia sclerotiella	0	-	87	2.5 - 3.9	0	-	89	6.4 - 9.7
Linnemannia sp nov	209	0 - 1.6	1256	1.8 - 3.4	209	**0 - 0.4**	1267	**7.9 - 9.8**
Linnemannia zychae	0	-	86	1.6 - 3.0	0	-	88	7.0 - 10.7
Lobosporangium transversale	1	**0.6**	172	**7.8 - 9.2**	0	-	76	7.7 - 16.6
Lunasporangiospora chienii	0	-	82	3.3 - 3.9	0	-	83	13.8 - 16.0
Lunasporangiospora selenospora	0	-	87	0.2 - 3.6	0	-	74	13.2 - 16.4
Modicella reniformis	0	-	78	7.4 - 8.3	0	-	78	11.6 - 15.9

Table 2.9 (cont'd)

Mortierella alpina	1024	0 - 6.0	2511	0.4 - 6.0	2115	0 - 7.9	2615	2.7 - 15.3
Mortierella antarctica	0	-	89	2.3 - 4.3	0	-	87	4.3 - 14.2
Mortierella bisporalis	0	-	86	3.0 - 4.9	0	-	69	10.1 - 16.4
Mortierella indohii	0	-	77	0.7 - 5.1	0	-	75	1.0 - 15.6
Mortierella polycephala	29	0.2 - 2.1	547	0.4 - 5.7	41	**0 - 1.2**	628	**3.6 - 15.7**
Mortierella reticulata					0	-	79	7.8 - 16.7
Necromycomortierella dichotoma	5	**0 - 0.8**	270	**3.1 - 4.8**	5	**0 - 1.2**	277	**12.3 - 14.5**
Podila clonocystis	29	0.2 - 2.2	544	0.1 - 3.8	29	0 - 5.2	549	0 - 14.0
Podila epicladia	29	0.2 - 1.6	547	0.2 - 3.8	29	0 - 2.4	547	0 - 13.8
Podila epigama	0	-	83	2.9 - 4.4	0	-	89	12.3 - 15.3
Podila horticola	48	0 - 2.5	722	0.2 - 4.1	71	0 - 6.4	799	0.1 - 16.4
Podila minutissima	928	0 - 1.6	2121	0.1 - 3.7	929	0 - 5.0	2122	0 - 14.1
Podila verticillata	135	0.2 - 4.2	1130	1.1 - 4.6	146	0 - 15.0	1119	4.1 - 17.6
Lichtheimia corymbifera	0	-	68	14.2 - 15.1	0	-	69	26.5 - 27.7
Mucor circinelloides	0	-	66	14.2 - 15.0	0	-	74	25.2 - 27.1
Umbelopsis ramanniana	0	-	77	12.6 - 13.5	0	-	73	26.1 - 27.7

Table 2.9 (cont'd)

	ITS			
	Intraspecific		Interspecific	
Species	*n*	Min% - Max%	*n*	Min% - Max%
Actinomortierella ambigua	0	-	225	1.6 - 18.7
Actinomortierella capitata	1	0	500	1.3 - 18.9
Actinomortierella wolfii	0	-	225	1.9 - 18.4
Benniella erionia	0	-	225	2.5 - 21.8
Benniella sp nov1	0	-	225	3.0 - 11.9
Benniella sp nov2	55	**0 - 0.3**	2108	**2.3 - 18.8**
Dissophora decumbens	0	-	225	2.8 - 19.5
Dissophora globulifera	1	**0.3**	500	**1.7 - 19.6**
Dissophora ornata	0	-	225	2.9 - 19.8
Entomortierella beljakovae	0	-	225	2.3 - 18.8
Entomortierella chlamydospora	0	-	225	1.6 - 20.8
Entomortierella echinospaera	0	-	225	3.9 - 19.8
Entomortierella lignicola	0	-	225	3.1 - 19.7
Entomortierella parvispora	19	0.2 - 7.6	1313	2.9 - 21.7
Gamsiella multidivaricata	0	-	225	2.2 - 17.3
Gamsiella stylospora	0	-	225	2.3 - 19.3
Gryganskiella cystojenkinii	0	-	225	3.5 - 21.5
Gryganskiella fimbricystis	0	-	225	3.8 - 20.4
Linnemannia acrotona	0	-	225	0 - 20.8
Linnemannia amoedoidea	1	0	500	1.2 - 19.1
Linnemannia camargensis	5	0.2 - 4.5	773	1.2 - 19.3
Linnemannia elongata	2351	0 - 3.4	11169	1.2 - 21.2
Linnemannia exigua	0	-	225	1.2 - 19.2
Linnemannia gamsii	55	0 - 3.2	2108	2.5 - 19.0
Linnemannia hyalina	305	0 - 11.2	4628	1.9 - 21.8
Linnemannia nantahalensis	0	-	225	1.9 - 18.7
Linnemannia schmuckeri	0	-	225	1.9 - 19.3
Linnemannia sclerotiella	0	-	225	1.9 - 19.0
Linnemannia sp nov	209	0 - 0.2	3893	1.2 - 20.8
Linnemannia zychae	0	-	225	1.9 - 20.3
Lobosporangium transversale	1	0.1	500	0.6 - 18.2
Lunasporangiospora chienii	0	-	225	1.2 - 9.1
Lunasporangiospora selenospora	1	2.4	500	1.9 - 8.8
Modicella reniformis	0	-	225	2.3 - 17.8
Mortierella alpina	1559	0 - 6.5	9468	1.2 - 21.3
Mortierella antarctica	0	-	225	3.1 - 19.9
Mortierella bisporalis	0	-	225	2.5 - 20.3
Mortierella indohii	0	-	225	0.3 - 20.4

Table 2.9 (cont'd)

Mortierella polycephala	29	0 - 0.8	1580	0 - 21.2
Mortierella reticulata	0	-	225	1.6 - 18.3
Necromycomortierella dichotoma	5	**0 - 0.2**	773	**3.5 - 19.4**
Podila clonocystis	29	0 - 2.7	1580	0 - 21.2
Podila epicladia	29	0 - 2.3	1580	0.7 - 21.4
Podila epigama				
Podila horticola	55	0 - 0.8	2108	0.2 - 21.2
Podila minutissima	755	0 - 2.5	6948	0 - 21.8
Podila verticillata	109	0 - 14.5	2885	1.9 - 21.3
Lichtheimia corymbifera				
Mucor circinelloides				
Umbelopsis ramanniana				

Table 2.10 – Locus sequence variability at the genus level

Summary statistics from pairwise blastn analyses of the locus sequences as submitted to Genbank. Empty cells indicate that the locus was not recovered for any strains of any species in that genus. '*n*' = the number of non-self pairwise blastn analyses conducted, where '0' indicates that only one sequence was available to represent the genus and therefore no intrageneric analyses could be conducted. "Min% - Max%" = the range of percent sequence variability, where '0' means the sequences were identical. Values in bold indicate a meaningful difference between the maximum observed intrageneric variation and the minimum observed intergeneric variation.

	1870				**2451**				**4955**			
	Intrageneric		**Intergeneric**		**Intrageneric**		**Intergeneric**		**Intrageneric**		**Intergeneric**	
Genus	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%
Actinomortierella	2	2.1 - 2.1	287	0 - 18.0	0	-	93	19.1 - 21.5	11	0 - 12.3	474	3.7 - 21.1
Benniella	109	0 - 12.5	971	13.0 - 15.2	0	-	92	19.1 - 22.0	2	0 - 10.3	386	0.4 - 16.2
Dissophora	13	1.3 - 15.7	475	10.1 - 16.6	5	**1.6 - 2.0**	285	**15.8 - 20.2**	5	**2.1 - 2.4**	383	**13.6 - 15.8**
Entomortierella	9	5.5 - 14.0	676	0.1 - 17.3	29	**0 - 14.7**	557	**16.1 - 21.9**	59	0 - 16.8	1119	0 - 17.3
Gamsiella	0	-	194	11.9 - 14.1	1	14.5	190	15.0 - 20.3	1	12.8	190	11.7 - 15.8
Gryganskiella	0	-	94	13.0 - 14.0					1	8	190	0 - 9.6
Linnemannia	10700	0 - 12.0	582	0 - 11.9	4826	0 - 24.3	2988	17.4 - 24.6	10614	0 - 15.7	565	0 - 15.5
Lobosporangium	1	**0**	189	**16.6 - 17.7**								
Lunasporangiospora	5	**2.3 - 9.6**	285	**11.9 - 13.7**	5	**2.5 - 14.5**	285	**20.5 - 23.1**	1	**13.1**	188	**15.4 - 19.5**
Modicella	0	-	90	14.0 - 16.6	0	-	92	20 - 23.9	-	-	-	-
Mortierella	2639	0 - 14.1	3195	0 - 14.8	2930	0 - 20.5	2508	15.0 - 22.7	2996	0 - 16.6	3035	2.9 - 16.8
Necromycomortierella	0	-	192	0.1 - 10.3					0	-	192	0 - 13.9
Podila	3927	0 - 13.3	2601	0 - 13.9	1331	**0 - 12.2**	2325	**17.5 - 23.0**	4101	0 - 17.6	2423	0 - 17.6
Lichtheimia												
Mucor					0	-	90	30.4 - 35.1				
Umbelopsis					0	-	90	30.2 - 32.9				

Table 2.10 (cont'd)

	5512		5512		ef1a				rpb1			
	Intrageneric		Intergeneric		Intrageneric		Intergeneric		Intrageneric		Intergeneric	
Genus	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%	*n*	Min% - Max%
Actinomortierella	0	-	97	22.9 - 24.8	5	0.3 - 7.7	480	0.1 - 8.1	24	0 - 17.6	562	7.6 - 20.1
Benniella					55	**0 - 3.6**	729	**5.8 - 7.8**	107	0 - 12.8	974	11.6 - 18.0
Dissophora	1	2.2	192	17.6 - 19.1	6	3.0 - 6.5	481	2.9 - 7.6	19	1.8 - 13.5	468	12.6 - 16.3
Entomortierella					136	0 - 5.6	1735	1.7 - 5.6	217	0 - 17.6	1751	0 - 17.7
Gamsiella					5	0.7 - 4.2	286	3.3 - 5.3	1	0	190	13.9 - 16.1
Gryganskiella	1	14.3	192	18.4 - 20.8	1	2.7	289	1.4 - 5.3	5	**10.9 - 11.8**	287	**12.6 - 15.2**
Linnemannia	7739	**0 - 14.5**	1068	**15.1 - 19.7**	9597	0 - 4.6	1583	2.0 - 4.7	11287	0 - 12.8	95	0 - 11.1
Lobosporangium					1	**0.6**	189	**7.8 - 9.2**	0	-	95	0.2 - 16.6
Lunasporangiospora					0	-	192	0.2 - 3.9	1	**10.4**	190	**13.2 - 16.4**
Modicella					0	-	92	7.4 - 8.4	0	-	93	11.6 - 15.9
Mortierella	2029	0 - 19.2	3411	15.9 - 20.1	1697	0 - 6.0	3343	2.0 - 6.0	3126	0 - 16.5	3298	1.9 - 16.7
Necromycomortierella	0	-	96	15.5 - 17.5	5	**0 - 0.8**	285	**3.1 - 4.8**	5	**0 - 1.2**	287	**12.3 - 14.5**
Podila	4159	0 - 16.8	2270	15.9 - 21.1	4193	0 - 4.2	2431	0.1 - 4.6	4486	0 - 16.3	2238	1.9 - 17.6
Lichtheimia					0	-	88	14.2 - 15.1	0	-	90	25.9 - 27.7
Mucor					0	-	87	13.4 - 15.0	0	-	88	25.1 - 27.1
Umbelopsis					0	-	90	12.6 - 13.5	0	-	88	25.9 - 27.7

Table 2.10 (cont'd)

	ITS			
	Intrageneric		Intergeneric	
Genus	*n*	Min% - Max%	*n*	Min% - Max%
Actinomortierella	19	0.0 - 17.7	1353	0.0 - 19.1
Benniella	89	0.0 - 18.8	2668	2.3 - 22.1
Dissophora	19	0.3 - 18.4	1353	0.6 - 20.1
Entomortierella	209	0.0 - 20.2	3933	1.6 - 22.0
Gamsiella	1	11.4	540	2.2 - 19.3
Gryganskiella	5	7.5 - 8.7	813	3.5 - 22.1
Linnemannia	12209	0.0 - 17.1	18525	0.0 - 21.8
Lobosporangium	1	**0.1**	540	**0.6 - 18.2**
Lunasporangiospora	5	2.4 - 6.3	813	1.2 - 9.9
Modicella	0	-	265	2.3 - 18.8
Mortierella	3191	0.0 - 20.4	12585	1.2 - 21.4
Necromycomortierella	5	0.0 - 0.2	813	3.5 - 19.4
Podila	3659	0.0 - 18.8	13225	0.5 - 21.8
Lichtheimia				
Mucor				
Umbelopsis				

Table 2.11 – Mortierellaceae speceis chatacteristics

The current and proposed classification, synonyms, geographic distribution, ecology, endobacteria, and spore morphologies of Mortierellaceae species represented in this study.

New Name	Basionym/ Synonym	Habitat	Distribution	Sporangio-spores	Chlamydo-spores	Zygo-Spores/ Mating	BRE or MRE	Refer-ences	Myco-bank #
Actinomortierella ambigua	*Mortierella ambigua*	Soil & Dung	North America, Asia, New Zealand	Hyaline, smooth-walled, oblong	Frequent, globose with large oil globule, brown	Present, Hetero-thallic	BRE	Young, 1985 Watanabe, 2002 Takashima et al. 2018	MB308266
Actinomortierella capitata	*Mortierella capitata Mortierella vesiculosa Actinomortierella vesiculosa Carnoya capitata*	Cultivated Soil, Pillbug Gut, & Dung	North America, Asia, Europe	Spherical, leaving sporangio-phore as sticky mass	Unknown	Hyaline (Morpho-logically) Hetero-thallic	MRE	Degawa & Tokumasu, 1997	MB308267
Actinomortierella wolfii	*Mortierella wolfii*	Decaying Hay & Animal Lung	North America, Asia, New Zealand	Short-cylindrical, double memrane	Sometimes abundant, numerous amoeba-like appendages	Unknown		Gams, 1977 Domsch et al. 1980	MB308269
Lunasporangio-spora selenospora	*Mortierella selenospora*	Soil, Mushroom compost	Europe, Indonesia	Smooth, lunate	Scarce, terminal	Unknown	MRE	Gams, 1976 Takashima et al. 2018	MB833724
Lunasporangio-spora chienii	*Mortierella chienii Mortierella umbellata Actinomortierella umbellata*	Cultivated & Forest Soil	North America, Asia	Reniform, smooth, double walled, lunate	Absent	Unknown	BRE	Chien, 1972 Takashima et al. 2018	MB833681

Table 2.11 (cont'd)

Podila clonocystis	*Mortierella clonocystis*	Soil	Europe	Subglobose, smooth	Two types: 1) small, globose; 2) consisting of broadened hyphal branches, repeatedly dichotomous	Unknown		Gams, 1976 Takashima et al. 2018	
Podila epicladia	*Mortierella epicladia*	Forest Soil	Europe, South America	Globose, smooth	Scarce, lemon-shaped	Unknown		Gams, 1976 Young, 1985 Watanabe, 2002 Takashima et al. 2018	
Podila epigama	*Moriterella epigama*	Dung & Compost	Europe, Asia, Australia	Fusoid with rounded ends	Absent	Abundant Homo-thallic		Gams, 1976 Gams, 1977	
Podila horticola	*Mortierella horticola*	Soil, Roots of herbaceous plants	Cosmo-politan	Single spored, spinulose sporangia	Unknown	Unknown	BRE	Gams, 1977 Domsch et al. 1980 Takashima et al. 2018	
Podila humilis	*Mortierella humilis*	Forest & Grassland Soil, esp Acidic; Compost	Europe, Asia, North America, New Zealand	Single spored, finely verrucose sporangia	Unknown	Naked, Hetero-thallic	BRE	Domsch et al. 1980 Takashima et al. 2018	

Table 2.11 (cont'd)

Podila minutissima	*Mortierella minutissima*	Agricultural & Forest Soil; Semisaprophytic mycophile	Europe, New Zealand, North America, Australia	Hyaline, globose	Absent	Hyaline, globose, smooth, small, Hetero-thallic	MRE	Kuhlman, 1969 Kuhlman, 1972 Gams, 1977 Rudakov, 1978 Domsch et al. 1980 Takashima et al. 2018	MB833767
Podila verticillata	*Mortierella verticillata Mortierella marburgensis Haplosporangium fasciculatum Haplosporangium attenuatissimum*	Soil, root, stump	Asia, North America, Europe, Australia	Conidia. globose, smooth	Unknown	Naked, Hetero-thallic	BRE MRE	Kuhlman, 1969 Kuhlman, 1972 Gams, 1977 Domsch et al. 1980 Watanabe, 2002 Takashima et al. 2018	
Necromortierella dichotoma	*Mortierella dichotoma*	Mouse dung; Necrotrophyic mycophile	Germany	Unknown	Unknown	Unknown		Rudakov, 1978	MB833726
Gryganksiella cystojenkinii	*Mortierella cystojenkinii*	Agricultural Soil	Europe	Ellipsoidal to cylindrical, smooth	Abundant, globose, thick-walled, light brown	Unknown		Gams, 1976	MB833858

Table 2.11 (cont'd)

Gryganksiella fimbricystis	*Mortierella fimbricystis*	Bog	South America	Ellipsoidal to cylindrical, smooth	Abundant, aerial, intercalary, ochre-orange, densely covered with fimbriate appendages	Unknown		Gams, 1976 Young, 1985	MB833859
Linnemannia acrotona	*Mortierella acrotona*	Soil	India	Unknown	Unknown	Unknown		Gams, 1976	MB833769
Linnemannia amoeboidea	*Mortierella amoeboidea*	Soil	North America, Europe	Elongate ellipsoidal smooth	Abundant, either: large, light brown, blunt appen-dages; small, smooth	Unknown		Gams, 1976	MB833770
Linnemannia camargensis	*Mortierella camargensis Haplosporangium gracile*	Soil	Europe	Sporangioles	Unknown	Unknown		Gams, 1977	
Linnemannia elongata	*Moriterella elongata Mortierella rishikesha Mortierella debilis*	Agricultural Soil	Cosmo-politan	Eongated, central oil droplet	Brown, thick walled	Present, Hetero-thallic	BRE MRE	Kuhlman, 1969 Kuhlman, 1972 Gams, 1977 Rudakov, 1978 Domsch et al. 1980 Takashima et al. 2018	MB833768

Table 2.11 (cont'd)

Linnemannia exigua	*Moriterella exigua* *Mortierella indica* *Mortierella sterilis*	Agricultural & Forest Soil, Crop Plant Rhizosphere	Europe, India, New Zealand	Cylindrical	"Amoeba-like" globose with irregular radiating hyphae	Unknown		Gams, 1977 Domsch et al. 1980 Watanabe, 2002	
Linnemannia gamsii	*Mortierella gamsii* *Mortierella spinosa* *Mortierella mutabilis*	Forest Soil, Bat Carcass	Japan, Europe, North America, Australia	Globose to slightly ellipsoidal	Small, regular	Rare, Globose, Hetero-thallic	BRE MRE	Kuhlman, 1975 Gams, 1977 Domsch et al. 1980 Takashima et al. 2018	
Linnemannia hyalina	*Mortierella hyalina* *Hydrophora hyalina* *Mortierella candelabrum var. minor* *Mortierella hygrophila* *Mortierella hygrophila var. minuta*	Facultative Biotrophic Mycophile; Soil, Roots, Basidiocarp, Decaying Plant Material, Dung	Europe, India, China, North America, Antarctica	Hyaline, subgolobse or ellispoidal	Ellipsoidal, solitary	Unknown assumed hetero-thallic		Gams, 1977 Rudakov, 1978 Domsch et al. 1980 Young, 1985 Watanabe, 2002	MB833682
Linnemannia nantahalensis	*Mortierella nantahalensis*	Soil	North America	Spherical, thick walled, yellow in mass	Absent	Unknown		Chien, 1971	
Linnemannia schmuckeri	*Mortierella schmuckeri*	Soil	Mexico, India, Wyoming	Present	Unknown	Unknown		Young, 1985	
Linnemannia sclerotiella	*Mortierella sclerotiella*	Mouse Dung	Europe, Asia	Ellipsoidal to subglobose, minute striate orna-mentation	Abundant, globose, ochraceous	Unknown		Gams, 1977	

Table 2.11 (cont'd)

Linnemannia sp nov		Soil	Australia, North America			Unknown			
Linnemannia zychae	*Mortierella zychae Mortierella brachyrhiza*	Ant Pellet, Decaying Wood, Bog, Horse Manure	Europe, Africa, New Zealand, North America, Puerto Rico	Ellipsoid	In chains or clusters	Hyaline, sub-globose to globose, uncov-ered Hetero-thallic		Kuhlman, 1969 Kuhlman, 1972 Watanabe, 2002	
Mortierella alpina	*Mortierella oblatispora Mortierella acuminata Mortierella renispora Mortierella monospora Mortierella thaxteri*	Water fungi & Truffle, Soil, Vermiculture, Alga, Plant Detritus, Fish Kidney & Air Bladder	Asia, Europe, North America, Australia	Ellipsoid, smooth, hyaline	Scarce	Hyaline, smooth, wall with three distinct layers; suspen-sors hetero-gamous, Hetero-thallic	BRE MRE	Dixon-Stewart, 1932 Gams, 1977 Kuhlman 1975 Domsch et al. 1980 Watanabe, 2002 Takashima et al. 2018	MB170280
Mortierella antarctica		Soil, Root Tip, Fish Air Bladder	Antarctica, USA, Europe	Present	Unknown	Unknown		Westerdijk Fungal Biodiversity Institute	MB317880

Table 2.11 (cont'd)

Mortierella bisporalis	*Haplosporangium bisporale*	Facultative Biotrophic Mycophile, Truffle	Europe	Strongly verrucose and ridged, sporangia one or two-spored	Unknown	Unknown		Thaxter, 1914 Gams, 1977 Rudakov, 1978 Domsch et al. 1980 Young, 1985 Watanabe, 2002	MB258541
Mortierella indohii		Agricultural Soil, Animal Dung	Asia, Europe, Africa, North America	Absent	Stylospores	Partially invested, Hetero-thallic		Chien et al. 1974	MB317900
Mortierella polycephala	*Mortierella polygonia* *Mortierella canina* *Mortierella echinulata* *Mortierella crystallina* *Mortierella angusta* *Mortierella vantieghemi* *Mortierella vantieghemii* *Mortierella raphani* *Mortierella vantieghemi var. raphani* *Mortierella lemonnieri*	Soil, Mouse & Rabbit Dung, Bat Cave, Bear Pen Soil, Mushroom	Europe, India, North & South America	Ovoidal to irregular	Stylospores spherical, verrucose to echinulate	Present, Homo-thallic or hetero-thallic		Domsch et al. 1980 Gams, 1977 Young, 1985	MB145769

Table 2.11 (cont'd)

Mortierella reticulata		Bear Pen Soil, Soil, Mouse Dung, Forest Soil	North & South America, Europe	Reticulated	Submerged, smooth	Unknown		Young, 1985	MB236117
Entomortierella beljakovae	*Mortierella beljakovae*	Root, Soil, & Ant Pellet	North America & Europe	Short ellipsoidal to subglobose, smooth	Abundant, solitary or in chains or irregular clusters, globose, thick-walled, ochraceous	Smooth and thick-walled, Hetero-thallic		Gams, 1977	MB833729
Entomortierella chlamydospora	*Mortierella chlamydospora Azygozygum chlamydosporum*	Soil, Roots	Japan, Europe, North America	Absent	Spiny, often aerial	Naked, one large suspensor Homo-thallic		Watanabe, 2002	
Entomortierella echinosphaera	*Mortierella echinosphaera*	Soil, Rotting Roots, Vermiculture	North America, Europe, Malaysia	Present	Intercalary or terminal, sometimes blunt spines	Unknown		Watanabe, 2002	
Entomortierella lignicola	*Mortierella lignicola Haplosporangium lignicola Mortierella sepedonioides*	Decaying Wood, Termite Nests	North & South America, Europe	Conidia/stylospores, spines short & stout	Unknown	Unknown		Kuhlman, 1969 Watanabe et al. 1998	

Table 2.11 (cont'd)

Entomortierella parvispora	*Mortierella parvispora Mortierella gracilis*	Soil, Decaying Wood, Needle Litter in Ant Mound	Asia, Europe, North America, Antarctica, Brazil	Globose, smooth	Absent	Globose, Hetero-thallic	BRE	Kuhlman, 1972 Gams, 1977 Domsch et al. 1980 Young, 1985 Takashima et al. 2018	
Lobosporangium transversale	*Echino-sporangium transversalis*	Soil	North America	Irregularly shaped	Unknown	Absent	MRE	Malloch, 1967	MB488122
Gamsiella multidivaricata	*Mortierella multidivaricata*	Decaying Wood	Asia	Sporangia two-spored, sporangio-phores branch repeatedly	Globose, ornamented, usually terminal	Unknown		Benjamin, 1978 Young, 1985	MB488121
Gamsiella stylospora	*Mortierella stylospora*	Soil, Rabbit Dung, Bear Pen Soil	North America, Europe, New Zealand	Stylospores single on unbranched aerial hyphae	Few, terminal	Present, Homo-thallic		Dixon-Stewart, 1932 Young, 1985	MB833728
Modicella reniformis			South America	Unknown	Unknown	Unknown	MRE	Ger-demann & Trappe, 1974	MB317772
Benniella erionia		Soil	Australia, North America			Unknown	MRE		MB833779
Benniella sp nov 1		Soil	North America			Unknown			
Benniella sp nov 2		Soil	Uganda			Unknown	MRE		

Table 2.11 (cont'd)

Dissophora decumbens		Forest Litter	North America	Globose to angular or irregular in shape	Terminal or intercalary, often clustered	Unknown		Thaxter, 1914	MB160412
Dissophora globulifera	*Mortierella globulifera Mortierella ericetorum*	Forest Litter, Agricultural Soil	Japan, Europe	Globose to subglobose, echinulate, hyaline	Globose to irregular, smooth, submerged, thin-walled	Globose, Hetero-thallic		Turner, 1956 Kuhlman, 1972	MB833727
Dissophora ornata		Forest Soil	South America	Isodiametric, elongate or irregulary lobate	Globose, thin-walled hyphal swellings, intercalary or terminal, sometimes in nests	Unknown		Veerkamp & Gams, 1983	MB135572

Table 2.12 - A comparison of historic Mortierellaceae phylogenies

A comparison of the Mortierellaceae phylogenies generated based on the species included in this study, Wagner et al. (2013), Petkovits et al. (2011), and the taxonomic groupings of Linnemann and Gams published in 1977 - 1989.

This Study	Species Not Included	Wagner (2013)	Petkovitz (2011)	Gams (1977-1989) + Gamsiella & Dissophora
Actinomortierella ambigua		5 - strangulata & wolfii	N/A	Actinomortierella
Actinomortierella capitata *Actinomortierella wolfii*			11 /wolfii	
				Spinosa
Lunasporangiospora selenospora		1 - selenospora & parvispora	12 /selenospora	Hygrophila
Lunasporangiospora chienii			N/A	Spinosa
	Mortierella macrocystis *Mortierella jenkinii* *Mortierella pulchella*		N/A	Hygrophila
			N/A	Spinosa
			9 /parvispora	
	Mortierella alliacea		N/A	Alpina
Podila clonocystis *Podila epigama* *Podila minutissima*		2 - verticillata-humilis	8 /verticillata-humulis	Hygrophila
Podila epicladia *Podila horticola* *Podila humilis* *Podila verticillata*				Spinosa Stylospora
Necromycomortierella dichotoma		1 - selenospora & parvispora	9 /parvispora	Hygrophila
Gryganksiella cystojenkinii				Spinosa
Gryganksiella fimbricystis			N/A	
Linnemanniella acrotona *Linnemanniella exigua* *Linnemanniella gamsii* *Linnemanniella nantahalensis*		7 - gamsii	1 /gamsii	
Linnemanniella amoeboidea				Simplex
Linnemanniella camargensis *Linnemanniella schmuckeri*		7 - gamsii	1 /gamsii	Schmuckeri

Table 2.12 (cont'd)

Linnemanniella elongata *Linnemanniella hyalina* *Linnemanniella sclerotiella* *Linnemanniella zychae*			Hygrophila
Linnemanniella sp nov	N/A		
Mortierella claussenii	7 - gamsii	N/A	Schmuckeri
Mortierella bainieri *Mortierella sarnyensis* *Mortierella armillariicola*		N/A 1 /gamsii	Hygrophila
Mortierella zonata			Stylospora
Mortierella alpina	6 - alpina & polycephala	10 /alpina	Alpina
Mortierella antarctica *Mortierella bisporalis*		2 /polycephala	Mortierella
Mortierella indohii			(isolated sp)
Mortierella polycephala			Mortierella
Mortierella reticulata		N/A	
Entomortierella parvispora	1 - selenospora & parvispora	9 /parvispora	Spinosa
Entomortierella beljakovae	3 - lignicola	6 /lignicola	Hygrophila
Entomortierella chlamydospora			(isolated sp)
Entomortierella echinosphaera			(isolated sp)
Entomortierella lignicola			Stylospora
Mortierella gemmifera *Mortierella khulmanii*			Hygrophila
Lobosporangium transversale	5 - strangulata & wolfii	7 /strangulata	N/A
Mortierella stangulata			Simplex
Gamsiella multidivaricata	4 - globulifera & angusta	5 /mutabilis	Gamsiella
Gamsiella stylospora			Stylospora
Dissophora globulifera		4 /globulifera	Simplex
Dissophora decumbens		3 /angusta	Dissophora
Dissophora ornata			Simplex/Dissophora
Mortierella angusta			Simplex

Table 2.12 (cont'd)

Mortierella echinula		N/A	Hygrophila
Modicella malleola *Modicella reniformis*		N/A	
Benniella erionia *Benniella* sp nov 1 *Benniella* sp nov 2		N/A	

CHAPTER 3. *MORTIERELLA ELONGATA* STIMULATES AERIAL GROWTH, SEED PRODUCTION, AND RESPONSES TO AUXIN, ETHYLENE, AND REACTIVE OXYGEN SPECIES IN ARABIDOPSIS THALIANA

Authors & Contributions

1. Natalie Vandepol - experiment design & completion, data collection, data analysis, & writing
2. Julian Liber - generation of cured fungal isolates, qPCR, root harvesting
3. Jason Matlock - statistical analysis of plant biomass data
4. Gregory Bonito - research support, experiment design

Introduction

Microbial promotion of plant growth has great potential to improve agricultural yields and protect plants against pathogens and/or abiotic stresses, while also relieving economic and environmental costs of crop production (Li et al. 2018; Bedini et al. 2018). Agriculturally important metrics pertaining to plant growth promotion include aerial biomass, root biomass, root architecture, seed number, seed size, and flowering time. One group of plant beneficial microbes is early-diverging filamentous fungi, which have been implicated in assisting plants in the colonization of land (Field et al. 2015). There are three main guilds of plant mutualistic fungi relevant to this study: arbuscular mycorrhizal (AM) fungi, ectomycorrhizal (EM) fungi, and non-mycorrhizal (NM) endophytic fungi. For the purpose of this study, NM root endophytes are defined as fungi that are found inside healthy plant roots but do not make any characteristic mycorrhizal structures. Most of these fungi are thought to promote plant growth primarily by providing water and mineral nutrients, and sometimes secondarily by precluding infection by pathogens and/or priming and regulating plant defense responses (Hooker et al. 1994). However, the signaling mechanisms and fungal symbiotic structures are very distinct between these functional guilds, largely because most EM and NM associations represent convergent evolution on a phenotype, rather than a shared evolutionary mechanism of interaction (Tedersoo et al. 2010).

Mortierellaceae are soil fungi in the subphylum Mortierellomycotina; they are closely related to Glomeromycotina (AMF) and Mucoromycotina, some of which are EM fungi (James et al. 2006; Spatafora et al. 2016). Plant associations with *Mortierella* have been recorded since the early 1900s and are broadly considered NM plant associates (Stiles, 1915; Bisby et al. 1935; Domsch et al. 1980). Research on several *Mortierella* species have been published this past decade, however, the extent of the plant growth promotion (PGP) phenotype(s) and the underlying mechanism(s) of association are still not well understood.

Recent inoculation studies of *Mortierella* on plant roots showed that these fungi elicit a strong plant growth promoting (PGP) phenotype on a broad range of plant hosts (Li et al, 2018; Ozimek et al. 2018; Johnson et al. 2019). Maize plants inoculated with *Mortierella elongata* had increased plant height and dry aerial biomass and analysis of phytohormone levels indicated high levels of abscisic acid and the auxin IAA (indole-3-acetic acid) in response to *M. elongata* (Li et al. 2018). In contrast, Arabidopsis inoculated with *M. hyalina* also showed increased total leaf surface area and aerial dry biomass, with reduced levels of abscisic acid and no stimulation of auxin-responsive genes (Johnson et al. 2019). *Mortierella antarctica* increases the growth of winter wheat by producing phytohormones IAA and gibberellic acid and the enzyme ACC (1-aminocyclopropane-1-carboxylate) deaminase, which degrades ACC, a precursor to the phytohormone ethylene (Ozimek et al. 2018). In this study, we have focused on the symbiosis between *M. elongata* and Arabidopsis, as both organisms have reference genomes (and transcriptomes) available, and their lifestyles and growth requirements are conducive to research conditions. We measured PGP of aerial growth at early and late life stages, seed production, and used RNA sequencing to characterize differentially expressed plant genes in response to fungal treatments.

One additional question pertaining to *Mortierella* has to do with whether endohyphal bacteria that colonize these fungi impact their PGP phenotype. Although the incidence of endobacteria within isolates of *Mortierella* is quite low (<10%), a diversity of bacteria including *Mycoavidus*

cysteinexigens and *Mycoplasma*-related endobacteria (MRE) are known to colonize mycelium of diverse species across seven of the eight established Mortierellaceae phylogenetic clades (Ohshima *et al.* 2016; Uehling *et al.* 2017; Desirò *et al.* 2018; Takashima *et al.* 2018). Many species, such as *M. elongata*, can harbor both *M. cysteinexigens* and MRE, however, there appears to be a single lineage of endobacteria within any particular isolate (Desirò *et al.*, 2018). Both MRE and a lineage of *Burkholderia*-related endobacteria (BRE) closely related to *Mycoavidus cysteinexigens* (i.e. *Ca.* Glomeribacter) are found in the Glomeromycotina and their impact on the fungal-plant interaction has been characterized (Bonfante & Desirò, 2017). In this study, we used two isolates of *M. elongata*, NVP64 and NVP80, to better understand mechanisms underlying *M. elongata* symbioses with plants. These isolates are colonized by *Mycoavidus cysteinexigens* (NVP64) and MRE (NVP80), designated as NVP64wt and NVP80wt because they are the wild-types of these strains. We generated "cured" isogenic lines of each isolate, NVP64cu and NVP80cu, where the endobacteria were removed though antibiotic treatments, to determine whether either endobacterium has an impact on the plant-fungal symbiosis.

Materials & Methods

Plant and fungal culturing

Growth media

Fungal strains were cultured in malt extract broth [MEB: 10 g/L Malt Extract (VWR), 1 g/L Bacto Yeast Extract (Difco, Thomas Scientific; New Jersey, USA)], malt extract agar [MEA: 10 g/L Malt Extract, 1 g/L Bacto Yeast Extract, 10 g/L Bacto Agar (Difco)], and Kaefer Medium [KM: 20 g/L D-Glucose, 2 g/L Peptone, 1 g/L Yeast Extract, 1 g/L Bacto Casamino Acids (Difco), 2 mL/L Fe-EDTA [2.5 g $FeSO_4*7H_2O$, 3.36 g Na_2EDTA, 500 mL water], 50 mL/L KM Macronutrients [12 g/L $NaNO_3$, 10.4 g/L KCl, 10.4 g/L $MgSO_4*7H_2O$, 30.4 g/L KH_2PO_4], 10 mL/L KM Micronutrients [2.2 g/L $ZnSO_4*7H_2O$, 2.2 g/L H_3BO_3, 0.16 g/L $CuSO_4*5H_2O$, 0.5 g/L $MnSO_4*H_2O$, 0.16 g/L $CoCl_2*5H_2O$, 0.11 g/L $(NH_4)_6Mo_7O_{24}*4H_2O$], pH 6.5 with 10 N KOH, and supplemented

with Thiamine (1 mg/L) and Biotin (0.5 mg/L) after autoclaving and cooling to 60°C]. Sterilized seeds were germinated on Murashige & Skoog medium [1xMS: 4.4 g/L Murashige and Skoog medium (Sigma Aldrich; Missouri, USA), pH 5.7 w/ KOH, and 10 g/L agar (Sigma, product# A1296)]. Plant-fungal experiments were conducted on Plant Nutrient Medium [PNM: 0.5 g/L KNO_3, 0.49 g/L $MgSO_4*7H_2O$, 0.47 g/L $Ca(NO_3)_2*4H_2O$, 2.5 mL/L Fe-EDTA, 1 mL/L PNM Micronutrients [4.3 g/L Boric Acid, 2.8 g/L $MnCl_2*4H_2O$, 124.8 mg/L Cupric Sulfate, 287.5 mg/L $ZnSO_4*7H_2O$, 48.4 mg/L $Na_2MoO_4*2H_2O$, 2.4 mg/L $CoCl_2*6H_2O$], 10 g/L agar (Sigma, product# A1296), autoclaved and the pH adjusted with 2.5 mL/L 1M H_2KPO_4 before pouring 22-24mL per 100mm^2 square plate (with grid)].

To generate a fungal substrate suitable for inoculating potting mix, white millet (Natures Window; Michigan, USA), horticultural perlite (PVP Industries, Inc; Ohio, USA), and pearled barley (International Foodsource; New Jersey, USA) were each soaked overnight in DI water. The millet and barley were each boiled in fresh DI water on a hotplate until the grains began to break open, then removed from the hotplate and drained of excess water. When prepared, millet and barley expand to about 150% and 300% of the dry volume, respectively. The boiled millet, boiled barley, and perlite were mixed in a 2:1:1 v:v:v ratio. For each treatment, 600 mL of this “millet mix” was placed into a gusseted Unicorn bag (Unicorn Bags, type 10T; Texas, USA) and autoclaved for 45 minutes, allowed to rest overnight under a sterile hood and autoclaved again for 45 minutes.

To generate sterile SureMix-based plant growth substrates, SureMix Perlite (Michigan Growers Products; Michigan, USA) substrate was saturated with deionized water, which was measured and placed into autoclavable bags to ensure the correct volume would be available, 1 bag for each experimental treatment. Potting mix bags were autoclaved 45 minutes on a liquid cycle, stored at room temperature for 3-7 days, autoclaved again for 45 minutes on a liquid cycle, cooled to room temperature, and rinsed through with 3L MilliQ water (18 MΩ·cm). Potting mix rinsing was performed on a dish cart covered with a double layer of window screen mesh and wrapped in a funnel fashioned of garbage bags to direct water into a floor drain, all sterilized with

Bleach and rinsed with MilliQ water.

Arabidopsis Seed Sterilization & Germination

Arabidopsis thaliana Col-0 CS70000 were obtained from the Arabidopsis Biological Resource Center. Seeds were germinated and grown for three generations in a grow room. Bulk seed was collected from the third generation and screened to homogenize seed size with 350 µm and 250 µm sieves (VWR, Pennsylvania, USA), retaining the middle fraction.

Arabidopsis seeds were divided from the screened stock into 1.5mL Eppendorf tubes using a 200 seed spoon, with up to 1200 seeds per tube. Seeds were surface sterilized by: 1) washing in 800 µL 70% Ethanol for 20 seconds, 2) discarding the ethanol, 3) washing in 400 µL 20% bleach (Clorox Performance, 8.3% Sodium Hypochlorite, Clorox, California, USA) for 30 seconds, 4) quenching with 1 mL sterile water, 5) discarding the liquid, 6) repeating steps 4 & 5 three times, and 7) resuspending in 500 µL sterile water.

Surface sterilized seeds were plated on 1xMS using a p1000 and sterile water, 16 seeds per plate in rows of 3, 4, 5, and 4, with about 1cm between seeds and rows (**Fig. 3.1a**). We germinated at least 5 times as many seeds as were needed for the experiment to allow greater control of seedling size.

1xMS germination plates were cold stratified for 2 days in the dark at 4°C to synchronize germination, then allowed to germinate and grow for 5 or 10 days, depending on the experiment, in a Percival I-36LLVL growth chamber at 103-118 µmol light with 16 hr day & 8hr night, 22°C, ambient humidity. Light levels were measured using an LI-250A light meter (LI-COR, Nebraska, USA).

Potting Mix Experiments

Millet Inoculum

Each fungal strain was grown in 4-6 250 mL Erlenmeyer flasks with 75 mL of MEB for 2 weeks. Colonized medium was poured out into an autoclaved beaker and the mycelium collected with sterile tweezers, coarsely chopped in a sterile petri dish, and added to sterile millet mix bags. The

bags were lightly mixed, sealed in two places with an impulse sealer, and the fungi allowed to colonize the spawn for 2 weeks.

Arabidopsis Growth Conditions

Five days after germination, Arabidopsis seedlings were transplanted from 1xMS plates to plug trays of autoclaved and rinsed SureMix and moved to a Bio Chambers AC-40 growth chamber with 16hr day, 8hr night, 22°C, ambient humidity. Seedlings were grown in plugs for 11 days (16 days after germination). The soil plugs and seedling roots were treated with Zerotol 2.0 (BioSafe Systems, Connecticut, USA), an algaecide, bactericide, and fungicide containing Hydrogen Peroxide & Peroxyacetic Acid. The Zerotol was applied as a soil drench for 15 minutes, rinsed three times with distilled water, and transplanted into $4in^3$ pots with SureMix mixed with the appropriate millet mix treatment. Each treatment was contained in a separate waterproof tray with an 18 pot capacity (3 rows of 6 pots). Using seventeen pots per treatment left an empty spot for watering. Four days after transplanting, seedlings were treated with 2L of Peters 20-20-20 fertilizer mixed at 1/8th strength (0.1tsp/L) in MilliQ water. Thereafter, plants were watered with MilliQ water as needed.

Above Ground Biomass

At 34 days after transplanting and inoculation (50 days after germination), all treatments were observed to have ripening siliques, necessitating harvesting to avoid excessive loss of seed biomass during plant handling. Twelve plants per treatment were harvested by cutting the roots at the potting mix line and trimming and/or folding the aerial parts into tared envelopes (Top Flight no.10 Security Envelope, Strip & Seal). Fresh weight was recorded immediately after harvesting was complete. Plants were dried at room temperature (20-22 ˚C) for 2 weeks and re-weighed for the dry biomass. All envelope and plant biomass measurements were taken on a Mettler Toledo PG2002-S scale.

Seed Collection

Five plants were randomly selected for seed collection. ARACON tubes (Arasystem, Belgium)

were installed over the rosette. When the remaining plants were harvested for biomass, these five plants were moved to a drying room for two weeks. Dry plant material was collected and stored in wax paper bags until processing. Seeds were isolated from plant material by manually massaging the bags to release seeds, filtering through a Rösle Stainless Steel Fine Mesh Tea Strainer (Wire Handle, 3.2-inch, model# 95158) to remove large plant debris, repeatedly passing over copier paper, and picking out remnant plant matter with tweezers. Cleaned seeds were collected in tared 2mL Eppendorf tubes and weighed on a Mettler Toledo AB104-S/FACT scale. To determine average seed mass, approximately 14mg of seeds per sample were weighed on an ultrasensitive balance, adhered to a piece of white paper, scanned, and counted by image analysis in ImageJ (Appendix D.2).

Statistical Analysis

Since the data were extremely non-normal, we performed Wilcox ranked sum tests. Between NVP64cu v. NVP64wt, NVP80cu v. NVP80wt, and NoMillet v. Uninoculated, we used two-tailed tests. Between each fungal treatment and the Uninoculated, we performed one-tailed tests with the alternative hypothesis being “less” or “greater” as appropriate. Data analysis and visualization was conducted in R using the ggpubr and ggsignif packages (Ahlmann-Eltze, 2019; Kassambara, 2019).

Agar-Based Experiments

Transplanting & Inoculation

We based the design of these experiments on the methodology used by Johnson et al. (2019). Arabidopsis seeds were surface sterilized and germinated as described previously. Ten days after germination, seedlings were categorized into three approximate seedling size “categories”: too small, too big, and average. Three “average” seedlings were transplanted to each mPNM plate such that the cotyledons aligned with the top line of the plate grid and the roots were not covered by the grid pattern (**Fig. 3.1b**). Each plate was numbered as it was populated with seedlings so that plates could be assigned to treatments serially (e.g., 1-A, 2-B, 3-C, 4-A, 5-B, 6-C, 7-A, etc.),

to homogenize variation and bias in seedling size throughout the transplant procedure. Plates were inoculated by transferring two 5mmx5mm squares of Kaefer Medium, sterile or colonized by the appropriate fungal culture, between the three seedlings.

Root Length

After transplanting and inoculation, seedlings and fungi were left undisturbed for one day to adhere to the media and minimize the likelihood of movement during handling. The plates were then imaged on an Epson scanner at 1200 dpi using Home mode and default settings (**Fig 3.1b**). Images were processed in ImageJ v.1.52p, using the 13 mm grid on the plates as a scale, the freehand line tool to trace the roots, and the measuring tool to determine starting root length of each seedling.

Growth Chamber

Light levels were measured using an LI-250A light meter (LI-COR) at 9 different points on each of the four shelves in the growth chamber (**Table 3.1**). To homogenize variability in environmental conditions across treatments, plates were distributed between light level regions and the lower three shelves as evenly as possible and their location in the chamber recorded. Each of the shelves accommodate 3 rows of 15 plates, with 5 plates assigned to each of the 9 zones on the shelf (**Fig. 3.2**).

Bolting Panel

To determine whether bolting time was affected by fungal colonization, PNM plates with 10 day old Arabidopsis seedlings were inoculated and monitored daily for evidence of bolting, which was defined as visible elongation of the emerging inflorescence away from the rosette (**Fig. 3.3**). As each plant bolted, the date was noted on the plate.

Harvesting Aerial Plant Material

The aerial portion of each plant was cut away from the roots and placed into a folded “envelope” made from weigh paper and dried in a 65°C drying oven for 48 hours. The envelopes of dried plants were stored in empty tip boxes and double bagged with Ziplock bags to prevent

reabsorption of atmospheric water before weighing. Dry plants were weighed on a DeltaRange XP26 ultrasensitive balance (Mettler Toledo; Ohio, USA).

Curing Fungi of Endobacteria

M. elongata lines NVP64wt and NVP80wt were cured of their endobacteria by repeated culturing in media containing antibiotics, a protocol adapted from Uehling et al. (2017). Fungi were transferred between MEB and MEA supplemented with 1 g/L Bacto Peptone (Difco), 100 µg/mL ciprofloxacin, 50 µg/mL kanamycin, 50 µg/mL streptomycin, and 50 µg/mL chloramphenicol. Each transfer was performed by transplanting a 1-4 mm^2 piece of tissue from the outer edge or surface of the mycelium with a Nichrome inoculating loop and submerging the tissue under the agar surface or broth to maximize contact of the growing hyphae with the antibiotics. Transfers were performed every 3 or 4 days, alternating agar and broth substrate, for a total of 7 transfers in antibiotic media.

Following antibiotic curing, tissue from the original and newly-cured lines, as well as the wild-type line, were cultured on antibiotic-free 60 mm MEA plates with an autoclaved cellophane sheet placed atop the agar. After 13 days of incubation, fungal tissue was scraped off the cellophane and DNA extracted using a CTAB-based chloroform extraction protocol (Supplementary Methods - CTAB-based DNA extraction protocol; Doyle, 1991).

Statistical Analysis

We conducted statistical analyses in R v.3.6.0 using the tidyverse v1.3.0, lme4 v1.1-21, lmerTest v3.1-1, car v3.0-6 packages (R Core Team, 2013; Bates et al. 2015; Kuznetsova, Brockhoff, & Christensen, 2017; Fox & Weisberg, 2019; Wickham et al. 2019). Bolting data were visualized as boxplots and visibly non-normal. We used the Kruskal-Wallis test to examine differences in bolting age between treatments (Kruskal & Wallis, 1952). Aerial dry weight data were visualized as boxplots and assessed as approximately normal and homoskedastic. We used analysis of variance (ANOVA) and linear models to examine differences in dry weight within each experimental dataset to determine the effects of environmental factors tested by each experiment.

Based on the results of these tests, we constructed a linear mixed model of the combined dry weight data from the two agar experiments, specifying treatment and seedling root length as fixed effects and experiment (Media Panel & Cured Panel) and plate (to account for three plants measured per plate) as nested grouping factors:

$$DryWeight \sim Treatment + RootLength + (1 \mid Experiment) + (1 \mid Plate)$$

We used the emmeans v1.4.4 package to perform pairwise comparisons of the model estimates for each treatment (Lenth, 2020). The estimated marginal mean, confidence interval, and significance groups were extracted for graphical summarization.

RNA Sequencing & Differential Gene Expression

Root Harvesting & Storage

The root material for the RNAseq experiment was collected from the plants generated in the Agar experiments (**Fig. 3.4**). Before collecting the aerial parts of the plants for biomass assays, five plates were selected from each treatment on the basis of similar light levels within the chamber. For each selected plate, two RNAse-zap treated, DEPC water rinsed, autoclaved steel beads were placed in one RNAse-free 1.5mL Eppendorf tube, handled with gloves treated with RNAse-zap. Eppendorf tubes were placed in an autoclavable tube box, open and upright, the box wrapped in foil and autoclaved for 25 minutes on a dry cycle. After autoclaving, wearing RNAse-zap treated gloves, the Eppendorf tubes were carefully removed from the box, closed, and labeled with the numbers of the plates from which the roots were to be collected.

During harvest, each plate was removed individually from the chamber, opened, and the roots collected with forceps and a scalpel. The roots were immediately placed in a cold Eppendorf tube and flash frozen in liquid nitrogen. The time between removing the plate from its place in the chamber to freezing the Eppendorf tube and roots did not exceed 30 seconds. The forceps and scalpel were soaked in 10% bleach between samples and excess liquid wicked off by a paper towel before contacting the roots. The Eppendorf tubes of root samples were stored at -80°C prior to extracting RNA.

RNA Extraction

Tissue was homogenized by three 30 second bursts at 30Hz in a TissueLyzer II (Qiagen; Germany), with 30 second rests in liquid nitrogen between each burst. RNA was extracted using a Qiagen RNEasy Plant Mini Kit, employing 450µL Buffer RLT lysis buffer (with 10µL β-ME per 1mL Buffer RLT), an on-column DNAse digest (RNase-Free DNase Set, Qiagen), and eluting 2x with 50µL RNAse free water. A 5µL aliquot was set aside to perform an initial quantification using a NanoDrop. Samples with less than 75µg/mL were concentrated by ethanol precipitation as described below. RNA was quantified and quality checked using BioAnalyzer (MSU RTSF). All RNA samples had RIN scores >9.0.

RNA Ethanol Precipitation

Low concentration RNA extractions were amended with 10 µL 3 M Sodium Acetate and then 300 µL ice cold 100% ethanol, vortexed briefly to mix, and precipitated at -20°C overnight. RNA was pelleted by centrifuging for 30 min at full speed at 4°C. The RNA pellet was washed with 200 µL ice cold 70% EtOH and centrifuged for 10 min at full speed at 4°C. The supernatant was discarded and the pellet air-dried for 15 min on the bench before being resuspended in 30-50 µL RNAse-free water. A 5 µL aliquot was taken for quantity and quality analysis and the remainder stored at -80°C.

Library Preparation & Sequencing

Libraries were prepared using the Illumina TruSeq Stranded mRNA Library Preparation Kit with the IDT for Illumina Unique Dual Index adapters following manufacturer's recommendations. Completed libraries were QC'd and quantified using a combination of Qubit dsDNA HS and Agilent 4200 TapeStation High Sensitivity DNA 1000 assays. The libraries were pooled in equimolar amounts and the pool quantified using the Kapa Biosystems Illumina Library Quantification qPCR kit. This pool was loaded onto an Illumina NextSeq 500/550 High Output flow cell (v2.5) and sequencing performed in a 1x75 bp single read format using a NextSeq 500/550 High Output 75 cycle reagent kit (v2.5). Base calling was done by Illumina Real Time

Analysis (RTA) v2.4.11 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v2.19.1.

qPCR

Primer sets for qPCR were designed using 16S rRNA gene sequences of *M. elongata* NVP64 and NVP80 endobacteria with the IDT PrimerQuest® Tool for 2 primers and intercalating dye (**Table 3.2**). Primer sets were verified using wild-type DNA samples, for which a standard curve was created with dilutions from 10^0 to 10^{-4} and efficiencies were within 90-110%. Absolute copy number calibration was not performed because only presence/absence validation was required. cDNA was synthesized for qPCR quantification using the LunaScript RT SuperMix Kit (New England Biolabs; Massachusetts, USA). qPCR reactions were composed of 7.5 µL Power SYBR Green PCR Master Mix (ThermoFisher Scientific; Massachusetts, USA), 5.5 µL nuclease-free water, 0.25µL each primer, and 1.5 µL of undiluted template. The reaction cycle was 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min with a fluorescence measurement. A melting curve was performed following amplification: 95°C for 15 sec and 60°C for 15 sec, then a 20 min ramp up to 95°C, followed by 95°C for 15 sec. At least two reactions were performed per sample and primer combination.

Sequence Analysis

Raw, demultiplexed reads were quality trimmed and filtered using Trimmomatic v.0.38 (Bolger, Lohse, & Usadel, 2014). A combined reference transcriptome was constructed from the Arabidopsis Thaliana Reference Transcript Dataset 2 (AtRTD2_19April2016.fa, accessed 10/21/2019) and Mortierella elongata AG77 (Morel2_GeneCatalog_transcripts_ 20151120.nt.fasta.gz, project 1006314, accessed 10/21/2019) (Zhang et al. 2016; Uehling et al. 2017). This combined reference transcriptome was indexed in Salmon v0.11.3 and used to quasi-map the trimmed reads to transcripts (Patro et al. 2017).

DGE Analysis

A transcript-to-gene (tx2gene) table was constructed in R v.3.6.0 using only Arabidopsis gene

annotations (AtRTD2_19April2016.gtf.txt, accessed on 01/12/2020), since fungal reads were extremely rare in the dataset (R Core Team, 2019; Zhang et al. 2016). Salmon quants.sf files were imported into R using tximport (type=salmon; Soneson et al. 2015). Differential gene expression analysis was carried out with both the EdgeR package and the DESeq2 package in R (Robinson et al. 2010; McCarthy et a., 2012; Love et al. 2014). Gene expression was computed for each treatment across the three biological replicates, with the control treatment specified as the reference level in the experimental design matrix. Differentially expressed genes were identified by contrasting each fungal treatment against the control. In DESeq2, gene lists from each comparison were filtered by adjusted p-value of 0.05 and an absolute value of log2 fold change (LFC) cutoff of 0.585, which corresponds to a fold change in expression of 1.5. We generated volcano plots of these pairwise comparisons using the EnhancedVolcano package in R (Blighe et al. 2019). In EdgeR, the gene list encompassed all four fungal treatments with a single p-value for each gene, so the EdgeR gene list was filtered by overall p-value and whether at least one fungal treatment LFC meeting the LFC cutoff (Robinson, McCarthy, & Smyth, 2010; McCarthy, Chen, & Smyth, 2012). The DESeq2 gene list was filtered to include only genes also present in the EdgeR gene list. Since DESeq2 provided p-values for each comparison, we used the log2-fold change and adjusted p-value of the DESeq2 analyses to compose our final DEG table. Gene ontology was assigned by referencing TAIR and UniProtKB annotation databases and synthesizing the most detailed and supported annotations (Berardini et al. 2015; UniProt Consortium, 2019).

Results

Potting Mix Experiments

Mortierella elongata *increased mature Arabidopsis aerial dry biomass*

All fungal treatments had significantly higher aerial dry biomass than the uninoculated millet control. Aerial dry biomass of full-grown Arabidopsis plants was not significantly different between

NVP64cu and NVP64wt or between NVP80cu and NVP80wt, but was significantly higher in the no-millet control than the uninoculated millet control (**Fig. 3.5**).

Mortierella elongata *impacted Arabidopsis seed production*

As with the aerial biomass, total seed mass was significantly higher in the no-millet control plants as compared to the uninoculated millet control. Both NVP80cu and NVP80wt were significantly higher than the uninoculated millet control (**Fig. 3.6**). In terms of endobacteria, no significant differences in seed mass were found between NVP64wt/cu, nor between NVP80wt/cu isogenic isolate pairs (**Fig. 3.7**). Unlike total seed mass, the average seed mass of the uninoculated millet control was significantly higher than the no-millet control and NVP64cu. There were no significant differences between uninoculated millet control, NVP80cu, and NVP80wt treatments.

Given the potential that sufficient seeds in the fungal treatments could be smaller due to incomplete development, rather than total reduction in seed size, we set out to determine whether this might be visible in histograms of individual seed areas from the image analysis. This would be represented by a bimodal histogram with peaks representing immature and mature seeds. No clear bimodality could be seen in most treatments (**Fig. 3.8**). The observed slight curve topography more likely represents variation in the mean seed size between samples as opposed to consistent bimodality across all samples. From the total seed mass and the average seed mass, we calculated the total seed number and found no significant differences between NVP64cu and NVP64wt or between NVP80cu and NVP80wt (**Fig. 3.9**). However, NVP80cu, NVP80wt and the no-millet control each had significantly higher total seed number than the uninoculated millet control.

Agar Experiments

Mortierella elongata *did not impact the timing of Arabidopsis bolting*

The Kruskal-Wallis Test was conducted to examine the age at which plants bolted according to fungal treatment. No significant differences (Chi square = 4.92, $p = 0.296$, df = 4) were found

among the five treatments. The mean age at which an inflorescence could first be seen to elongate from the rosette was 22 days, 12 days post transplanting and inoculation (DPI). Therefore, we harvested all further agar experiments at 12 DPI to prevent bolting from affecting dry weight data, which differed from the 7 day co-cultivation time used by Johnson et al. (2019).

Mortierella elongata *increased young Arabidopsis aerial dry biomass*

We expected that several environmental factors could potentially impact our observation of how Arabidopsis responds to *M. elongata*. These included the (1) starting size of the plant; (2) local light level, (3) medium on which the fungus was cultured, and (4) process by which the fungi were cured of their endobacteria. We determined that there was no statistically significant correlation between light level and harvested plant dry weight in any of the treatments (**Table 3.3**). We performed linear modeling of the dry weights as a function of medium, treatment, and interaction between those, and determined there were no significant differences in harvested plant dry weight based on media ($F_{1,\ 110}$=0.966, p=0.328; **Table 3.4**) and no significant interaction between medium and treatment ($F_{4,110}$=0.331, p=0.857). Analysis of variance found no statistically significant differences in mean harvested plant dry weight, between three independently generated cured lines (L0, L1, and L2) of *M. elongata*, for both NVP64 ($F_{2,42}$=0.443 p=0.645) and NVP80 ($F_{2,42}$=1.966, p=0.153), suggesting that differences between wild-type (wt) and cured (cu) strains are likely due to the presence/absence of endobacteria, rather than accumulated mutations from the antibiotic passaging protocol. Analysis of variance in seedling root length showed that the mean seedling root length was consistent between treatments of each experiment ($F_{4,755}$=0.953, p=0.433), but differed significantly between experiments ($F_{1,755}$=267.3, p=2e-16), with no significant interaction effect ($F_{4,755}$=0.541, p=0.706). Preliminary linear model analysis showed a significant positive correlation between seedling root length and harvested plant dry weight, with no significant differences between the slope of this correlation across experiments or treatments (**Table 3.5**). We fit a linear mixed model of combined aerial dry weight data from both experiments as a function of treatment and seedling root length. Results of this

model can be seen in **Table 3.6**. The estimated marginal means of aerial dry weight was significantly higher in all four fungal treatments compared to the control, but there were no significant differences between fungal treatments (**Fig. 3.10**).

All Mortierella elongata *strains colonize Arabidopsis roots evenly*

We used the cycle number at which the fluorescent signal of the qPCR probe exceeded the threshold level to calculate the ratio of *M. elongata* RNA to Arabidopsis RNA in each reaction. This ratio represents the degree of fungal colonization of plant roots. There were no significant differences in this ratio between any of the fungal treatments ($p>0.1$) and each lineage of endobacteria was detected only in the wild-type strains (**Table 3.7**; **Fig. 3.11**).

Differential Gene Expression

Molecular Results

Sequencing returned an average of 34.7 million (30.5-37.7M) reads per sample, with an average of 97.64% (97.22-97.85%) mapping rate to the combined reference transcriptome. Of the mapped reads, an average of 99.82% (98.64-99.99%) mapped to plant transcripts (**Table 3.8**).

Arabidopsis differentially expressed genes in response to Mortierella elongata

We conducted initial RNAseq data exploration in DESeq2 to confirm consistent gene expression profiles between biological replicates of each condition (**Fig. 3.12**). We noted that all four fungal treatments clustered together away from the control and that there was no observed clustering by isogenic strain (NVP64 or NVP80) or by cured/wild-type. Indeed, NVP64cu and NVP80wt seem to be the most similar.

DESeq2 identified a total of 465 genes that were differentially expressed and met LFC and adjusted p-value cutoffs in at least one of the four fungal treatments as compared to the control. Of these, there were 301 DEGs in NVP64cu v. Control, 135 in NVP64wt v. Control, 142 in NVP80cu v. Control, and 213 in NVP80wt v. Control (**Fig. 3.13**). EdgeR identified 679 genes as being differentially expressed at a collective adjusted p-value threshold, and at least one sample

meeting the LFC cutoff. There were 376 DEGs in NVP64cu v. Control, 240 in NVP64wt v. Control, 282 in NVP80cu v. Control, and 319 in NVP80wt v. Control. When only considering genes present in both the EdgeR and DESeq2 DEG lists, our analyses identified 385 DEGs (**Table 3.9**; **Fig. 3.14**). Since DESeq2 provided p-values for each comparison, we used the log2-fold change and adjusted p-value of the DESeq2 analyses to filter the expression patterns in the final DEG list.

Thirty-four plant genes were differentially expressed when inoculated with any/all of the four fungal treatments as compared to the uninoculated control, 55 were significantly altered in expression in three fungal treatments, 114 were significantly altered in two fungal treatments, and 182 DEGs were significantly altered in only one fungal treatment (**Table 3.9**). Of the included LFC values, all treatments were consistent in the trend of being either up- or down-regulated as compared to the control, with one exception (**Table 3.9**).

Defense response genes

Thirty-four DEGs were broadly involved in plant defense against other organisms. Nine were specifically related to defense against bacteria, eight of which were upregulated. These included *FLS2* and *CML12*, which are essential to the perception of an innate immune response to bacterial flagellin and *CML12* also participates in regulating the activity of an auxin efflux protein (Bender & Snedden, 2013). Three cysteine-rich receptor-like protein kinases (*CRK10*, *CRK18*, and *CRK31*) were up-regulated. The functions of CRKs are not well understood, though several members of this protein family, including *CRK10*, have been shown to respond to pathogens and/or oxidative stress (Rayapuram et al. 2012). The down-regulated gene was *euonymus lectin S3* (*EULS3*), which is involved in ABA-mediated stomatal closure in response to bacterial pathogens (Van Hove et al. 2015).

Twelve DEGs were involved in defense responses to fungus, 7 down-regulated and 5 up-regulated. Foure of these, *dl4875c*, AT4G22214, AT4G22217, and *CAPE3*, were significant in all four treatments. The first, *dl4875c*, is a down-regulated ubiquitin-protein transferase involved in regulating defense response (Mukhtar et al. 2011). The second and third are predicted to be

members of the defensin-like protein family, which are involved in killing non-self cells. The fourth is an up-regulated CAP (Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1 protein) superfamily protein of unknown function. Of the other defense genes, *CEP1* is a down-regulated protease that supports the final stages of programmed cell death during fungal infection (Höwing et al. 2014). *TI1* is another down-regulated member of the defensin-like protein family involved in defense responses to fungi (Chassot et al. 2007). The other four up-regulated DEGs were *HR3*, *F18O19.27*. *HR3* is expressed in response to fungal infection, though it does not confer resistance to fungal pathogens (Xiao et al. 2007).

Nine DEGs were identified as defense responses via hormone signaling or metabolism. These included *AZI1*, *AZI3*, and *EARLI1*, which are collectively involved in salicylic acid signaling and priming of both systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Cecchini et al. 2015). Unexpectedly, *AZI1* was up-regulated while the other two were down-regulated. Transcriptional regulator of defense response *TDR1* was up-regulated and is involved in ethylene signaling in response to chitin (Riechmann & Meyerowitz, 1998; Libault et al. 2007). GDSL lipase *GLIP2*, also up-regulated, responds to pathogen attack, salicylic acid, jasmonic acid and ethylene by down-regulating auxin signaling (Lee et al. 2009).

Hormone Signaling Genes

In total, there were 36 DEGs primarily related to hormone signaling and 18 “cross-categorized” as hormone signaling in defense (9 DEGs), development (3 DEGs), metabolism (3 DEGs), or abiotic stress responses (3 DEGs).

In addition to *GLIP2* described earlier, twelve DEGs were related to auxin biosynthesis and/or signaling, 8 down-regulated and 4 up-regulated in plants grown with *M. elongata*. Four enzymes related to auxin biosynthesis (*NIT1*, *NIT2*, *GH3.17, GH3.7*, and *UGT74E2*) were down-regulated. *NIT1* and *NIT2* are nitrilases that mediate IAA production from indole-3-acetonitrile (IAN) (Grsic-Rausch et al. 2000; Pollmann et al. 2006). *GH3.17* conjugates amino acids to IAA (Staswick et al. 2005). *GH3.7* conjugates cysteine to chorismate, which is a precursor to salicylic acid and

aromatic amino acids, depending on downstream modifications (Mano & Nemoto, 2012; Holland et al. 2019). The cysteine-chorismate conjugate is a precursor to aromatic amino acids, including tryptophan, which is a biosynthetic precursor to IAA (Pieck et al. 2015; Holland et al. 2019). *UGT74E2* glycosylates the auxin indole-3-butyric acid (IBA), which has activity independent of IAA and can be interconverted to IAA (Tognetti et al. 2010). Two auxin efflux carrier proteins (*PILS3* and *PILS5*) were down-regulated; these proteins regulate intracellular IAA concentration and are themselves regulated by brassinosteroid signaling and abiotic environmental stimuli, such as light and temperature (Sun et al. 2019). The ethylene- and auxin-responsive genes *ARGOS* and *SAUR76* were up-regulated.

In addition to *ARGOS*, *SAUR76* and *TDR1*, seven DEGs were related to ethylene signaling. The ethylene biosynthesis enzyme *ACS7* was down-regulated (Riechmann & Meyerowitz, 1998; Huang et al. 2013; Prasch & Sonnewald, 2013). Ethylene responsive transcription factor *RAP2-9* and three ethylene response factors (*ERT2*, *ERF59*, and *ERF73*), the first three of which were up-regulated and the last was down-regulated. *ERT2* is a negative regulator of ethylene-activated signailing pathways (Sakai et al. 1998). *ERF73* and *ERF112* are ethylene-responsive transcription factors. *ERF59* is a point of cross-talk between the ethylene and jasmonic acid (JA) signaling pathways (Pré et al. 2008).

Three DEGs were found to be related to jasmonic acid synthesis and signaling: *JOX2*, *RGLG3*, and *WRKY51*. The first two were down-regulated and the last was up-regulated. *JOX2* hydroxylates JA, repressing the JA signaling pathway (Smirnova et al. 2017). *RGLG3* is an upstream modulator of JA signaling, potentially by mediating SA suppression of JA signaling (Zhang et al. 2015). *WRKY51* is one of the proteins that mediate SA repression of JA signaling (Gao et al. 2011).

Three DEGs were found to be involved in abscisic acid pathways (*NCED3*, *MYB74*, and *KIN1*), all down-regulated (Kurkela & Franck, 1990; Sato et al. 2018). *NCED3* is a key enzyme in ABA biosynthesis. We also identified *GIM2* as an up-regulated DEG in our dataset. *GIM2*

negatively regulates ABA sensitivity and gibberellin biosynthesis (Li et al. 2019).

Two wall-associated signaling kinases, *WAK1* and *WAK2*, and two genes that regulate *WAK1* activity, *GRP3* and *GRP3S*, were up-regulated in fungal treatments (Park et al. 2001; Brutus et al. 2010). *GRP3* is expressed in response to ABA, SA, and ethylene (de Olivera et al. 1990). *WAK1* functions in defense responses via salicylic acid signaling (Brutus et al. 2010).

Three DEGs were related to brassinosteroid signaling, a putative squalene epoxidase (*SQE4*) was down-regulated and baurol synthase *BARS1* and brassinosteroid-induced positive cell growth regulator *ARL* were up-regulated (Hu et al. 2006; Lodeiro et al. 2007; Rasbery et al. 2007).

Abiotic Stress Response Genes

Fifty-five DEGs were identified as having functions related to abiotic stress responses. Fifteen DEGs were related to hypoxia and/or oxidative stress, 6 of which were down-regulated and 9 up-regulated. Seven DEGs were related to drought and/or cold/salt stress, all of which were down-regulated. Eleven DEGs were identified as responsive to iron, potassium, and phosphate deficiency, all but one down-regulated. Finally, four DEGs are broad responses to ABA and many other abiotic stresses, one is a hypothetical protein (*T9L3_30*), and eight are classified as RmlC-like cupins superfamily proteins, seven of which were up-regulated.

Development

We identified 30 DEGs related to plant development. Five down-regulated DEGs (*MRN1*, *MRO*, *THAS1*, *THAH*, and *THAD*) compose two operon-like gene sets involved in root development (Field & Osbourn, 2008; Field et al. 2011; Go et al. 2012; Johnson, 2012). Six DEGs were related to general growth regulation, three up-regulated and three down-regulated. Twelve DEGs were annotated as either regulating flowering or only expressed during the flowering life stage, ten of which were down-regulated, including a transcription factor involved in gibberellin signaling.

Metabolism

One hundred and nine DEGs were related to metabolism, some with tenuous or conflicting

annotations related to hormone signaling or defense pathways. Ten DEGs were related to toxin catabolism, 9 of which were various glutathione S-transferases, 7 of which were down-regulated. There were also 14 DEGs related to protein modification. Eleven DEGs were transporters of various nutrients and metabolites, including amino acids, nitrate, toxins, and cations, almost all of which were down-regulated. Ten DEGs were related to amino acid metabolism and/or nitrogen metabolism, six down-regulated and four up-regulated.

Unknown

There were 77 DEGs with no broad process or pathway classification, 35 down-regulated and 42 up-regulated. Eight were transmembrane proteins or described as having extracellular activity. Notably, 15 DEGs were significant in at least 3 of the 4 fungal treatments and therefore of consistent importance.

Discussion

In this study, we sought to molecularly characterize symbiosis between plants and *M. elongata*. We specifically assayed aerial plant growth, seed production, and differential gene expression in Arabidopsis plants responding to several different *M. elongata* strains. We also tested whether two different fungal strains, each associated with (or cured of) a different bacterial endosymbiont, had significantly different phenotypes for each tested metric of plant growth. Finally, we used RNA-seq to identify plant genes that are differentially expressed during Arabidopsis-*M. elongata* symbiosis in order to begin describing the mechanism of interaction.

Mortierella elongata promotes Arabidopsis growth independent of endobacteria

We found that Arabidopsis inoculated with any of our four fungal treatments had increased aerial growth compared to the uninoculated controls, whether harvested before or after flowering. These results are corroborated by recent studies of *M. elongata* inoculated maize, where *M. elongata* increased the height and dry aerial biomass of maize in V3-V5 early vegetative stages, which corresponds to when maize has begun relying on photosynthesis and the environment for

resources, rather than seed resources (Li et al. 2018; Abendroth et al. 2011). We were surprised to see that neither BRE nor MRE had a significant impact on plant growth in either experimental system, although NVP80wt showed a weak trend towards smaller plants than NVP80cu in the potting mix experiment (**Fig. 3.5**).

Our seed production data also indicate that fungal treatments had a strong effect on Arabidopsis seed size and total seed number. NVP80cu and NVP80wt both had significantly higher total seed number than the uninoculated millet controls, whereas neither NVP64cu nor NVP64wt were significantly different from uninoculated control. Although 5 replicates were used, additional replication might have strengthened the statistically weak trend of NVP64wt toward higher seed number. Average seed size was unexpectedly higher in the uninoculated millet control plants compared to most of the fungal treatments (**Fig. 3.7**). This may represent a fitness strategy in which plants grown under stressful conditions create fewer, larger seeds to increase offspring fitness, whereas healthy plants can produce a higher number of smaller seeds because they will each need fewer starting resources to survive and reproduce (Sadras, 2007; Breen & Richards, 2008).

The potting mix experiments demonstrated that uninoculated grains in control treatments not only invite colonization by environmental contaminants, but the grains have a strong, consistent negative impact on plant growth. Preliminary studies of *Mortierella* interacting with millet plants using millet-based spawn suggest that some of this effect may be due to allelopathic compounds in the grains, as millet plants were observed to be much less affected by a millet-based spawn than Arabidopsis (data not shown). Comparisons between uninoculated spawn and inoculated spawn treatments, neglecting to include a no-spawn control, could potentially bias results toward stress mitigation and not neutral environment plant growth promotion. Another challenge of the potting mix-based experiments was deciding at what point to conclude the experiment, since the highly stressed uninoculated spawn control plants matured much sooner than the other treatments. The difficulty of handling maturing Arabidopsis without significant loss of seeds and

siliques necessitated harvesting all plants for aerial biomass before the plants completed seed production and senesced. This meant that the plant life stage could not be controlled in the biomass and seed production data.

The potting mix experiment was necessary and technically sufficient to collect data about seed production but may have benefited from an increased sample size for seed collection rather than harvesting those plants for aerial biomass. The agar system was more suited to assay early life-stage aerial growth and root gene expression. Now that *M. elongata* has been shown to impact plant growth, more extensive experiments can be justified to further explore plant-fungal interactions. The agar system is well suited for high-throughput assays of plant and fungal knock-out mutants to further isolate important genes and pathways involved in this symbiosis. An improved potting mix system, with a grain-free inoculation protocol, would be ideal to non-destructively track plant growth over time and to construct a more detailed description of how *M. elongata* affects plant growth and development.

Mortierella elongata may regulate Arabidopsis defense and abiotic stress responses

A number of plant hormones mediate the initiation and maintenance of plant-microbe symbioses, including auxins (most commonly IAA), jasmonates/jasmonic acid (JA), salicylic acid (SA), abscisic acid (ABA), ethylene (ET), and brassinosteroids (BRs). These hormones can be produced by both the plant and microbial symbionts and are often required to appropriately suppress and redirect the plant defense response in order for the microbe to establish symbiosis. The regulation and importance of each hormone depends on the type of interaction, pathogenic vs. beneficial, bacterial vs. fungal, and both species of plant and microbe that are interacting. For example, ethylene suppresses AMF colonization, but promotes EM colonization (Chanclud & Morel, 2016; Foo et al. 2016; Splivallo et al. 2009). Similarly, the beneficial non-mycorrhizal fungi *M. hyalina* and *S. indica* stimulate plant production of jasmonic acid and salicylic acid, respectively, when initiating symbiosis with Arabidopsis (Meents et al. 2019; Vahabi et al. 2015). While this study did not include direct measurement of phytohormone levels, we did identify

several DEGs related to the biosynthesis and signaling of ethylene, auxin, and abscisic acid.

Auxin and Root Development

Many fungi synthesize and secrete auxin, which have been shown to impact plant growth. *M. verticillata* and *M. antarctica* both synthesize IAA and were shown to improve winter wheat seedling growth (Ozimek et al. 2018). The genome of *M. elongata* (strain AG77) has the key genes of IAA synthesis and maize roots inoculated with *M. elongata* had a 37% increase in IAA concentration (Li et al. 2018). Our study found that *M. elongata* suppressed Arabidopsis auxin biosynthesis genes (*NIT2* and *GH3.7*), but up-regulated several auxin-responsive genes. Since 1) Arabidopsis auxin biosynthesis is being down-regulated, 2) auxin synthesis is generally self-inhibitory in plants, and 3) auxin response genes are up-regulated, we hypothesize that the Arabidopsis roots are responding to *M. elongata*-derived auxins (Salehin et al. 2015). In contrast, Arabidopsis auxin-related genes did not respond to initial or established *M. hyalina* colonization, even though Arabidopsis roots had a 3-fold increase in IAA concentration during initial colonization as compared to control roots (Johnson et al. 2019; Meents et al. 2019). Moreover, some of this IAA was again fungal in origin, as the *M. hyalina* mycelium alone had a significantly higher IAA concentration than the tested pathogenic fungi (Meents et al. 2019). Their assay did show a very brief response to auxin that quickly dissipated to background gene expression levels. They hypothesized that Arabidopsis roots did respond to fungal auxin very briefly, but the auxin response was likely interrupted by other plant hormones/elicitors. Indeed, JA acts antagonistically to auxin responses and *M. hyalina* was found to produce high levels of JA in pure culture and initially stimulates significant accumulation of JA in Arabidopsis roots (Meents et al. 2019). However, JA levels were not elevated several days after initial colonization, indicating that these hormone responses are not maintained throughout the Arabidopsis-*M. hyalina* symbiosis (Johnson et al. 2019). Since we found increased auxin responsive gene expression during well-established symbiosis, our data suggest *M. elongata* employs a different phytohormone regulatory strategy than is indicated in *M. hyalina*.

Enhanced aerial plant growth by auxin-producing microbes is attributed to improved root structure, particularly lateral root growth, but assessing the impact of Mortierellaceae fungi on plant root development is not so straightforward (Li et al. 2018). Johnson et al. (2019) found that *M. hyalina* had a slight, but significant negative impact on Arabidopsis root dry biomass compared to uninoculated plants and identified three root development (*SHR, CPC, and AHP6*) genes that responded to *M. hyalina* as opposed to the plant pathogen *Alternaria brassicae*. These genes were not among the DEGs identified in our study. However, we did find that the entire operon-like gene set related to thalianol biosynthesis and metabolism was downregulated by *M. elongata* (Field & Osbourn, 2008). Thalianol-related metabolites are predicted to function in promoting root development, but the mechanism is still under investigation (Field & Osbourn, 2008). Future research is needed to determine how each of these fungi impact Arabidopsis root development and how that relates to increased aerial plant growth.

Ethylene (ET)

Ethylene is a plant hormone involved in maturation, senescence, and response to biotic and abiotic stress. Decreasing the level of ethylene in plant tissues generally promotes plant growth. The role of ET in plant response to pathogens is well characterized and includes increased ET biosynthesis and signaling through a single conserved pathway, which includes proteins in the *TDR1* family (Broekaert et al. 2006). However, the origin and role of ET in the initiation of beneficial plant-fungal symbioses is specific to the fungi involved. For instance, elevated ET appears to promote colonization by ectomycorrhizal fungi, but inhibits colonization by AMF (Chanclud & Morel, 2016; Foo et al. 2016; Splivallo et al. 2009). Moreover, the ET signaling pathway is known to have multiple points of crossover with other hormone signaling pathways, including JA and cytokinin, some of which occur through the ERF family of transcription factors, including *TDR1* (Broekaert et al. 2006). In our study, we found that Arabidopsis colonized by *M. elongata* had down-regulated ACC synthase *ACS7*, which synthesizes the metabolite 1-amino-cyclopropane-1-carboxylate (ACC), which is a precursor to ethylene. However, some genes

related to ethylene signaling were up-regulated in response to *M. elongata*. Since ET biosynthesis is downregulated in Arabidopsis roots in response to *M. elongata*, it is possible that related response genes are being up-regulated via other hormone pathways, although there were only three DEGs specifically associated with JA signaling in our dataset and they were each significant in only one fungal treatment.

Abscisic Acid (ABA), Abiotic Stress, & Reactive Oxygen Species (ROS)

In general, genes related to ABA and abiotic stress are down-regulated by *M. elongata*. These include the ABA synthesis enzyme *NCED3* and responses to drought, cold, salt, iron deficiency, potassium deficiency, phosphorous deficiency, and heavy metal tolerance. Many plant growth promoting fungi are thought to transport water and nutrients to plants, particularly phosphorus. Mortierellaceae species are known to solubilize phosphate and improve its uptake in plants (Zhang et al. 2011). Considering the availability of potassium, phosphorus, and iron in the PNM growth medium, it is striking that so many genes related to deficiencies of these nutrients were down-regulated compared to the control plants. There were two main exceptions to this reduction in abiotic stress: oxidative stress responses and a group of RmlC-like cupins superfamily proteins whose function is unknown.

ROS are a common plant defense response to both beneficial and pathogenic microbes (Nath et al. 2017). Both *M. hyalina* and *M. elongata* stimulate ROS-responsive genes, though the two ROS-responsive genes specifically tested by Johnson et al. (2019) were not among the DEGs in our dataset. Six of the up-regulated oxidative stress genes are peroxidases. One is a raffinose synthase. Raffinose is thought to act as an osmoprotectant and ROS scavenger (Nishizawa et al. 2008). Finally, we observed down-regulation of uridine diphosphate glycosyltransferase *UGT74E2*, which responds to ROS and drought to convert the auxin IBA to IBA-Glc (Tognetti et al. 2010). ROS also stimulates conversion of IAA to IBA. Increased expression of *UGT74E2* further sequesters IBA and prevents oxidation back to IAA (Tognetti et al. 2012). While no *UGT74E2* suppression or deletion mutant phenotypes have been reported, overexpression of

UGT74E2 leads to increased sensitivity to ABA (Tognetti et al. 2010). In summary, we observe down-regulation of auxin synthesis, ABA synthesis and signaling, and an important gene connecting the ROS, ABA, and auxin pathways. From this, we infer that *M. elongata* stimulates ROS responsives genes, but these responses are isolated from other hormone pathways and limited to peroxidases and antioxidants.

Calcium Signaling and Plant Defense

In addition to hormones, many plant-microbe interactions involve calcium signaling in plant roots (Yuan et al. 2017). *M. hyalina* symbiosis with Arabidopsis is activated by calcium signaling (Johnson et al. 2019). This calcium-signaling was required both for the plants to receive pathogen protection by *M. hyalina*, and for *M. hyalina* to colonize Arabidopsis roots, but the signaling-deficient plants still showed the wild-type aerial growth promotion. This suggests a calcium-signaling dependent defense response to limit the rate of root colonization by *M. hyalina*. Johnson et al. (2019) identified four Ca-signaling genes (*At3g47480, At3g03410, At5g23950*, and *At3g60950*) that specifically responded to *M. hyalina*, as opposed to the plant pathogen *Alternaria brassicae*. These genes were not among the DEGs identified in our study. However, we did note up-regulation of the calcium-signaling gene *CML12* identified in our RNA-seq experiment, which is induced by both stress and hormone signals, including auxin, touch, darkness, oxidative stress, and herbivory (McCormack & Braam, 2003; Cho et al. 2016).

DEG analyses show that *M. elongata* stimulated several general, fungal, and bacterial defense-related genes in Arabidopsis roots. However, we also noted suppression of genes involved in programmed cell death and production of defensin-like proteins meant to kill cells of invading organisms. As such, these defense responses could indicate both regulation of *M. elongata* colonization and a priming of the plant innate immune response, explaining the elevated expression of definitively bacterial defense genes like *FLS2*. As noted in maize-*M. elongata* symbioses, *M. elongata* may curate auxin levels to colonize maize roots to suppress systemic defense through the salicylic acid pathway (Li et al. 2018). Further, this active microbial regulation

of the plant immune response can promote plant growth in a field environment by limiting further resource allocation to defense when attacked by pathogens (Kazan & Manners, 2009; Li et al. 2018).

The role of phytohormones in fungi

While it is well established that fungi can manipulate, and often produce, phytohormones to orchestrate plant responses as described here and in Chapter 1, the effects of phytohormones on fungi are not understood. Studies of plant hormone impacts on fungal growth and development are currently limited to a few plant pathogens and AM fungi. Generally, exogenous ethylene promotes fungal spore germination and mycelial growth (Lockhart et al, 1968; El-Kazzaz et al, 1983; Kepczynska, 1989, 1993). Exogenous ethylene is required for spore germination in species of *Alternaria*, *Botrytis*, *Pennicilium*, and *Rhizopus* and often promotes mycelial growth (Kepczynska, 1989). It is worth noting that these species infect fruit and likely evolved this requirement to ensure spore germination in the presence of ripening fruit, limiting the relevance of those findings to mechanisms employed by root-associated fungi (El-Kazzaz et al, 1983). Gryndler et al (1998) found that exogenous auxin (IAA) repressed hyphal growth of two AM fungi, *Glomus fistulosum* and *G. mosseae*, at biologically relelvant concentrations, but abscisic acid (ABA) and cytokinins had no perceivable effect until applied in concentrations very high, non-physiological concentrations. The current model of phytohormone regulaiton of AM fungi suggests that 1) SA inhibits pre-symbiotic growth; 2) ethylene, JA, and cytokinins inhibit symbiotic fungal growth inside plant roots; and 3) auxin, JA, and ABA promote the formation and function of arbuscules within plant root cells (Pozo et al, 2015). It is unclear how these relationships and regulatory systems apply to the growth, development, or plant associations of *M. elongata*.

Future Directions

In this study, we measured plant growth and productivity at early and late life stages after a stable symbiosis had been established. It is important to note that the mechanism by which *M. elongata* maintains symbiosis with Arabidopsis may be very different from that required to initiate

and establish symbiosis, as in the case of JA levels in the Arabidopsis-*M. hyalina* symbiosis or transient stress responses in Arabidopsis during *S. indica* infection (Johnson et al. 2019; Meents et al. 2019; Vahabi et al. 2015). Future research may illuminate mechanisms of extremely early stages of interactions between *M. elongata* and plants.

Our results indicate that *M. elongata* may affect Arabidopsis root architecture and development. When designing plant-fungal symbiosis experiments, it may be important to conduct phytohormone tests in a medium/system that shields roots and “below-ground” interactions from light to prevent any fungal production of 2-keto- 4-methylthiobutyric acid (KMBA) to ethylene that might not take place in a natural system (Splivallo et al. 2009; Chagué, 2010). It would be highly informative to conduct shared-media assays that test whether direct contact is required for this symbiosis. A spent-medium assay could also be used to test whether constitutively produced metabolites from one organism trigger a response in the other to initiate interaction.

We were unable to analyze the fungal transcriptome due to extremely low read abundance. It might be possible to use a microbiome enrichment kit or other technique to isolate or increase the proportion of fungal RNA. This could allow co-expression network analysis and significantly improve our understanding of the fungal response to the plant, rather than just the plant response to the fungus.

The hormone signaling pathways discussed here are each composed of scores to hundreds of genes. Our differential gene expression analysis yielded only ~45 genes related to hormone signals, with at most 10-12 DEGs in any one hormone signaling pathway, as in the cases of auxin and ethylene. While it is concerning to have so small a portion of these signal pathways represented, we are encouraged in our focus on auxin and ethylene as potentially important mediators due to the presence of key biosynthesis and response genes in our dataset. We present these data as the basis for future experiments and not as conclusive evidence of a proposed mechanism.

Conclusions

In conclusion, our data show that *M. elongata* promotes aerial plant growth and also affects seed production. This plant phenotype was found to be independent of whether *Mortierella* symbionts were colonized by MRE or BRE endohyphal bacterial symbionts. We hypothesize that the mechanism of plant-fungal symbiosis involves fungal production of auxin and stimulation of the ethylene and ROS response pathways. Future research is needed to test these hypotheses and further characterize the fungal side of this symbiosis.

Figures & Tables

Figure 3.1 – Arabidopsis seedlings used in plant-fungal interaction assays

Panel a) 10-day old seedlings on 1xMS germination plates and b) 11-day old seedlings and blocks of media (colonized by fungi in fungal treatments or sterile in uninoculated control treatments) as arranged on PNM plates for the agar-based plant-fungal interaction experiments.

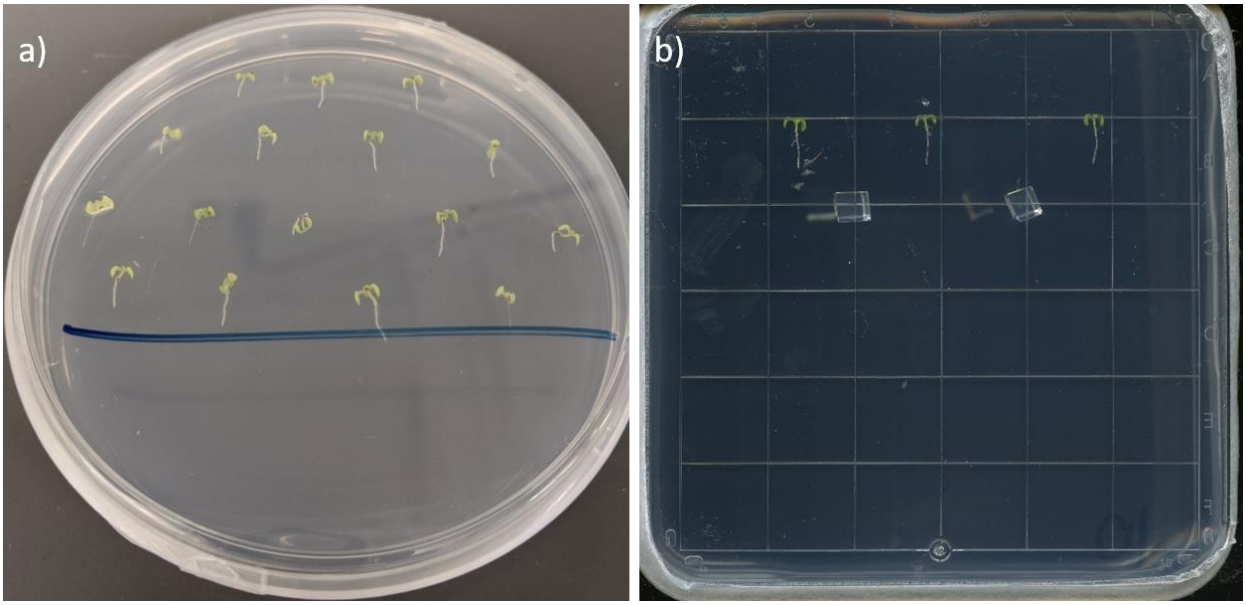


Table 3.1 - A map of the light levels in the growth chamber

Arabidopsis seeds germination and Arabidopsis-*M. elongata* interaction studies were conducted on agar plates. These were incubated in a Percival growth chamber. Each shelf in the chamber was divided into nine regions and the light level in each region was measured using an LI-250A light meter (LI-COR) with the chamber door closed to ensure realistic conditions. Light levels on the middle and bottom shelves were measured after arranging agar plates on the above shelf/shelves.

Shelf	Row	Zone	Light Level (umol)
Top	Front	Left	103
		Middle	112
		Right	104
	Middle	Left	104
		Middle	113
		Right	104
	Back	Left	107
		Middle	116
		Right	109
Middle	Front	Left	112
		Middle	116
		Right	111
	Middle	Left	111
		Middle	116
		Right	110
	Back	Left	113
		Middle	118
		Right	112
Bottom	Front	Left	109
		Middle	114
		Right	110
	Middle	Left	108
		Middle	113
		Right	108
	Back	Left	109
		Middle	114
		Right	109

Figure 3.2 – Agar plates with Arabidopsis plants in the growth chamber

Arabidopsis seeds germination and Arabidopsis-*M. elongata* interaction studies were conducted on agar plates. These were incubated in a Percival growth chamber. Plates were stacked on a gentle angle to encourage smooth directional root growth along the agar surface.

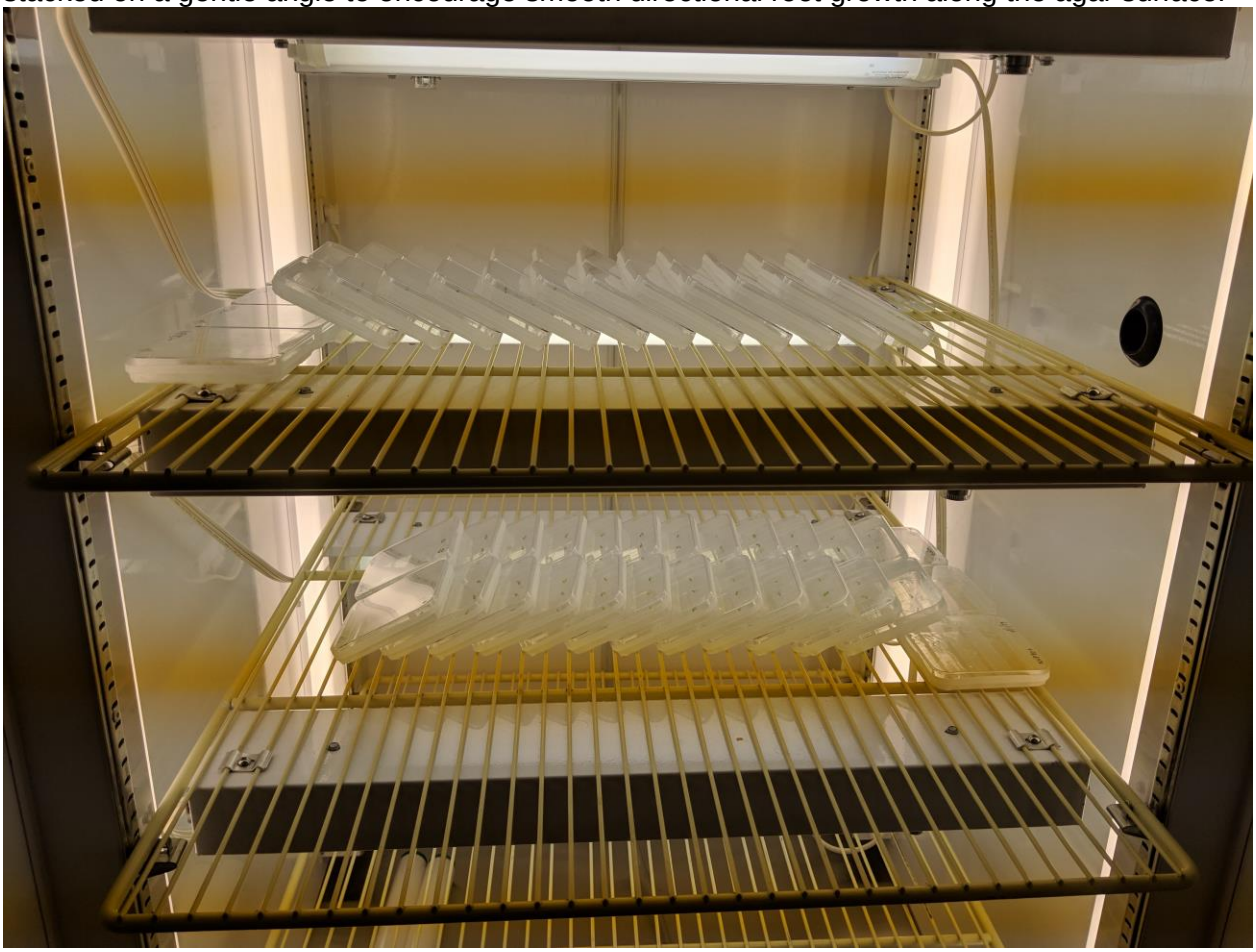


Figure 3.3 – **Bolting phenotype**

The arrow indicates the elongation of the Arabidopsis inflorescence away from the rosette of leaves which was considered to indicate “bolting.”

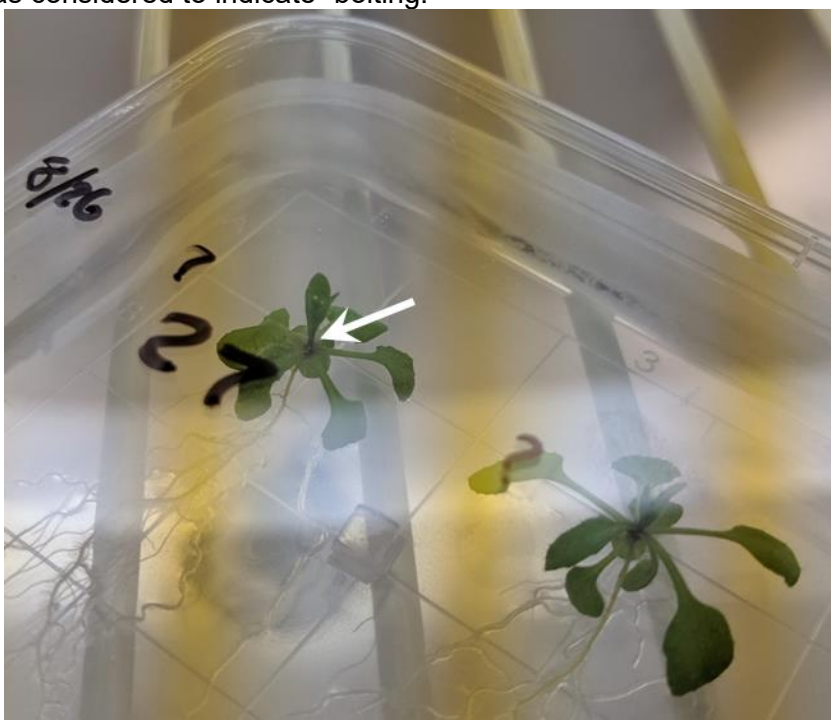


Figure 3.4 – Arabidopsis plants at the time of harvest for aerial biomass assay

Ten days after germination, Arabidopsis seedlings were transplanted from 1xMS germination plates to these PNM plates and inoculated with small blocks of Kaefer Medium, either colonized by fungi (left) or sterile (right). The Arabidopsis (and fungi, when applicable) grew on PMN plates for 12 days, at which point these pictures were captured and the plants harvested for aerial biomass assays.

Table 3.2 - **qPCR primer sets**

The qPCR primer sets used to quantify fungal colonization of plant roots and check for BRE/MRE in cured and wild-type fungal strains and fungus-colonized plant roots.

Target Organism	Target Gene	Name	Sequence
Arabidopsis thaliana	Glyceraldehyde-3-phosphate dehydrogenase C2 (*GAPC2*)	qGAPDH-F1	CATGACCACTGTCCACTCTATC
		qGAPDH-R1	CACCAGTGCTGCTAGGAATAA
Mortierella elongata	RNA polymerase II large subunit (*RPB1*)	qRPB1-F2	TCACCAAGTTCATCACCATCTC
		qRPB1-R2	AAGCCCGTCATGGGTATTG
Mycoavidus cysteinexigens (L. elongata BRE)	16S	MycAvi_16S_F1	TCAACCTGGGAACTGCATAC
		MycAvi_16S_R1	CGGTGTTCCTCCACATATCTAC
Mollicutes-related endobacteria (L. elongata MRE)	16S	NVP80MREq_16S_F1	CCTGAAAGAAGCTGGTGATACT
		NVP80MREq_16S_R1	TGACTGCCTTCGCCTTTATT

Figure 3.5 - **Aerial dry biomass of Arabidopsis plants grown in sterile potting mix**

Treatments refer to the composition of the potting mix. The untreated control (NoMillet) contrasted treatments where the sterile potting soil was mixed 97:3 v:v with sterile millet mix (Uninoculated), or millet mix inoculated with one of four *Mortierella elongata* strains (NVP64cu, NVP64wt, NVP80cu, or NVP80wt). Arabidopsis was grown to maturity and the aerial biomass harvested and dried. Colors correspond to treatment, horizontal brackets and numbers indicate pairwise Wilcox ranked sum tests and the resulting p-value. N=12 for all treatments. Between NVP64cu v. NVP64wt, NVP80cu v. NVP80wt, and NoMillet v. Uninoculated, we used two-tailed tests. Between each fungal treatment and the Uninoculated, we performed one-tailed tests with the alternative hypothesis “greater”.

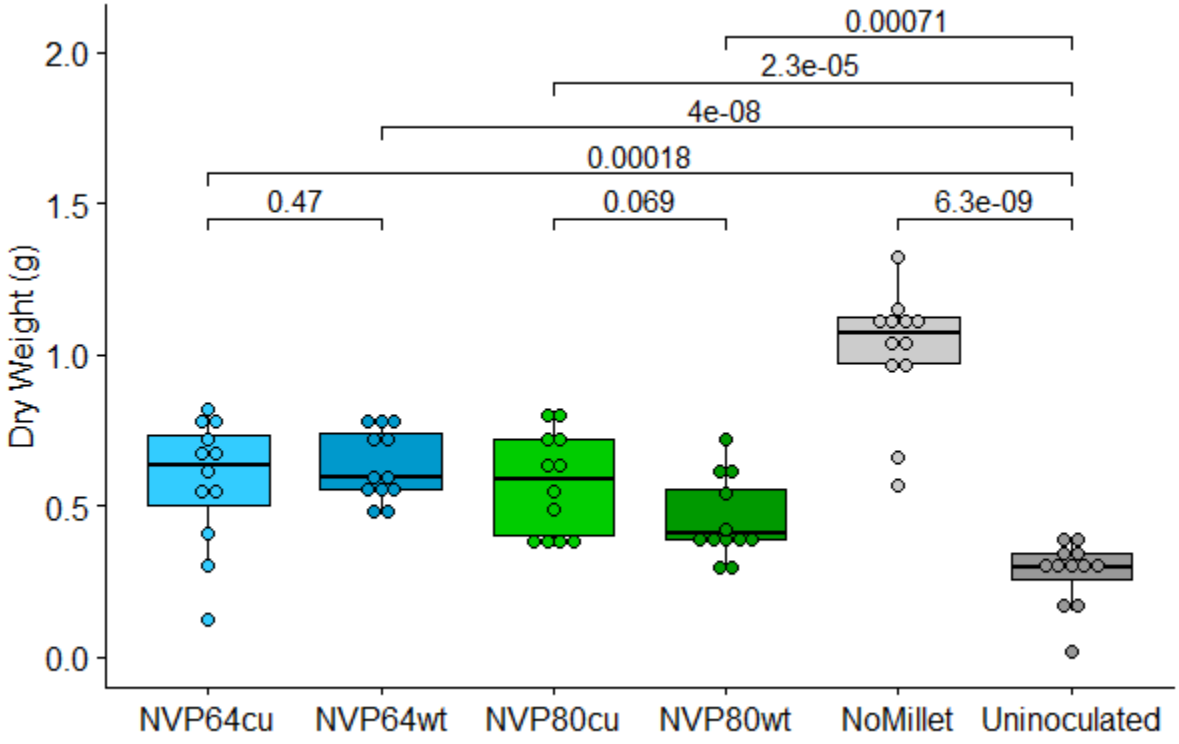


Figure 3.6 – Total mass of Arabidopsis seed

Treatments refer to the composition of the potting mix in which Arabidopsis plants were grown. The untreated control (NoMillet) contrasted treatments where the sterile potting soil was mixed 97:3 v:v with sterile millet mix (Uninoculated), or millet mix inoculated with one of four *Mortierella elongata* strains (NVP64cu, NVP64wt, NVP80cu, or NVP80wt). Arabidopsis was grown to maturity and the seeds collected by Aracon tubes. N=5 for all treatments. Colors correspond to treatment, horizontal brackets and numbers indicate pairwise Wilcox ranked sum tests and the resulting p-value.

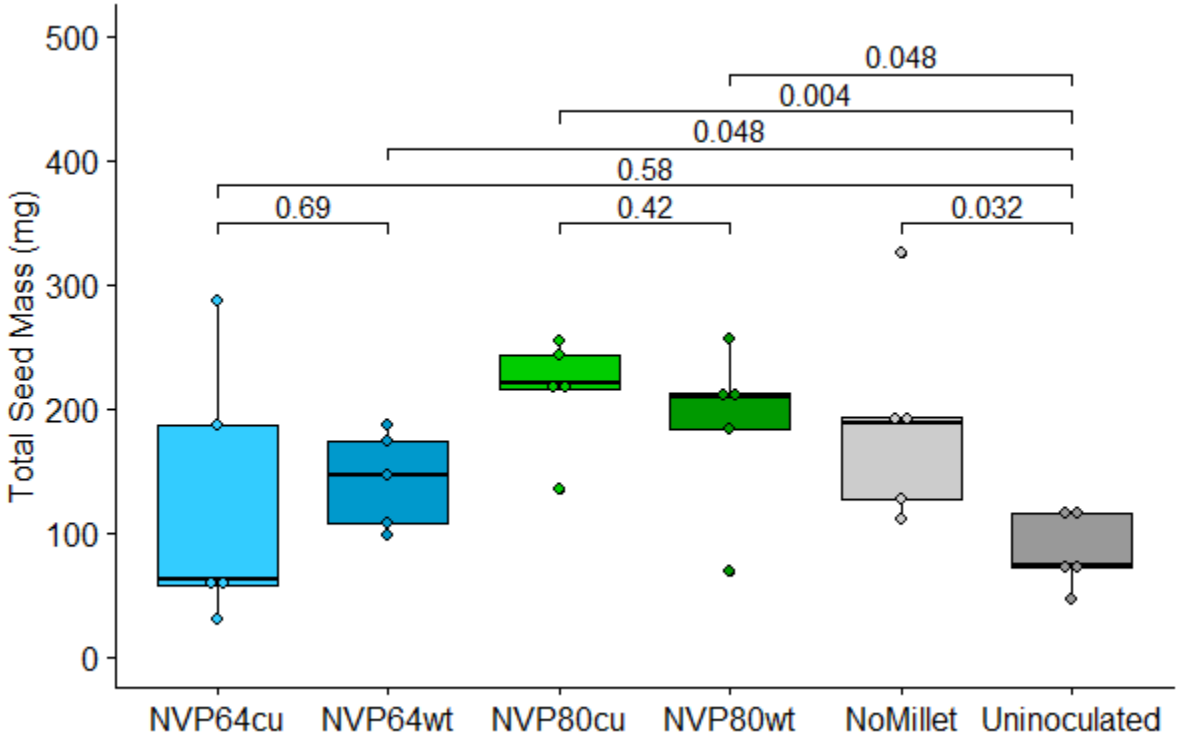


Figure 3.7 – Average Arabidopsis seed mass

Treatments refer to the composition of the potting mix. The untreated control (NoMillet) contrasted treatments where the sterile potting soil was mixed 97:3 v:v with sterile millet mix (Uninoculated), or millet mix inoculated with one of four *Mortierella elongata* strains (NVP64cu, NVP64wt, NVP80cu, or NVP80wt). Arabidopsis was grown to maturity and the seeds collected by Aracon tubes. Average seed mass was determined by weighing and then counting a subset of seeds taken from the total seed mass. N=5 for all treatments. Colors correspond to treatment, horizontal brackets and numbers indicate pairwise Wilcox ranked sum tests and the resulting p-value.

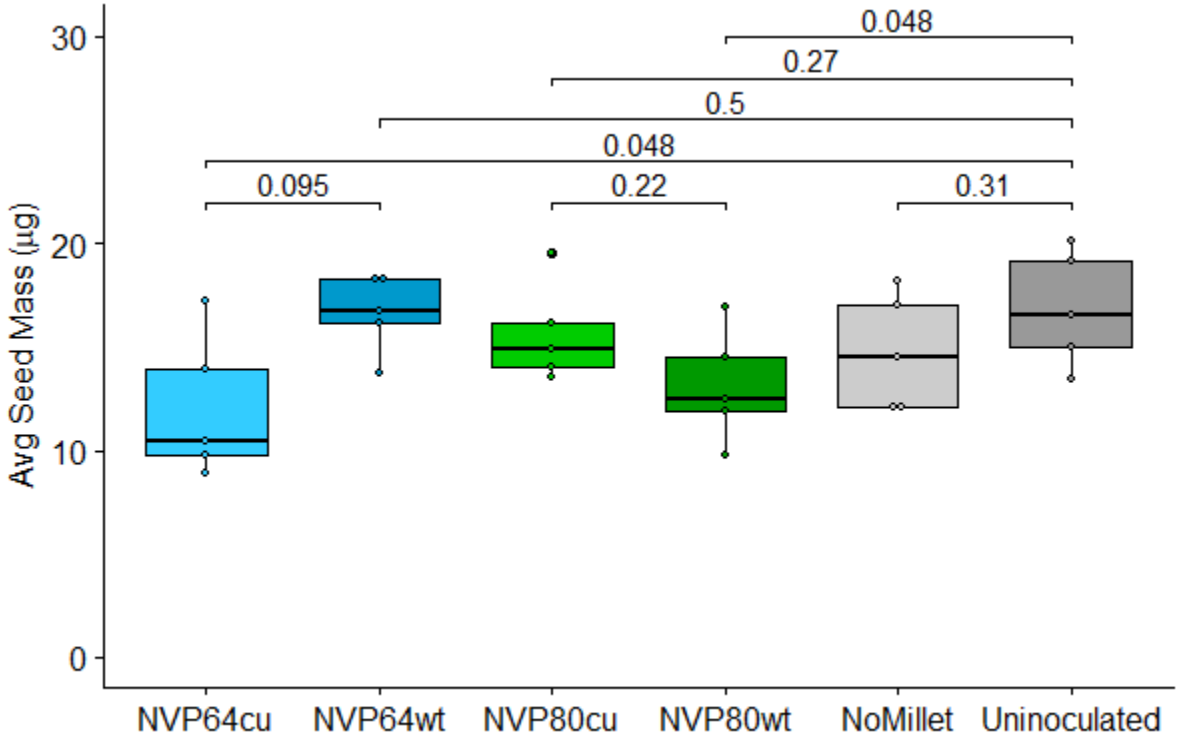


Figure 3.8 - **Density histogram of Arabidopsis seed image area**

"Strain" refers to the composition of the potting mix in which Arabidopsis plants were grown. The untreated control (NoMillet) contrasted treatments where the sterile potting soil was mixed 97:3 v:v with sterile millet mix (Uninoculated), or millet mix inoculated with one of four *Mortierella elongata* strains (NVP64cu, NVP64wt, NVP80cu, or NVP80wt). Arabidopsis was grown to maturity and the seeds collected by Aracon tubes. A subset of seeds from 5 samples per treatment were adhered to white paper and imaged using an Epson scanner. The x-axis indicates the pixel count of each individual seed scanned for each treatment, with samples pooled within treatments. The vertical dashed lines indicate the mean pixel area for seeds in each treatment.

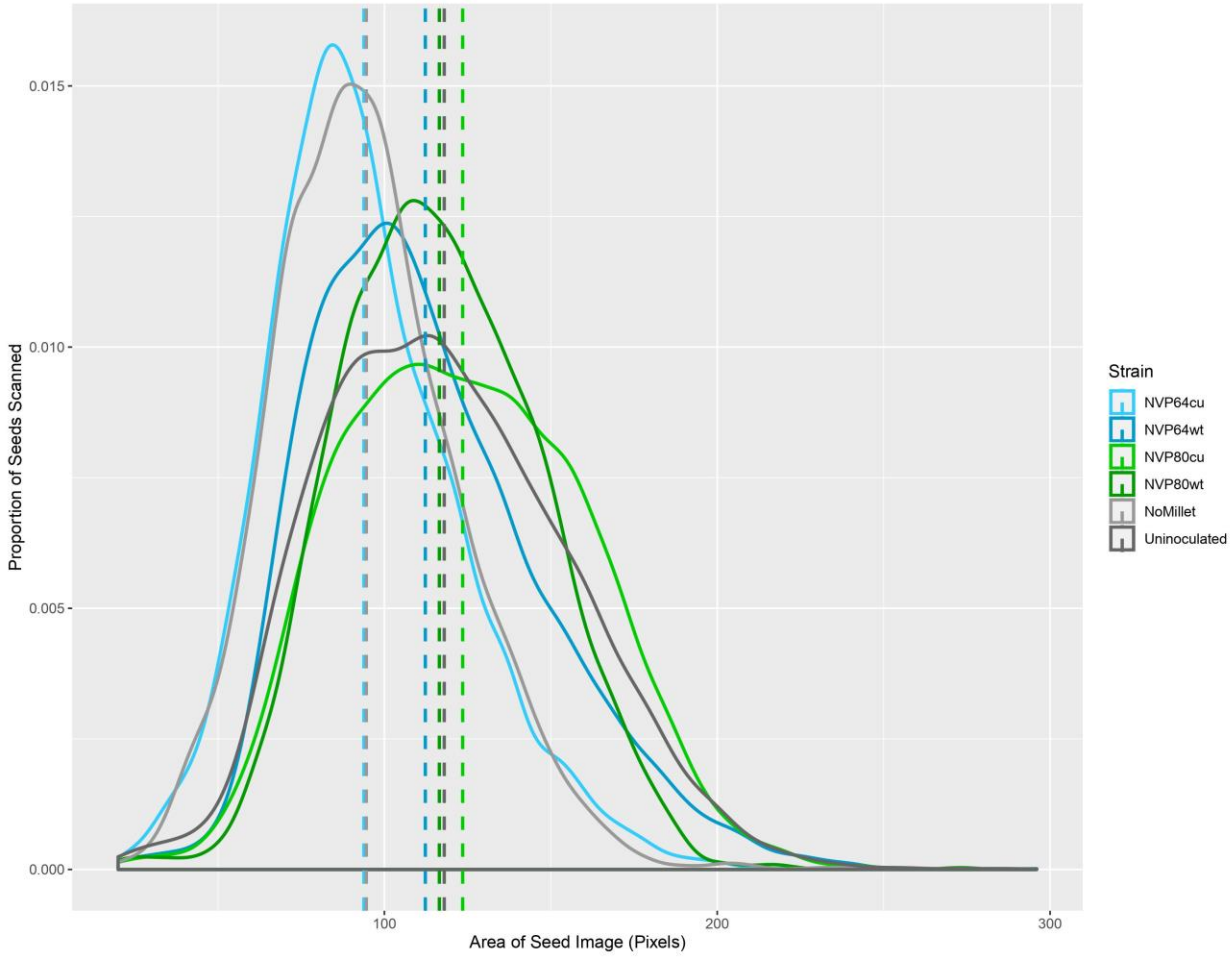


Figure 3.9 – Total seed number produced by Arabidopsis

Treatments refer to the composition of the potting mix. The untreated control (NoMillet) contrasted treatments where the sterile potting soil was mixed 97:3 v:v with sterile millet mix (Uninoculated), or millet mix inoculated with one of four *Mortierella elongata* strains (NVP64cu, NVP64wt, NVP80cu, or NVP80wt). Arabidopsis was grown to maturity and the seeds collected by Aracon tubes. Average seed mass was calculated by dividing total seed mass by average seed mass for each sample. N=5 for all treatments. Colors correspond to treatment, horizontal brackets and numbers indicate pairwise Wilcox ranked sum tests and the resulting p-value.

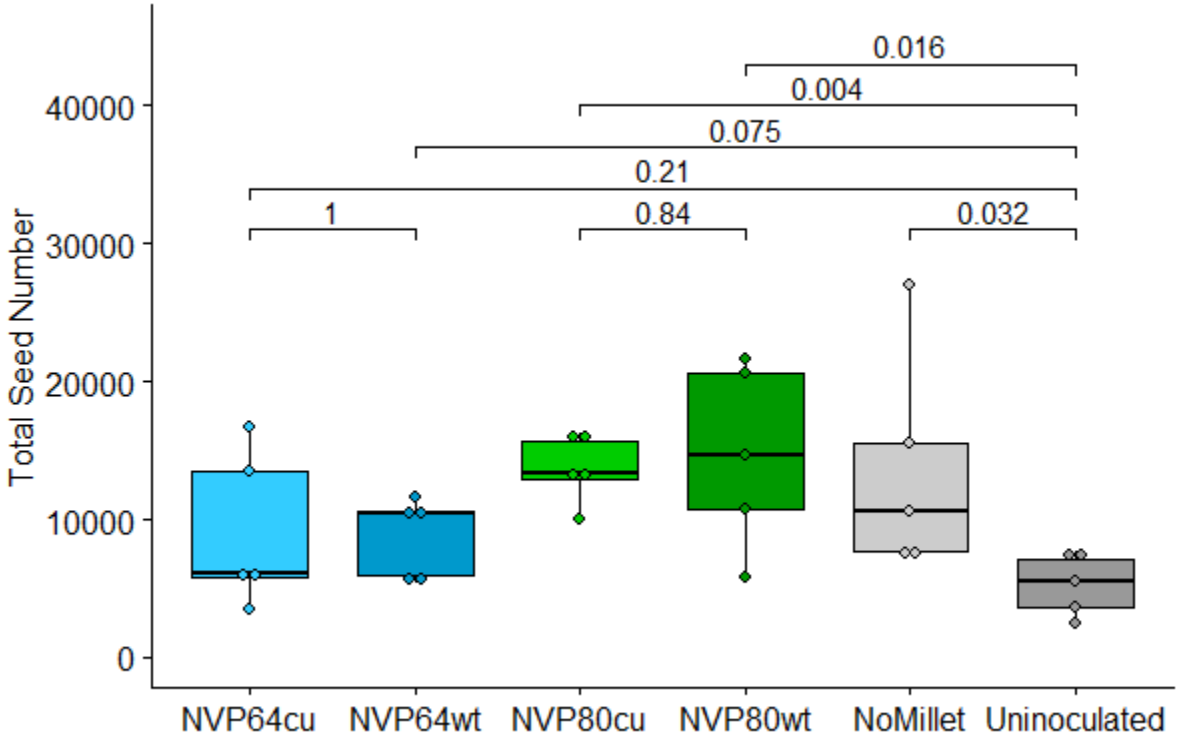


Table 3.3 - **Linear modeling of Arabidopsis aerial dry weight as a function of light level**

The aerial dry biomass of Arabidopsis plants harvested from agar-based Arabidopsis-*Mortierella elongata* interaction experiments and modeled as a function of light level. Medium indicates the composition of the medium on which *M. elongata* was cultured: KM = Kaefer Medium; MEA = Malt Extract Agar. Treatment indicates the strain of *M. elongata* with which Arabidopsis was inoculated or the uninoculated control.

Medium	Treatment	Trend Slope	SE	DF	0.95 Conf. Limit
KM	NVP64cu	0.00	0.02	100	-0.030 - 0.037
	NVP64wt	0.01	0.02	100	-0.027 - 0.049
	NVP80cu	0.03	0.02	100	-0.009 - 0.062
	NVP80wt	0.01	0.02	100	-0.030 - 0.049
	Control	-0.02	0.02	100	-0.055 - 0.010
MEA	NVP64cu	-0.01	0.02	100	-0.055 - 0.027
	NVP64wt	0.03	0.02	100	-0.017 - 0.075
	NVP80cu	0.01	0.02	100	-0.034 - 0.057
	NVP80wt	0.01	0.02	100	-0.036 - 0.054
	Control	-0.03	0.02	100	-0.067 - 0.013

Table 3.4 – **Linear modeling of Arabidopsis aerial dry weight as a function of treatment and medium**

The aerial dry biomass of Arabidopsis plants from agar-based Arabidopsis-*Mortierella elongata* interaction experiments, modeled as a function of treatment (control v. different strains of *Mortierella elongata*), the medium on which the fungi had been cultured, and any interaction between those terms. We also conducted pairwise comparisons within treatments of the estimate marginal means (EMMs) for each inoculating medium.

Treatment	**Medium**	**Estimated Marginal Mean (EMM)**				**Contrasts between Media**				
		EMM	SE	DF	0.95 Conf. Limit	ΔEMM	SE	DF	t.ratio	*p*
NVP64cu	KM	2.22	0.075	110	2.05 - 2.39	0.027	0.11	110	0.248	0.80
	MEA	2.19	0.081	110	2.01 - 2.38					
NVP64wt	KM	1.96	0.075	110	1.79 - 2.13	0.035	0.11	110	0.320	0.75
	MEA	1.92	0.081	110	1.74 - 2.11					
NVP80cu	KM	2.06	0.075	110	1.89 - 2.23	0.044	0.11	110	0.398	0.69
	MEA	2.01	0.081	110	1.83 - 2.20					
NVP80wt	KM	2.10	0.075	110	1.93 - 2.27	-0.018	0.11	110	-0.161	0.87
	MEA	2.11	0.081	110	1.93 - 2.30					
Control	KM	1.84	0.075	110	1.67 - 2.01	0.154	0.11	110	1.393	0.17
	MEA	1.69	0.081	110	1.50 - 1.87					

Table 3.5 - **Linear modeling of Arabidopsis aerial dry weight as a function of starting seedling root length**

The aerial dry biomass of Arabidopsis plants from agar-based Arabidopsis-*Mortierella elongata* interaction experiments, modeled as a function of seedling starting root length. There were no significant differences in the slope of the relationship of starting root length to final aerial dry weight across experimental rounds or treatments.

Experiment	Treatment	Slope	SE	DF	0.95 Conf. Limit	Signif. Group
Media Panel	NVP64cu	0.1254	0.047	1034	-0.013 - 0.263	a
	NVP64wt	0.1578	0.0424	1034	-0.033 - 0.282	a
	NVP80cu	0.1185	0.0425	1034	-0.006 - 0.243	a
	NVP80wt	0.1614	0.0403	1034	-0.043 - 0.28	a
	Control	0.1348	0.0427	1034	-0.009 - 0.26	a
Cured Panel	NVP64cu	0.0885	0.0182	1034	-0.035 - 0.142	a
	NVP64wt	0.0796	0.0347	1034	-0.022 - 0.181	a
	NVP80cu	0.0721	0.019	1034	-0.016 - 0.128	a
	NVP80wt	0.1471	0.0363	1034	-0.040 - 0.254	a
	Control	0.0615	0.0291	1034	-0.024 - 0.147	a

Table 3.6 - **Linear mixed modeling of Arabidopsis aerial dry weight**

To account for having measurements for three plants per agar plate and two independent repetitions of the agar-based interaction experiment, experimental round and plate were treated as random/grouping effects. The starting root length and experimental treatment were fixed effects, where the uninoculated control treatment was estimated as the intercept.

Fixed Effects					
	Estimate	**Std.Error**	**df**	**t-value**	***p***
(Intercept)	0.581	0.098	8.502	5.92	2.7E-04
treatment=NVP64cu	0.601	0.068	230.9	8.79	3E-16
treatment=NVP64wt	0.565	0.076	232.6	7.41	2E-12
treatment=NVP80cu	0.650	0.068	230.8	9.52	<2E-16
treatment=NVP80wt	0.681	0.076	231.9	8.93	<2E-16
Root Length	0.122	0.008	514.1	16.06	<2E-16
Random effects					
	Name	**Variance**	**Std.Dev.**	**# of Groups**	
Plate	(Intercept)	0.074	0.273	255	
Experiment	(Intercept)	0.005	0.072	2	
Residual	0.117	0.342	-	-	
EMM Pairwise Comparisons					
Contrast	**estimate**	**SE**	**df**	**t.ratio**	***p***
Control - NVP64cu	-0.6005	0.069	250	-8.7	<.0001
Control - NVP64wt	-0.5654	0.0763	249	-7.41	<.0001
Control - NVP80cu	-0.6498	0.0689	249	-9.43	<.0001
Control - NVP80wt	-0.6807	0.0762	248	-8.93	<.0001
NVP64cu - NVP64wt	0.0351	0.0689	250	0.509	0.9864
NVP64cu - NVP80cu	-0.0494	0.0573	249	-0.86	0.9108
NVP64cu - NVP80wt	-0.0803	0.069	250	-1.16	0.7717
NVP64wt - NVP80cu	-0.0844	0.0689	250	-1.23	0.7363
NVP64wt - NVP80wt	-0.1153	0.0763	249	-1.51	0.5555
NVP80cu - NVP80wt	-0.0309	0.0689	249	-0.45	0.9916

Figure 3.10 – *Mortierella elongata* colonization of Arabidopsis increased aerial dry weight in agar-based interaction experiments

The estimated marginal mean of Arabidopsis aerial dry weight modeled as a function of starting root length and treatment, which included the uninoculated control and four strains of *M. elongata*. The degrees of freedom for each comparison were approximated using the kenward-roger method and the *p*-values adjusted for multiple comparisons using the Tukey method for comparing a family of 5 estimates. Letters indicate significantly different groups with an alpha value of 0.05. Exact values can be found in Table 3.6.

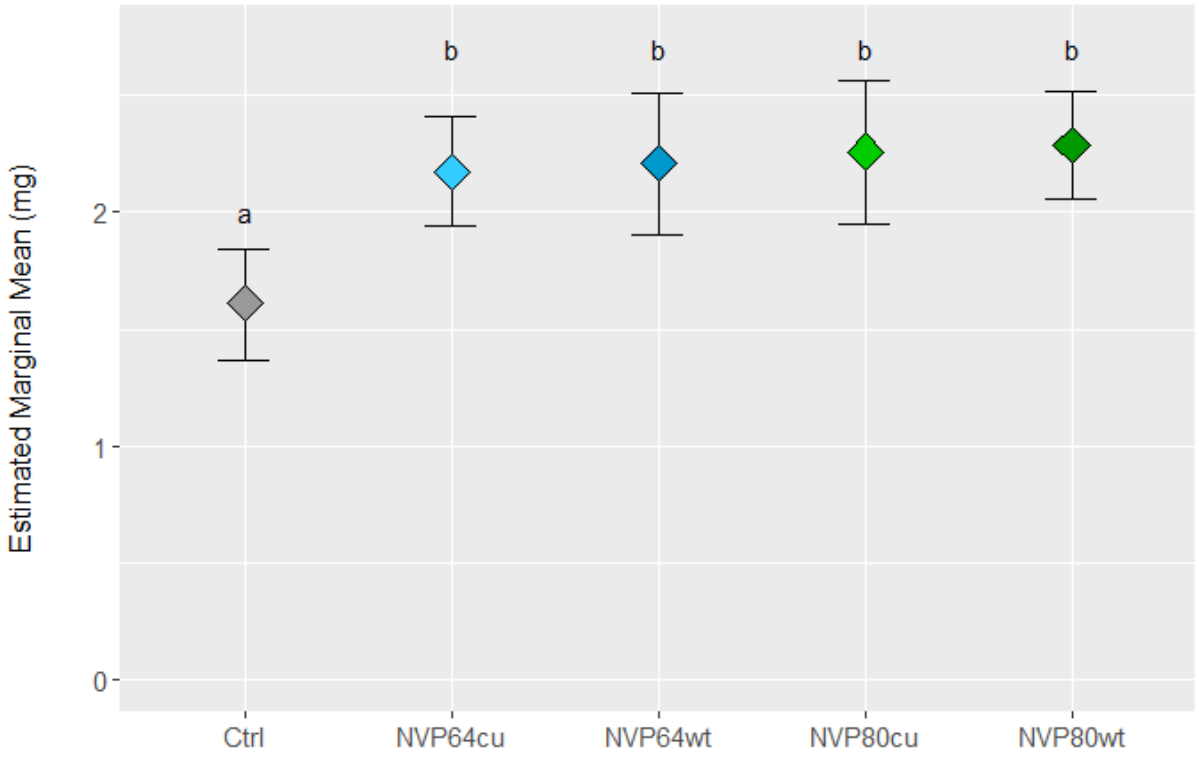


Table 3.7 – **qPCR of plant, fungal, and endobacterial genes from RNA**

Values indicate the qPCR cycle number at which amplification reached the threshold of detection for each locus tested in each cDNA library from the Arabidopsis root RNA samples used in the RNAseq experiment. Dash = not tested. Arabidopsis *GADPH* and *M. elongata* RPB1 are single copy genes. The bacterial 16S gene is multicopy, which was necessary for detection, since these endobacteria are very low abundance in the fungal hyphae.

Strain	Sample Number	Arabidopsis (GADPH)	M. elongata (RPB1)	MRE (16S)	BRE (16S)
NVP64cu	118	17.44	30.77	-	-
	108	17.28	33.29	-	-
	48	16.13	29.51	-	-
NVP64wt	94	16.19	29.05	-	34.71
	64	16.25	30.07	-	36.07
	24	16.34	29.47	-	34.76
NVP80cu	36	16.34	24.83	-	-
	106	16.06	25.88	-	-
	46	16.21	27.65	-	-
NVP80wt	22	16.56	31.68	18.31	-
	82	16.69	30.69	15.13	-
	102	15.96	26.67	11.8	-

Figure 3.11 – *Mortierella elongata* strains equivalently colonized Arabidopsis roots

RNA was extracted from Arabidopsis roots colonized by *M. elongata*, pooled from all three plants on each agar plate, from three plates per treatment. The inferred ratio of fungal:plant cDNA is based on the qPCR results and standard curves for each qPCR primer set. Since Arabidopsis *GADPH* and *M. elongata* RPB1 are single copy genes, the ratio of fungal and plant template provides a normalized estimate of fungal colonization of plant roots.

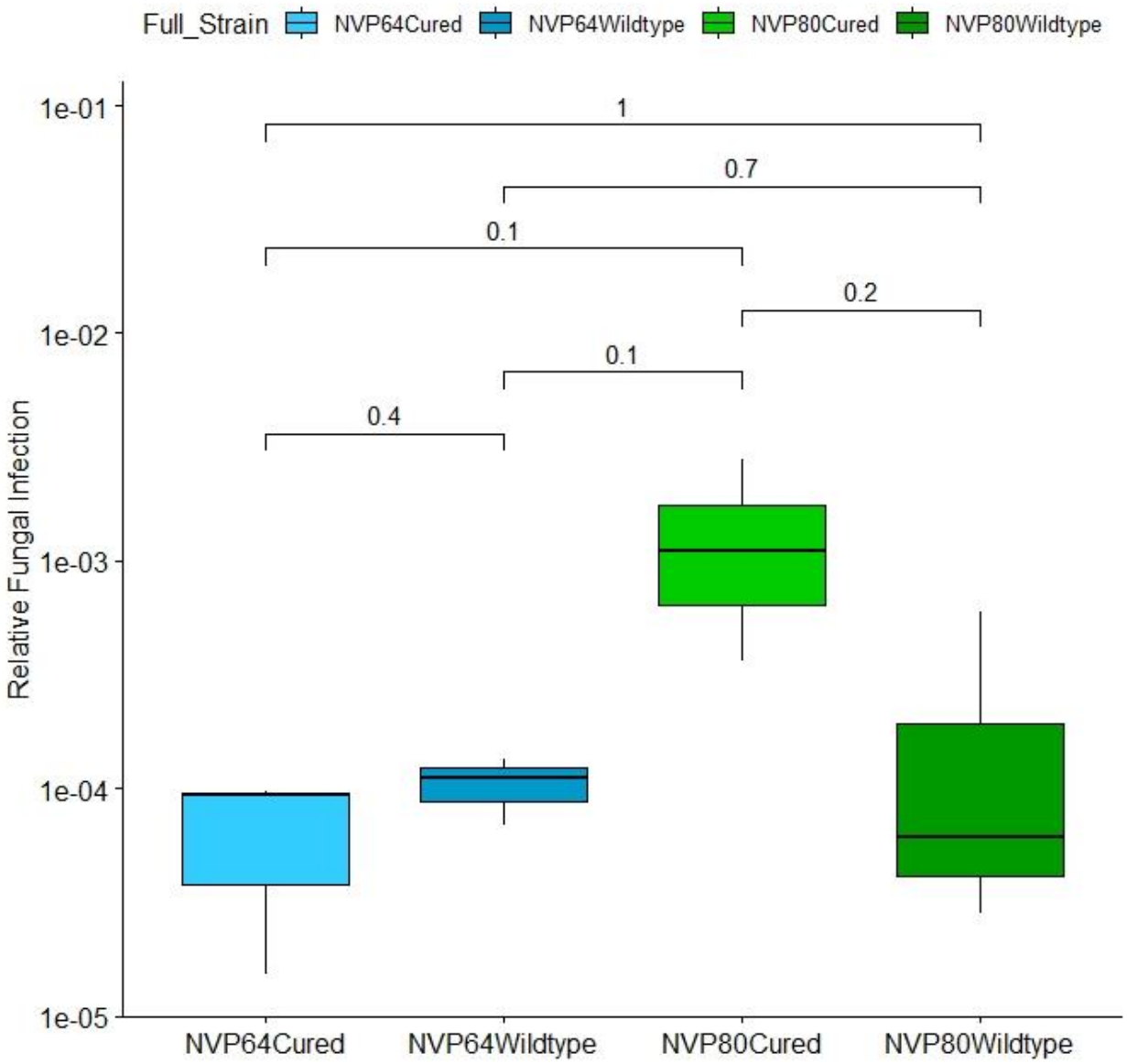


Table 3.8 – **Molecular results of RNA sequencing run**

The number of sequenced reads passing initial filtration by the sequencer, the percentage of those reads that mapped to the combined reference transcriptome, and the proportion of mapped reads that mapped to plant or fungal transcripts.

Treatment	Sample Number	Sequenced Reads	Mapping Rate	Mapped to Arabidopsis	Mapped to *M. elongata*
NVP64cu	48	37,420,119	97.66%	99.960%	0.040%
	108	36,495,398	97.67%	99.989%	0.011%
	118	37,754,726	97.78%	99.965%	0.035%
NVP64wt	24	37,276,912	97.58%	99.955%	0.045%
	64	32,066,022	97.72%	99.967%	0.033%
	94	30,536,173	97.70%	99.946%	0.054%
NVP80cu	36	32,494,719	97.22%	98.647%	1.353%
	46	33,188,011	97.53%	99.710%	0.290%
	106	33,919,080	97.31%	99.395%	0.605%
NVP80wt	22	34,589,059	97.73%	99.981%	0.019%
	82	36,239,973	97.70%	99.964%	0.036%
	102	34,826,178	97.65%	99.808%	0.192%
Control	50	33,645,723	97.85%	99.996%	0.004%
	60	35,809,882	97.77%	99.996%	0.004%
	80	34,944,673	97.77%	99.996%	0.004%

Figure 3.12 – Principal component analysis of differential Arabidopsis gene expression

Arabidopsis root RNAseq data analyzed using DESeq2, sequenced from three biological replicates taken from each of the uninoculated control and fungal treatments inoculated with *Mortierella elongata*.

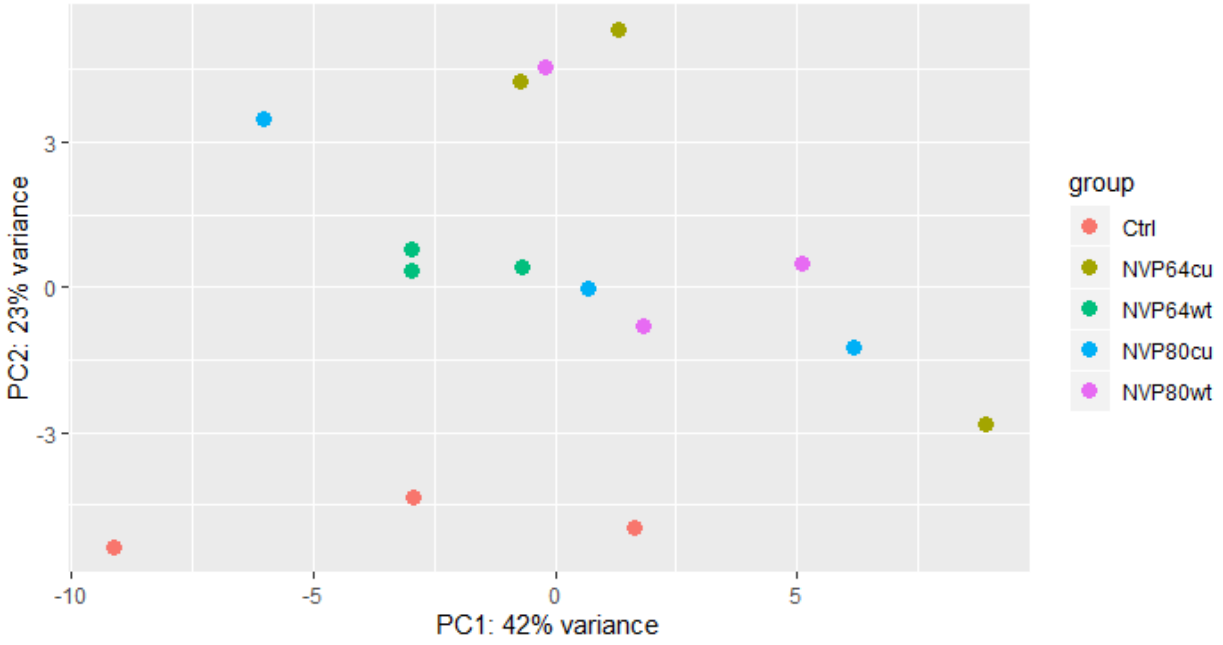


Figure 3.13 - **Volcano plots of differential gene expression**

Pairwise comparisons of normalized gene expression between fungal treatments and the uninoculated control, calculated from the DESeq2 analyses. Each point represents a gene, plotted by adjusted p-value and Log2 Fold Change (LFC) in expression between the fungal treatment and the control. Vertical dashed lines indicate the |LFC|=1 threshold and horizontal lines indicate the adjusted p-value threshold of 0.05 used to identify genes with significant changes in expression. Genes are colored by which of the LFC and p-value cutoffs were exceeded: gray = failed both; green = exceeded only the LFC cutoff, but not the p-value cutoff; blue = exceeded p-value cutoff, but not LFC; red = exceeded both cutoffs.

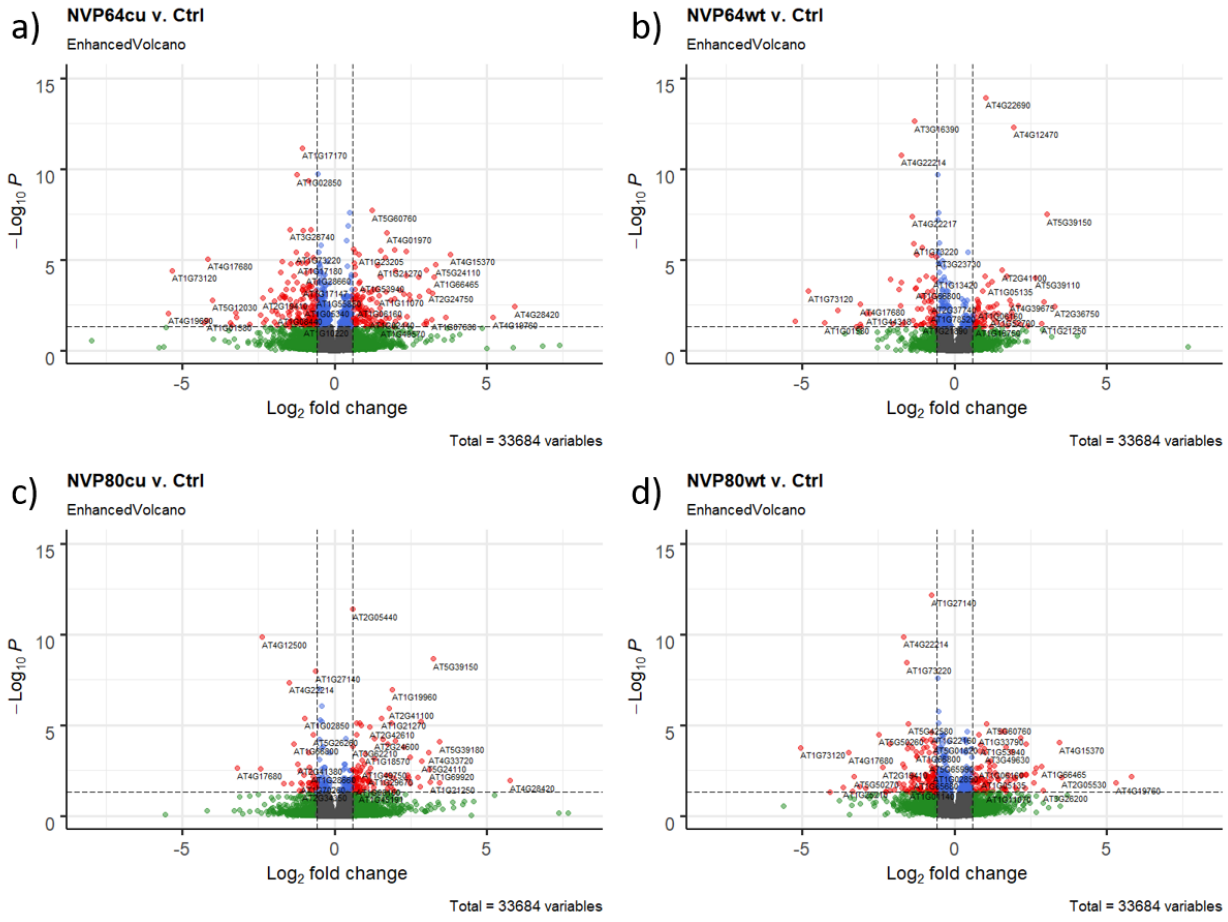


Table 3.9 - Arabidopsis genes differentially expressed in response to *Mortierella elongata*

Log 2 fold change (LFC) values were calculated by DESeq2 and filtered at $|LFC|=\log_2(1.5)=0.58$ and adjusted p-value = 0.05. Table is organized first by functional annotation, then by direction of regulation, and finally by the number of fungal treatments in which the gene was differentially expressed.

Functional Annotation			Log2 Fold-Change				Name	Gene
Broad	**Middle**	**Detail**	**NVP 64cu**	**NVP 64wt**	**NVP 80cu**	**NVP 80wt**		
Abiotic Stress	Al tolerance	aluminum activated malate transporter family protein	-1.88			-1.83	-	AT1G08440
Abiotic Stress	Broad	RESPONSIVE TO HIGH LIGHT 41	-0.99				RHL41	AT5G59820
Abiotic Stress	Broad	Sucrose synthetase			0.60	0.70	SUS1	AT5G20830
Abiotic Stress	Cold/Heat/Salt/ Drought	Annexin 7				-0.61	ANNAT7	AT5G10230
Abiotic stress	Cold/Salt/ Drought	ORGANIC CATION/CARNITINE TRANSPORTER5		-0.62			OCT5	AT1G79410
Abiotic Stress	Drought	Methylenetetrahydrofolate reductase family protein	-1.15			-1.06	POX2	AT5G38710
Abiotic Stress	Drought	sucrose synthase 3	-1.54				SUS3	AT4G02280
Abiotic Stress	Drought, cold, & salt	NAD(P)-linked oxidoreductase superfamily protein	-2.43			-2.14	AKR4C9	AT2G37770
Abiotic Stress	Drought, cold, & salt	NAD(P)-linked oxidoreductase superfamily protein	-1.49				-	AT5G62420
Abiotic Stress	Hypoxia	PHYTOGLOBIN 1			0.81	0.82	HB17	AT2G16060
Abiotic Stress	Hypoxia/ Oxidative Stress	F-box/RNI superfamily protein	-5.34	-4.79		-5.06	T18K17.22	AT1G73120
Abiotic Stress	Hypoxia/ Oxidative Stress	alpha/beta-Hydrolases superfamily protein	-1.72			-1.42	CXE6	AT1G68620
Abiotic Stress	Hypoxia/ Oxidative Stress	Peroxidase superfamily protein	2.26	1.92	1.84	1.98	PER28	AT3G03670
Abiotic Stress	Hypoxia/ Oxidative Stress	Stachyose synthase, Raffinose synthase 4	1.72	0.93	1.31	1.54	STS	AT4G01970
Abiotic Stress	Hypoxia/ Oxidative Stress	DNA polymerase epsilon catalytic subunit A	1.14		1.25	1.09	-	AT1G19530

Table 3.9 (cont'd)

Abiotic Stress	Hypoxia/ Oxidative Stress	Peroxidase superfamily protein	1.51			1.65	PER10	AT1G49570
Abiotic Stress	Hypoxia/ Oxidative Stress	Peroxidase superfamily protein	1.98				PER5	AT1G14550
Abiotic Stress	Hypoxia/Salt	HSP20-like chaperones superfamily protein 17.6A	-4.02	-3.84		-3.13	HSP17.7	AT5G12030
Abiotic Stress	Hypoxia/Salt	HSP20-like chaperones superfamily protein 17.8				-1.55	HSP17.8	AT1G07400
Abiotic Stress	Iron deficiency	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-1.64	-1.25		-1.68	BHLH101	AT5G04150
Abiotic Stress	Iron deficiency	ferric reduction oxidase 2	-4.20	-4.27			FRO2	AT1G01580
Abiotic Stress	Iron deficiency	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	-3.22	-3.09			ORG2	AT3G56970
Abiotic Stress	Iron deficiency	basic helix-loop-helix (bHLH) DNA-binding superfamily protein		-2.78		-2.26	ORG3	AT3G56980
Abiotic Stress	Iron deficiency	iron-regulated transporter 1	-5.46	-5.24			IRT1	AT4G19690
Abiotic Stress	Iron deficiency	nicotianamine synthase	-1.73			-1.61	NAS2	AT5G56080
Abiotic Stress	Iron deficiency	nicotianamine synthase	-1.52				NAS1	AT5G04950
Abiotic Stress	K deficiency	CBL-interacting protein kinase 9				-1.47	CIPK9	AT1G01140
Abiotic Stress	Metal/Ion Transport	DETOXIFICATION 43, FERRIC REDUCTASE DEFECTIVE 3, MANGANESE ACCUMULATOR 1	-0.94	-0.77	-0.65	-0.75	DTX43	AT3G08040
Abiotic Stress	Metal/Ion Transport	alkenal reductase	-1.60		-1.17		P1	AT5G16970
Abiotic Stress	Metal/Ion Transport	copper transporter 2	-2.80				COPT2	AT3G46900
Abiotic Stress	Metal/Ion Transport	cation/H+ exchanger 17	-1.61				CHX17	AT4G23700
Abiotic Stress	Metal/Ion Transport	STELAR K+ outward rectifier	1.48				SKOR	AT3G02850
Abiotic Stress	Oxidative Stress	Peroxidase 56	-0.64				PRX56	AT5G15180
Abiotic Stress	Oxidative Stress	Zinc-binding dehydrogenase family protein	-0.61				-	AT5G17000
Abiotic Stress	Oxidative Stress	Peroxidase 37	0.82		0.85	0.96	PRX37	AT4G08770

Table 3.9 (cont'd)

Abiotic Stress	Oxidative stress	peroxidase superfamily protein			0.68		-	AT2G18150
Abiotic stress	Oxidative stress	Peroxidase 49			0.72		-	AT4G36430
Abiotic Stress	P deficiency	ribonuclease 1	-1.99	-1.64		-1.67	RNS1	AT2G02990
Abiotic Stress	P deficiency	-				-1.17	G3Pp1	AT3G47420
Abiotic Stress	P deficiency	EXS (ERD1/XPR1/SYG1) family protein	0.97	0.87			-	AT2G03260
Abiotic Stress	Response to Cd	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	-1.44	-1.09	-1.21	-1.24	-	AT2G41380
Abiotic Stress	Salt	TONOPLAST INTRINSIC PROTEIN 2;3	-0.66		-0.82	-0.67	TIP2;3	AT5G47450
Abiotic Stress	Unspecified	hypothetical protein	-1.03		-0.59		T9L3_30	AT5G14730
Abiotic Stress	Unspecified	RmlC-like cupins superfamily protein		-3.21			-	AT5G38910
Abiotic Stress	Unspecified	RmlC-like cupins superfamily protein	2.34	3.03	3.23	2.35	-	AT5G39150
Abiotic Stress	Unspecified	RmlC-like cupins superfamily protein	2.46		1.93	2.23	-	AT5G38940
Abiotic Stress	Unspecified	RmlC-like cupins superfamily protein		2.66	2.47	2.00	MXF12.14	AT5G39110
Abiotic Stress	Unspecified	RmlC-like cupins superfamily protein	1.54	1.74	2.26		-	AT5G39160
Abiotic Stress	Unspecified	RmlC-like cupins superfamily protein		2.11	2.84		-	AT5G39120
Abiotic Stress	Unspecified	RmlC-like cupins superfamily protein		2.92	3.45		-	AT5G39180
Abiotic Stress	Unspecified	RmlC-like cupins superfamily protein				2.00	-	AT3G05950
Abiotic Stress/ Hormone	Broad/ABA	CBL-interacting serine/threonine protein kinase	-0.60			-0.69	CIPK14	AT5G01820
Abiotic Stress/ Hormone	Cold/Drought/ ABA	Dehydrin family protein		-0.78			-	AT4G38410
Abiotic Stress/ Hormone/ Signaling	Broad/ABA/ Calcium signal	annexin 4		-0.94	-0.70	-0.90	ANNAT4	AT2G38750
Abiotic Stress/ Metabolism	Hypoxia/ Protein Modification	AGC2 KINASE 1, Oxidative Signal Inducible 1	-0.91				AGC2-1	AT3G25250
Defense	Bacteria	euonymus lectin S3				-0.64	EULS3	AT2G39050
Defense	Bacteria	Calcium-binding EF hand family protein	1.38	1.57	1.78	1.63	CML12	AT2G41100
Defense	Bacteria	sigma factor binding protein 1	1.03	0.97	1.11	0.87	SIB1	AT3G56710

Table 3.9 (cont'd)

Defense	Bacteria	indole glucosinolate biosynthesis	0.90		1.01	0.80	MYB51	AT1G18570
Defense	Bacteria	cysteine-rich RLK (RECEPTOR-like protein kinase) 31	1.54		1.75	1.65	CRK31	AT4G11470
Defense	Bacteria	Leucine-rich receptor-like protein kinase family protein	1.50		0.94	1.18	FLS2	AT5G46330
Defense	Bacteria	CAP (Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1 protein) superfamily protein	2.13			1.71	F2J10.7	AT1G50050
Defense	Bacteria	Cysteine-rich receptor-like protein kinase			0.94		CRK10	AT4G23180
Defense	Bacteria	cysteine-rich receptor-like protein kinase		1.42			CRK18	AT4G23260
Defense	Fungus	SBP (S-ribonuclease binding protein) family protein	-4.15	-3.10	-3.20	-3.49	dl4875c	AT4G17680
Defense	Fungus	Defensin-like protein 99	-0.77	-1.74	-1.48	-1.68	-	AT4G22214
Defense	Fungus	Defensin-like protein 100	-0.62	-1.39	-0.77	-1.02	-	AT4G22217
Defense	Fungus	trypsin inhibitor protein 1	-2.34			-2.26	TI1	AT2G43510
Defense	Fungus	papain-like cysteine protease	-2.09			-2.49	CEP1	AT5G50260
Defense	Fungus	Defensin-like protein 96			-0.64		-	AT4G22230
Defense	Fungus	chitinase A	-1.07				CHIB1	AT5G24090
Defense	Fungus	CAP (Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1 protein) superfamily protein	2.77	2.17	3.08	2.63	CAPE3	AT4G33720
Defense	Fungus	Chitinase family protein	3.02		1.98	2.36	F18O19.27	AT2G43620
Defense	Fungus	homolog of RPW8 3	1.08		1.10	1.04	HR3	AT3G50470
Defense	Fungus	Lectin like protein induced by chitin			1.60		-	AT3G16530
Defense	Fungus	Defensin-like protein 98	0.61				-	AT4G22212
Defense/ Hormone	General/SA	priming the SAR and ISR responses	-0.86		-1.02		EARLI1	AT4G12480
Defense/ Hormone	General/SA	priming the SAR and ISR responses	-2.06		-2.44		AZI3	AT4G12490

Table 3.9 (cont'd)

Defense	General	nodulin MtN21-like transporter family negatively regulates resistance against biotrophic pathogens			-1.12		UMAMIT36	AT1G70260
Defense	General	UDP-glycosyltransferase 73B4	-0.90				UGT73B4	AT2G15490
Defense	General	Disease resistance-responsive (dirigent-like protein) family protein		-1.00			-	AT4G11210
Defense/ Hormone	General/SA	priming the SAR and ISR responses		1.94			AZI1	AT4G12470
Defense	General	Mannose-binding lectin family protein	0.68			0.78	-	AT1G33790
Defense/ Hormone	Auxin, JA, SA, Ethylene	GDSL lipase 2	0.83		0.70	0.86	GLIP2	AT1G53940
Defense/ Hormone	Fungus/ Ethylene	Ethylene-responsive transcription factor	1.94			2.04	TDR1	AT3G23230
Defense/ Hormone	SA dependent/ Oxidative Stress	UDP-Glycosyltransferase 73B3	-0.78				UGT73B3	AT4G34131
Defense/ Metabolism	General/ Secondary	UDP-Glycosyltransferase 73B2	-0.79				UGT73B2	AT4G34135
Defense/ Metabolism	General/ Secondary	ATP binding cassette G6	-0.71				ABCG6	AT5G13580
Defense/ Metabolism	SAR/ Secondary	Phytoalexin deficient 3		0.82			PAD3	AT3G26830
Development	Flowering	Agamous-like 19	-0.78		-0.71	-0.83	AGL19	AT4G22950
Development	Flowering	DNAJ heat shock N-terminal domain-containing protein		-0.88		-0.95	-	AT2G21510
Development	Flowering	UDP-Glycosyltransferase superfamily protein	-1.06			-0.88	UGT87A2	AT2G30140
Development	Flowering	SGNH hydrolase-type esterase superfamily protein		-1.71		-1.58	-	AT2G40250
Development	Flowering	hypothetical protein	-1.90			-1.97	F21F14.100	AT3G61930
Development	Flowering	UDP-glycosyltransferase 79B8				-0.64	UGT79B8	AT2G22930
Development	Flowering	myb domain protein 45	-1.17				MYB45	AT3G48920
Development	Flowering	expansin-like B1	-0.63				EXLB1	AT4G17030
Development	Flowering	glycosyl hydrolase 9B17	-1.34				GH9B17	AT4G39000

Table 3.9 (cont'd)

Development	Flowering	Late embryogenesis abundant (LEA) protein-like protein	0.78			0.75	-	AT1G54890
Development	Growth	Vascular-related unknown protein 2	-0.95			-0.83	VUP2	AT1G50930
Development	Growth	EXPANSIN B2		-1.74		-1.33	EXPB2	AT1G65680
Development	Growth	FANTASTIC FOUR 3		-0.67			FAF3	AT5G19260
Development	Growth	promotes cell growth in response to light	0.96	0.70	1.16	0.77	LSH10	AT2G42610
Development	Growth	xanthine dehydrogenase 2	0.82	1.00	0.91	0.75	XDH2	AT4G34900
Development	Growth	Regulates cortical microtubule organization		0.72	0.78		SP1L2	AT1G69230
Development	Transcription	Light sensitive hypocotyls 4	0.69	0.75	0.97	0.68	LSH4	AT3G23290
Development	Root	marneral oxidase	-0.76	-1.05	-0.87	-0.92	MRO	AT5G42590
Development	Root	Thalian-diol desaturase cytochrome P450, family 705, subfamily A, polypeptide 5	-1.65	-1.38	-1.42	-1.64	THAD1	AT5G47990
Development	Root	thalianol hydroxylase cytochrome P450, family 708, subfamily A, polypeptide 2	-1.16			-1.19	THAH	AT5G48000
Development	Root	Thalianol synthase 1	-1.93			-1.71	THAS	AT5G48010
Development	Root	marneral synthase		-3.06			MRN1	AT5G42600
Development	Seed	CYTOCHROME P450, FAMILY 78, SUBFAMILY A, POLYPEPTIDE 6	0.81		0.76	0.63	CYP78A6	AT2G46660
Development	Senescence	DNA/RNA Degradation				-1.41	BFN1	AT1G11190
Development	Senescence	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	0.93			1.01	-	AT3G49630
Development	Transcription Factor	Petal Loss	0.59		0.60		PTL	AT5G03680
Development		CURVATURE THYLAKOID 1C		0.59			CURT1C	AT1G52220
Development	Flowering	CLAVATA 1	0.64				CLV1	AT1G75820
Development/ Hormone	Broad/ SA/JA/ABA	EXTENSIN 1/4, OBP3-RESPONSIVE GENE 5		0.81			EXT4	AT1G76930

Table 3.9 (cont'd)

Development/ Hormone	Flowering/ Gibberellin	Transcription factor PRE4		-0.73			PRE4	AT3G47710
Hormone	ABA & Gibberellin	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	0.99	1.00		1.07	GIM2	AT2G36690
Hormone	ABA/SA/Eth	GLYCINE RICH PROTEIN 3		1.38		1.11	GRP3	AT2G05520
Hormone	Abscisic Acid	9-cis-epoxycarotenoid dioxygenase 3	-1.70				NCED3	AT3G14440
Hormone	Abscisic Acid	MYB domain protein 74				-0.61	MYB74	AT4G05100
Hormone	Abscisic Acid	Stress-induced protein KIN1	-1.26				KIN1	AT5G15960
Hormone	Auxin	nitrilase 2	-1.05		-0.80	-0.87	NIT2	AT3G44300
Hormone	Auxin/ Brassinosteroid	Auxin efflux carrier family protein	-0.95			-0.80	PILS5	AT2G17500
Hormone	Auxin	Nitrilase 1	-0.77			-0.78	NIT1	AT3G44310
Hormone	Auxin	Uridine diphosphate glycosyltransferase 74E2	-1.47				UGT74E2	AT1G05680
Hormone	Auxin	Acyl acid amido synthetase		-2.10			GH3.7	AT1G23160
Hormone	Auxin	IAA-amido synthase that conjugates Asp and other amino acids to auxin in vitro				-0.61	GH3.17	AT1G28130
Hormone	Auxin/ Brassinosteroid	Auxin efflux carrier family protein	-0.89				PILS3	AT1G76520
Hormone	Auxin	ZINC INDUCED FACILITATOR-LIKE 1	-0.70				ZIFL1	AT5G13750
Hormone	Auxin	Auxin response factor 20		0.76			ARF20	AT1G35240
Hormone	Auxin	big grain like 1	0.66				BGL1	AT1G69160
Hormone	Auxin, Ethylene	SAUR-like auxin-responsive protein family	1.34				SAUR76	AT5G20820
Hormone	Auxin, Ethylene	Auxin-Regulated Gene Involved in Organ Size	0.65				ARGOS	AT3G59900
Hormone	Brassinosteroid	squalene monooxygenase 2	-1.80	-1.76		-2.13	SQE4	AT5G24140
Hormone	Brassinosteroid	ATBS1(ACTIVATION-TAGGED BRI1 SUPPRESSOR 1)-INTERACTING FACTOR 1	-0.60				AIF1	AT3G05800
Hormone	Brassinosteroid	ARGOS-like protein	1.03		1.09	1.01	ARL	AT2G44080

Table 3.9 (cont'd)

Hormone	Brassinosteroid	baruol synthase 1	3.80		2.74	3.44	BARS1	AT4G15370
Hormone	Cytokinin	SULFOTRANSFERASE 4B		-0.71			ST4B	AT1G13420
Hormone	Cytokinin	UDP-glucosyl transferase 73C1		3.29			UGT73C1	AT2G36750
Hormone	Ethylene (Eth)	1-amino-cyclopropane-1-carboxylate (ACC) synthase 7	-1.01	-0.99	-0.85	-1.08	ACS7	AT4G26200
Hormone	Ethylene (Eth)	Ethylene-responsive transcription factor ERF112	-0.79				ERF112	AT2G33710
Hormone	Eth/JA	ethylene response factor	0.74	0.76	0.69	0.80	ERF59	AT1G06160
Hormone	Ethylene (Eth)	ETHYLENE RESPONSE 2	0.84		0.79	0.84	ERT2	AT3G23150
Hormone	Ethylene (Eth)	a member of the ERF (ethylene response factor) subfamily B-2 of ERF/AP2 transcription factor family	0.73			0.60	ERF73	AT1G72360
Hormone	Ethylene (Eth)	Integrase-type DNA-binding superfamily protein	1.94			2.04	TDR1	AT3G23230
Hormone	Ethylene (Eth)	ethylene-activated signaling pathway	1.49			1.44	RAP2.9	AT4G06746
Hormone	Jasmonic Acid	Jasmonic acid oxidase 2	-0.91				JOX2	AT5G05600
Hormone	Jasmonic Acid	RING DOMAIN LIGASE 3				-0.60	RGLG3	AT5G63970
Hormone	Jasmonic Acid	Involved in jasmonic acid inducible defense response			1.16		WRKY51	AT5G64810
Hormone	Signaling	wall-associated kinase 2	1.41	1.11	1.53	1.16	WAK2	AT1G21270
Hormone	Signaling	cell wall-associated kinase		2.86	3.14		WAK1	AT1G21250
Hormone	Signaling	glycine-rich protein 3 short isoform Regulates the function of the receptor protein kinase WAK1		1.79			GRP3S	AT2G05380
Hormone/ Development	Gibberellin/ ABA/ Flowering	Gibberellin-regulated protein 14		0.67	0.75	0.69	GASA14	AT5G14920
Hormone/ Metabolism	Cytokinin/ Secondary	Cytokinin-induced F-Box protein				-0.77	CFB	AT3G44326
Metabolism	Amino Acid	Glutamine dumper 2	-0.63			-0.61	GDU2	AT4G25760
Metabolism	Amino Acid	Transmembrane amino acid transporter family protein	-0.59			-0.84	AVT3	AT5G65990
Metabolism	Amino Acid	ACC Oxidase 1	0.73		0.71	0.81	ACO1	AT2G19590

Table 3.9 (cont'd)

Metabolism	Amino Acid	GLUTAMINE DUMPER 5	0.94			0.82	GDU5	AT5G24920
Metabolism	Amino Acid	D-Amino acid racemase 1	0.59				DAAR1	AT4G02850
Metabolism	Cell Wall	Cellulose synthase-like protein E1	-0.60				CSLE1	AT1G55850
Metabolism	Cell Wall	rhamnogalacturonan II specific xylosyltransferase	-0.70				RGXT3	AT1G56550
Metabolism	Cell Wall	xyloglucan endotransglucosylase/hydrolase 16		-0.61			XTH16	AT3G23730
Metabolism	Cell Wall	invertase/pectin methylesterase inhibitor superfamily	-0.63				PME60	AT5G51500
Metabolism	Cell Wall	xyloglucan endotransglucosylase/hydrolase 7	0.61		0.88		XTH7	AT4G37800
Metabolism	Cell Wall	Glycosyl hydrolase 9B13			0.60		GH9B13	AT4G02290
Metabolism	Cell Wall	XYLOGLUCAN ENDOTRANSGLUCOSYLASE/HYDROLASE 9			0.64		XTH9	AT4G03210
Metabolism	DNA Repair	NUDIX HYDROLASE HOMOLOG 18	-0.89				NUDT18	AT1G14860
Metabolism	Lipid	Lipid transfer-like protein VAS	-0.72			-0.67	VAS	AT5G13900
Metabolism	Lipid	UDP-3-O-acyl N-acetylglycosamine deacetylase family protein	-25.00				LPXC3	AT1G25054
Metabolism	Lipid	GDSL-like Lipase/Acylhydrolase superfamily protein;	0.97		1.10	1.01	GDSL1	AT1G29670
Metabolism	Lipid	Lipase class 3-related protein			2.80		-	AT5G24220
Metabolism	Methylation	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	-0.94				-	AT3G56080
Metabolism	Nitrogen	Nitrile specifier protein 3		-1.31		-0.61	NSP3	AT3G16390
Metabolism	Nitrogen	Glutamine synthetase cytosolic isozyme 1-1	-0.62			-0.64	GLN1-1	AT5G37600
Metabolism	Nitrogen	nitrate transport		-0.60			NPF1.2	AT1G52190
Metabolism	Nitrogen	Glutamine synthetase cytosolic isozyme 1-4		-0.71			GLN1-4	AT5G16570
Metabolism	Nitrogen	HIGH AFFINITY NITRATE TRANSPORTER 2.6	0.90				NRT2.6	AT3G45060
Metabolism	Oxidative Stress	Cyclin-dependent kinase inhibitor	-0.71			-0.69	-	AT3G20340

Table 3.9 (cont'd)

Metabolism	Phosphate/ Protein modification	PURPLE ACID PHOSPHATASE 17	-0.94			-0.89	PAP17	AT3G17790
Metabolism	Phosphate/ Protein modification	purple acid phosphate 8	-0.77				PAP8	AT2G01890
Metabolism	Protein modification	U-box domain-containing protein kinase family protein	-2.34			-2.36	PUB34	AT2G19410
Metabolism	Protein modification	Yippee family putative zinc-binding protein	-0.94			-0.90	-	AT3G55890
Metabolism	Protein modification	Eukaryotic aspartyl protease family protein		-0.87	-0.67		-	AT5G19110
Metabolism	Protein modification	RING/U-box superfamily protein				-1.48	-	AT4G00305
Metabolism	Protein modification	Zinc ion binding RING/U-box superfamily protein	-2.27				ATL35	AT4G09110
Metabolism	Protein modification	Sulfite exporter TauE/SafE family protein	1.57	1.87	1.79		-	AT4G21250
Metabolism	Protein modification	Eukaryotic aspartyl protease family protein	1.02			1.04	-	AT3G51340
Metabolism	Protein modification	ADP-ribosylation factor D1A	1.15				ARFD1A	AT1G02440
Metabolism	Protein modification	Leucine-rich repeat protein kinase family protein	3.00					AT1G51810
Metabolism	Protein modification	EP1-like glycoprotein 4 curculin-like (mannose-binding) lectin family protein	0.67				GAL2	AT1G78860
Metabolism	Protein modification	SMAX1-LIKE 8			0.67		SMXL8	AT2G40130
Metabolism	Protein modification	alpha-(1,6)-fucosyltransferase	0.69				-	AT5G28960
Metabolism	Redox	NAD(P)-binding Rossmann-fold superfamily protein	-1.04	-1.25	-1.33	-1.25	-	AT1G66800
Metabolism	Redox	NAD(P)-linked oxidoreductase superfamily protein	-0.79				-	AT1G60750

Table 3.9 (cont'd)

Metabolism	Redox	NAD(P)-binding Rossmann-fold superfamily protein				-0.72	-	AT1G64590
Metabolism	Redox	NAD(P)-binding Rossmann-fold superfamily protein	-0.71				-	AT2G29320
Metabolism	Redox	ALTERNATIVE OXIDASE 1A	-0.63				AOX1A	AT3G22370
Metabolism	Redox	Cyclopropane-fatty-acyl-phospholipid synthase			-0.60		-	AT3G23510
Metabolism	Redox	2-oxoglutarate (2OG) and Fe(II)-dependent oxygenase superfamily protein	1.22		1.11	1.15	F2A19.2	AT3G61400
Metabolism	Redox	Glutaredoxin 3	0.87		0.77	0.85	GRXS3	AT4G15700
Metabolism	Redox	Thioredoxin superfamily protein	1.14		1.19		GRXC14	AT3G62960
Metabolism	Redox	member of the CC-type glutaredoxin (ROXY) family		1.01		0.90	GRXS4	AT4G15680
Metabolism	Redox	Monothiol Glutaredoxin 5		0.98	0.85		GRXS5	AT4G15690
Metabolism	Secondary	beta glucosidase 11	-1.25		-0.99	-0.70	BGLU11	AT1G02850
Metabolism	Secondary	Aldolase superfamily protein		-2.88			HEMB2	AT1G44318
Metabolism	Secondary	S-adenosylmethionine decarboxylase proenzyme		-0.65			-	AT5G15948
Metabolism	Secondary	nitrilase 4	-1.49				NIT4	AT5G22300
Metabolism	Secondary	Tyrosine transaminase family protein	5.91		5.76	5.82	-	AT4G28420
Metabolism	Secondary	peroxidase superfamily protein	3.21			2.61	-	AT5G39580
Metabolism	Secondary	Terpenoid Synthase 12			0.60		TPS12	AT4G13280
Metabolism	Secondary	Terpene synthase 8	0.60				TPS08	AT4G20210
Metabolism	Starvation	senescence-associated family protein				-0.76	DUF581	AT1G22160
Metabolism	Toxin & Lipid	12-OXOPHYTODIENOATE REDUCTASE 2	-0.80				OPR2	AT1G76690
Metabolism	Toxin Catabolism	glutathione S-transferase TAU 25	-1.20		-0.76	-0.82	GSTU25	AT1G17180
Metabolism	Toxin Catabolism	glutathione transferase belonging to the tau class of GSTs			-0.63	-0.76	GSTU14	AT1G27140
Metabolism	Toxin Catabolism	glutathione S-transferase TAU 16	-0.87			-0.72	GSTU16	AT1G59700
Metabolism	Toxin Catabolism	glutathione transferase lambda 1	-3.25			-2.91	GSTL1	AT5G02780

Table 3.9 (cont'd)

Metabolism	Toxin Catabolism	Phenolic glucoside malonyltransferase 1	-1.37			-1.15	PMAT1	AT5G39050
Metabolism	Toxin Catabolism	glutathione S-transferase TAU 24	-1.06				GSTU24	AT1G17170
Metabolism	Toxin Catabolism	glutathione S-transferase TAU 22	-1.25				GSTU22	AT1G78340
Metabolism	Toxin Catabolism	GLUTATHIONE S-TRANSFERASE TAU 8	-0.85				GSTU8	AT3G09270
Metabolism	Toxin Catabolism	glutathione S-transferase TAU 12	2.33	2.41	3.12		GSTU12	AT1G69920
Metabolism	Toxin Catabolism	glutathione S-transferase F3			2.40		GSTF3	AT2G02930
Metabolism	Transport	organic cation/carnitine transporter1	-1.27	-1.34	-0.84	-1.56	OCT1	AT1G73220
Metabolism	Transport	DETOXIFICATION 22	-0.83			-0.83	DTX22	AT1G33090
Metabolism	Transport	Nodulin-like / Major Facilitator Superfamily protein			-1.06	-1.18	-	AT2G34350
Metabolism	Transport	ABC transporter family protein	-1.77			-1.37	ABCB15	AT3G28345
Metabolism	Transport	NITRATE TRANSPORTER 2.4	-0.75			-1.04	NRT2.4	AT5G60770
Metabolism	Transport	glycolipid transfer & ceramide transport	-1.19				GLTP2	AT1G21360
Metabolism	Transport	pleiotropic drug resistance 6	-0.68				ABCG34	AT2G36380
Metabolism	Transport	Amino acid transport				-1.11	AAT1	AT4G21120
Metabolism	Transport	Amino acid transport			-1.06		-	AT5G02170
Metabolism	Transport	multidrug and toxic compound extrusion & iron homeostasis under osmotic stress	1.20		0.92	0.98	DTX48	AT1G58340
Metabolism	Transport	ATP-BINDING CASSETTE B9, P-GLYCOPROTEIN 9			0.71		ABCB9	AT4G18050
Metabolism		UDP-3-O-acyl N-acetylglycosamine deacetylase family protein	-3.38			-3.66	LPXC5	AT1G25210
Metabolism		GDSL-motif esterase/acyltransferase/lipase	-0.93		-0.77		-	AT1G28660
Metabolism		Mannose-binding lectin superfamily protein	-4.30			-4.10	JAL12	AT1G52120
Metabolism		NADP-malic enzyme 1	-1.41			-1.41	NADP-ME1	AT2G19900
Metabolism		UDP-Glycosyltransferase superfamily protein	-0.75			-0.70	-	AT3G46700

Table 3.9 (cont'd)

Metabolism		Jacalin-related lectin 41		-0.84	-0.75		JAL41	AT5G35940
Metabolism		HXXXD-type acyl-transferase family protein	-1.15			-1.13	BAHD1	AT5G47980
Metabolism		Acyl-CoA N-acyltransferases (NAT) superfamily protein	-1.37			-1.52	-	AT5G67430
Metabolism		Uncharacterized protein family (UPF0497)	-1.03				CASPL1C1	AT4G03540
Metabolism		Tyrosine transaminase family protein		-1.17			-	AT4G23590
Metabolism		Rhamnogalacturonate lyase family protein		-0.86			-	AT4G37950
Metabolism		S-adenosylmethionine-dependent methyltransferase activity			-1.68		-	AT5G38780
Metabolism		Ankyrin repeat family protein	1.12	1.24	1.30	1.12	F12G12.13	AT2G24600
Metabolism		Ankyrin repeat family protein	1.97	1.27	1.74	1.70	F12G12.13	AT5G52710
Metabolism		Phosphoglycerate mutase family protein		0.92	0.72	0.71	-	AT1G09932
Metabolism		hydroxyproline-rich glycoprotein family protein	1.46	1.30		1.06	-	AT1G11070
Metabolism		NmrA-like negative transcriptional regulator family protein	0.62		0.62		-	AT1G19540
Metabolism		Plant invertase/pectin methylesterase inhibitor superfamily protein	0.79			0.59	-	AT1G23205
Metabolism		Thioredoxin superfamily protein	1.20			1.20	GRXS7	AT4G15670
Metabolism		S-adenosylmethionine-dependent methyltransferase activity				1.34	-	AT1G15125
Metabolism		nodulin MtN21-like transporter family protein	0.60				UMAMIT22	AT1G43650
Metabolism		a member of the glycerophosphodiester phosphodiesterase like (GDPD-like) family			0.94		SVL2	AT1G66970
Metabolism		alpha carbonic anhydrase 2				0.60	ACA2	AT2G28210
Metabolism		Plant invertase/pectin methylesterase inhibitor superfamily	1.07				PME25	AT3G10720

Table 3.9 (cont'd)

Metabolism		Serine carboxypeptidase-like 16	0.70				SCPL16	AT3G12220
Metabolism		Anthranilate phosphoribosyltransferase-like protein			0.92		MCTO13	AT5G03435
Metabolism		Berberine bridge enzyme-like 26			0.82		ATBBE26	AT5G44400
Metabolism/ Hormone	Toxin & Lipid/ SA response	12-OXOPHYTODIENOATE REDUCTASE 1	-0.87				OPR1	AT1G76680
Metabolism/ Hormone	Toxin Catabolism/ SA response	GLUTATHIONE S-TRANSFERASE 25, GLUTATHIONE S-TRANSFERASE TAU 7	-0.75				GSTU7	AT2G29420
P450		Terpenoid cyclases/Protein prenyltransferases superfamily protein	1.24	-1.83			TPS16	AT3G29110
P450		cytochrome P450, family 705, subfamily A, polypeptide 12	-1.33	-1.28	-1.15	-1.53	CYP705A12	AT5G42580
P450		cytochrome P450, family 89, subfamily A, polypeptide 5	-1.10			-0.66	CYP89A5	AT1G64950
P450		sterol 22-desaturase cytochrome P450, family 710, subfamily A, polypeptide 4	-3.43			-3.34	CYP710A4	AT2G28860
P450		CYTOCHROME P450, FAMILY 89, SUBFAMILY A, POLYPEPTIDE 2	-0.60				CYP89A2	AT1G64900
P450		cytochrome P450, family 72, subfamily A, polypeptide 8	-1.49				CYP72A8	AT3G14620
P450		CYTOCHROME P450, FAMILY 72, SUBFAMILY A, POLYPEPTIDE 13	-0.99				CYP72A13	AT3G14660
P450		CYTOCHROME P450, FAMILY 72, SUBFAMILY A, POLYPEPTIDE 15	-0.60				CYP72A15	AT3G14690
P450		Cytochrome P450 superfamily protein	-1.47				CYP81D11	AT3G28740
P450		cytochrome P450, family 706, subfamily A, polypeptide 1	1.30	1.03	1.41	1.23	CYP706A1	AT4G22690
P450		cytochrome P450, family 706, subfamily A, polypeptide 2	1.38	1.72	1.87	1.35	CYP706A2	AT4G22710
P450		cytochrome P450, family 71, subfamily B, polypeptide 22	3.02			2.92	CYP71B22	AT3G26200

Table 3.9 (cont'd)

P450		CYTOCHROME P450, FAMILY 735, SUBFAMILY A, POLYPEPTIDE 2	0.65				CYP735A2	AT1G67110
P450		putative obtusifoliol 14-alpha demethylase	0.60				CYP51A1	AT2G17330
P450		CYTOCHROME P450, FAMILY 709, SUBFAMILY B, POLYPEPTIDE 2		0.70			CYP709B2	AT2G46950
P450		cytochrome P450, family 705, subfamily A, polypeptide 3	2.13				CYP705A3	AT4G15360
Transcription	Senescence	NAC DOMAIN CONTAINING PROTEIN 59, ORE1 Sister 1	-0.81				NAC59	AT3G29035
Transcription	Transcription Factor	HOMEOBOX-LEUCINE ZIPPER PROTEIN 17	-0.79			-0.77	HB17	AT2G01430
Transcription	Transcription Factor	Protein RADIALIS-like 2 MATERNAL EFFECT EMBRYO ARREST 3	0.89		0.81	0.75	RL2	AT2G21650
Transcription		TCP INTERACTOR CONTAINING EAR MOTIF PROTEIN 2		-0.63	-0.60	-0.61	TIE2	AT2G20080
Transcription		myb domain protein 112	-1.01			-1.08	MYB112	AT1G48000
Transcription		zinc-finger protein 10		-0.76		-0.67	ZFP10	AT2G37740
Transcription		zinc finger (AN1-like) family protein	-1.43			-1.38	SAP12	AT3G28210
Transcription		WRKY DNA-binding protein 75	-1.21			-1.33	WRKY75	AT5G13080
Transcription		NAC DOMAIN CONTAINING PROTEIN 100	-0.82			-0.69	NAC100	AT5G61430
Transcription		WRKY DNA-binding protein 31	-1.26				WRKY31	AT4G22070
Transcription		basic helix-loop-helix DNA-binding superfamily protein	-0.72				BHLH118	AT4G25400
Transcription		RING/FYVE/PHD zinc finger superfamily protein	20.55	20.88	19.39	21.00	-	AT5G36670
Transcription		WRKY DNA-binding protein 30	3.32		2.87	2.68	WRKY30	AT5G24110
Transcription		Zinc-finger domain of monoamine-oxidase A repressor R1 protein	2.75			2.21	-	AT1G67270
Transcription		basic leucine-zipper 8		0.67			bZIP	AT1G68880
Transcription		WRKY DNA-binding protein 59			2.41		WRKY59	AT2G21900

Table 3.9 (cont'd)

Transcription		Plant invertase/pectin methylesterase inhibitor superfamily	1.39				PME20	AT2G47550
Transcription		a member of the DREB subfamily A-4 of ERF/AP2 transcription factor family	0.80				TINY2	AT5G11590
Transcription		ARABIDOPSIS MYB-RELATED PROTEIN 1	0.76				MYR1	AT5G18240
Transcription		WRKY DNA-binding protein 51			1.16		WRKY51	AT5G64810
Transcription/ Defense/ Hormone/ Signaling		MYB domain protein 30	-0.61				MYB30	AT3G28910
Signaling	Calcium	AFG1-like ATPase family protein				-0.62	-	AT4G30490
Signaling	Extracellular	represses plant growth, root development response to N starvation & broad hormone signaling		-1.22			CEP5	AT5G66815
Signaling	Protein modification	Leucine-rich repeat transmembrane protein kinase	1.65		1.06	1.30	T3M22.2	AT1G29740
Signaling	Protein modification	Leucine-rich repeat transmembrane protein kinase	0.75			0.67	-	AT1G29730
Signaling	Unspecified	P-loop containing nucleoside triphosphate hydrolases superfamily protein	1.23	0.80	0.73	1.06	MAE1.1	AT5G60760
Unknown	Extracellular	transmembrane protein		-1.23	-0.72	-0.74	AT14A	AT3G28290
Unknown	Extracellular	Bifunctional inhibitor/lipid-transfer protein/ seed storage 2S albumin superfamily protein	-1.71		-2.39	-1.53	-	AT4G12500
Unknown	Extracellular	transmembrane protein	3.26		2.47	2.85	-	AT1G66465
Unknown	Extracellular	transmembrane protein	1.99		2.00	1.86	-	AT3G60470
Unknown	Extracellular	TRAF-like family protein	1.97			1.56	-	AT3G20360
Unknown	Extracellular	transmembrane protein			1.11		-	AT5G44572
Unknown	Extracellular	transmembrane protein			1.28		-	AT5G48175

Table 3.9 (cont'd)

Unknown	Extracellular	exocyst subunit exo70 family protein A3	2.38				EXO70A3	AT5G52350
Unknown	ncRNA	SHORT OPEN READING FRAME 5	-1.01				SORF5	AT3G57157
Unknown		Vesicle transport protein	-0.76	-1.08	-0.85	-0.91	-	AT5G23840
Unknown		VQ motif-containing protein	-1.03	-0.76		-0.71	VQ1	AT1G17147
Unknown		Outer arm dynein light chain 1 protein	-1.45	-1.25		-1.34	T11I11.17	AT1G78230
Unknown		hypothetical protein	-1.24	-0.86		-0.92	-	AT4G33666
Unknown		Haloacid dehalogenase-like hydrolase superfamily protein	-0.70		-0.59	-0.62	-	AT5G02230
Unknown		Cysteine-rich repeat secretory protein 4	-0.80			-0.91	CRRSP4	AT1G63600
Unknown		conserved upstream opening reading frame relative to major ORF AT1G70780.1	-0.61			-0.63	CPuORF28	AT1G70782
Unknown		Pollen Ole e 1 allergen and extensin family protein	-1.12			-0.89	PRP1	AT2G47530
Unknown		Galactose oxidase/kelch repeat superfamily protein	-1.97	-2.04			F17A17.6	AT3G07720
Unknown		Remorin family protein	-0.72			-0.91	T2J13.220	AT3G48940
Unknown		hypothetical protein	-1.16			-1.11	-	AT4G37700
Unknown		NFU1 iron-sulfur cluster protein	-1.23			-1.18	-	AT5G07330
Unknown		TRAF-like family protein		-0.76	-0.72		F9D12.8	AT5G26260
Unknown		spastin, putative	-0.78			-0.87	-	AT5G46060
Unknown		Haloacid dehalogenase-like hydrolase (HAD) superfamily protein	-0.65			-0.69	-	AT5G59490
Unknown		cysteine-rich transmembrane module stress tolerance protein	-0.99				ATHCYSTM 1	AT1G05340
Unknown		hypothetical protein				-1.31	-	AT1G52342
Unknown		DUF538 family protein, putative				-0.61	DUF538	AT1G55265
Unknown		MICRORNA414, SHORT OPEN READING FRAME 16				-0.62	MIR414	AT1G67195
Unknown		Carbohydrate-binding X8 domain superfamily protein		-0.77			-	AT1G78520

Table 3.9 (cont'd)

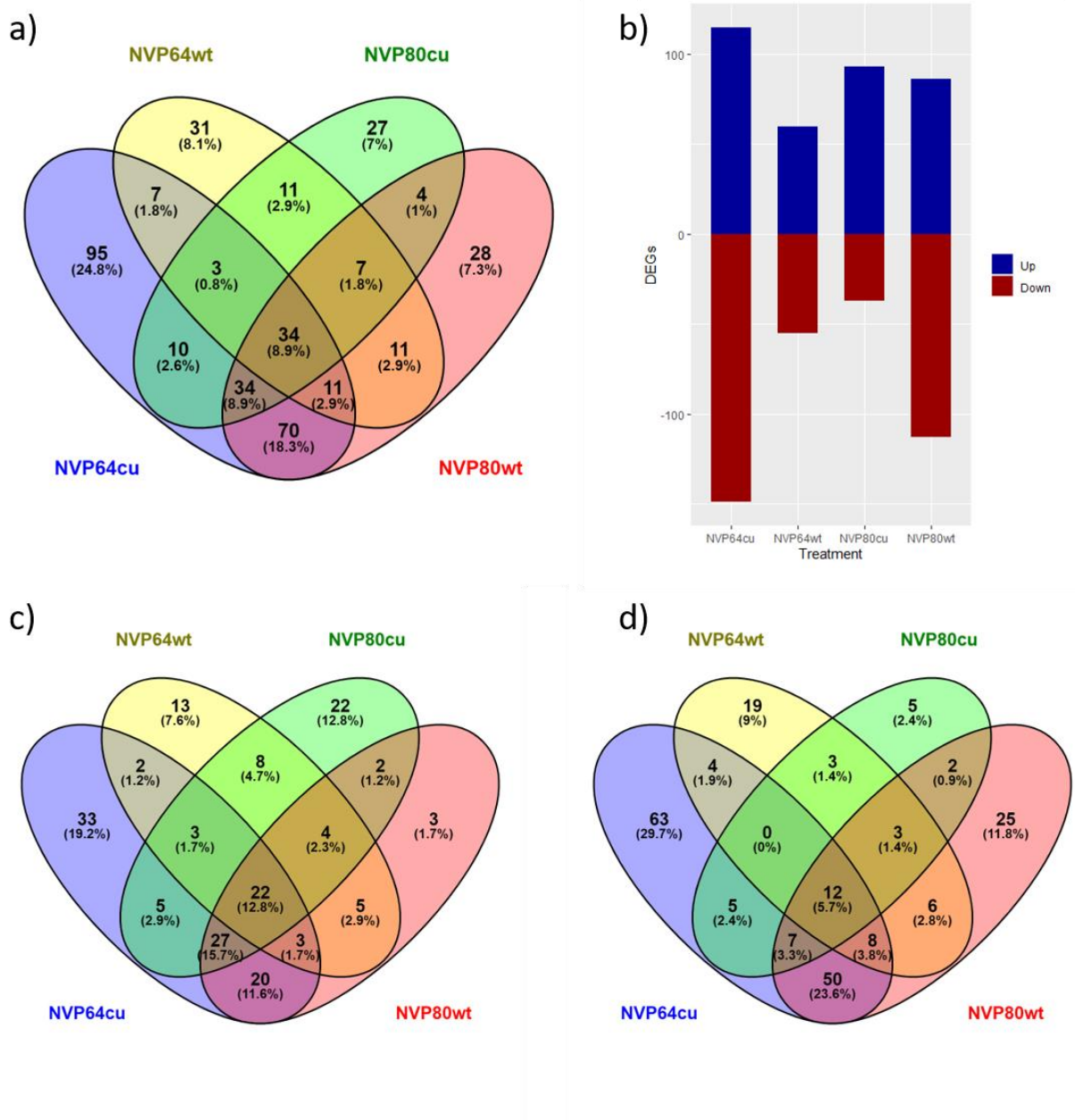
Unknown		maternal effect embryo arrest protein	-0.88				-	AT2G01008
Unknown		pseudogene	-1.62				-	AT2G07811
Unknown		D14-like 2, AB hydrolase-1 domain-containing protein				-0.62	DLK2	AT3G24420
Unknown		pseudogene				-1.25	-	AT3G56275
Unknown		U-box kinase family protein	-1.43				-	AT3G61410
Unknown		hypothetical protein	-0.71				-	AT4G04745
Unknown		hypothetical protein (DUF581)	-1.21				-	AT4G39795
Unknown		alpha/beta-Hydrolases superfamily protein				-1.39	-	AT5G42930
Unknown		Nucleotide-diphospho-sugar transferase family protein				-0.67	-	AT5G44820
Unknown		F-box/RNI-like/FBD-like domains-containing protein				-3.30	-	AT5G50270
Unknown		cotton fiber-like protein				-0.70	-	AT5G54300
Unknown		stress up-regulated Nod 19 protein	-0.85				-	AT5G61820
Unknown		GPI-anchored adhesin-like protein, putative	0.83	0.70	0.82	0.76	DUF547	AT1G16750
Unknown		LURP-one-like protein	2.48	2.03	2.41	2.35	DUF567	AT1G33840
Unknown		hypothetical protein	2.35	1.81	1.70	2.16	-	AT4G39675
Unknown		Ankyrin repeat family protein	1.85	1.87	1.82	1.76	-	AT5G54710
Unknown		CLAVATA 3/ESR (CLE)-like protein	1.52	1.28	1.59	1.14	CLE46	AT5G59305
Unknown		PLAC8 family protein	1.69		1.25	1.43	PCR6	AT1G49030
Unknown		pseudogene	1.58		1.94	1.84	-	AT1G58130
Unknown		Glycine-rich protein family	2.94		3.45	3.53	-	AT2G05530
Unknown		hypothetical protein		0.77	0.74	0.59	-	AT4G16008
Unknown		Unknown gene	0.72	0.75		0.72	-	AT5G01740
Unknown		pseudogene		1.10		0.88	-	AT1G05135
Unknown		short open reading frame 2	0.79			0.87	SORF2	AT1G11185
Unknown		Leucine-rich repeat protein family protein		0.71	0.89		-	AT1G49750

Table 3.9 (cont'd)

Unknown		alpha/beta-Hydrolases superfamily protein		1.17		1.00	-	AT1G52700
Unknown		pseudogene	3.09			2.33	-	AT2G24750
Unknown		Rhodanese/Cell cycle control phosphatase superfamily protein		0.71	0.76		STR9	AT2G42220
Unknown		GDSL-like Lipase/Acylhydrolase superfamily protein	1.27			1.15	K24A2.4	AT3G27950
Unknown		Embryo sac development arrest 32	0.68		0.60		EDA32	AT3G62210
Unknown		hypothetical protein		0.86	0.82		-	AT4G16000
Unknown		CBS domain protein	0.69	0.66			-	AT5G52790
Unknown		Ankyrin repeat family protein		1.75		1.69	-	AT5G54720
Unknown		hypothetical protein	1.20				-	AT1G13480
Unknown		decreased in response to Mn, increased by cytokinin			1.90		-	AT1G19960
Unknown		Leucine-rich repeat (LRR) family protein	0.84				-	AT1G33612
Unknown		P-loop containing nucleoside triphosphate hydrolases superfamily protein	3.63				IAN4	AT1G33900
Unknown		pseudogene	0.67				-	AT1G54660
Unknown		Glycine-rich protein family			0.60		GRP9	AT2G05440
Unknown		Glycine-rich protein family			0.63		-	AT2G05510
Unknown		E6-like protein			0.81		E6L1	AT2G33850
Unknown		Cysteine/Histidine-rich C1 domain family protein	1.69				-	AT2G43220
Unknown		hypothetical protein	0.97				-	AT3G46880
Unknown		TRAF-like family protein	0.75				-	AT4G00780
Unknown		Retrotransposon like protein		0.77			-	AT4G16870
Unknown		HXXXD-type acyl-transferase family protein		1.17			-	AT5G07860
Unknown		transmembrane protein		0.88			-	AT5G44574
Unknown		hypothetical protein	0.91				-	AT5G44585

Figure 3.14 - **Abundance of differentially expressed Arabidopsis genes**

Arabidopsis genes differentially expressed (DEGs) in roots colonized with *Mortierella elongata* as compared to the uninoculated control, identified usng DESeq2 with fold-change threshold of 1.5 and p-value threshold of 0.05. a) A Venn diagram of all DEGs in the final, filtered dataset. b) A bar graph of all DEGs, split between up- and down-regulated. c-d) Venn diagrams of c) up- and d) down-regulated DEGs identified for each fungal treatment.



CHAPTER 4. SYNTHESIS

Objectives

The goals of this dissertation research were three-fold: 1) to resolve the phylogeny of the Mortierellaceae using novel phylogenetic markers and phylogenomics. 2) To confirm and characterize plant growth promotion by *Mortierella elongata* in *Arabidopsis thaliana* and determine what affect fungal endosymbiotic bacteria may have on that phenotype. 3) To then elucidate the genetic basis of Mortierella-plant association using transcriptome sequencing. Two side projects aimed to a) to develop an *Agrobacterium tumefaciens*-mediated transformation system in *M. elongata* (Appendix B) and b) establish a mating system for *M. elongata* as a basis for identifying genetic regulators of sexual reproduction and how endobacteria might impact fungal mating (Appendix C).

Mortierellaceae Phylogeny & Taxonomy

In Chapter 2, I identified novel, phylogenetically informative, single-copy loci for which I developed family-specific primers. I used those primers to amplify target loci from over 300 Mortierellaceae isolates. In collaboration with Dr. Stajich, I also used low-coverage genome sequencing of over 60 representative Mortierellaceae isolates to recover over 400 genetic loci. I combined the amplicon and LCG datasets to generate a highly supported Mortierellaceae phylogeny. By combining these approaches, I was successful in resolving the phylogeny of Mortierellaceae into 13 monophyletic genera, 7 of which are newly proposed.

Of the non-ribosomal markers used in this study, the most universally informative locus was *RPB1*, which is already an established phylogenetic marker, and should probably supersede the ITS region for isolate identifications as reference sequences are accumulated. However, the *RPB1* locus was not entirely sufficient for resolving the full phylogeny and the other non-ribosomal loci proved necessary in discriminating between very closely related species, such as Locus 2451

in *Podila*. We selected from the full set of loci identified by our pipeline on the basis of similar primer melting temperature to *RPB1*, since we were multiplexing the primer sets. It is possible that some of the untested loci could be of similar value to *RPB1*. These loci, and more importantly the primers, were selected and designed from only three representative genomes. As additional *de novo* genomes become available, the locus selection process could be repeated and would probably yield a narrower selection of potentially suitable novel loci from which more broadly suitable primers could be designed.

This study simultaneously included novel species lineages and included limited established species diversity. This study was the first inclusion of *Modicella* in a detailed molecular study of the Mortierellaceae. Moreover, our expanded geographical sampling efforts identified a novel genus containing at least three novel species and one novel species in the *Linnamannia*. Even so, this study only included a total of 44 of the 125 described species in the Mortierellaceae. The type specimens for represented species were often not included, which limited our ability to resolve the occasional overlapping species groupings, such as *Podila humilis* and *P. verticillata*. In addition, some species were only represented by a few or one isolate, such as *Necromortierella dichotoma*. This limits our confidence in the placement of those species in the phylogeny. Despite the limitations of the ITS region for phylogenetics and species identification, we did use ITS sequences to estimate where most of the excluded species might fall in the proposed taxonomy.

I expect that the next revision of the Mortierellaceae phylogeny and taxonomy will employ either genome sequencing of additional species diversity or a second effort similarly combining phylogenomic analysis of a subset of isolates used to anchor an amplicon dataset including broader species diversity. Given the continuing advances in genome sequencing, the former approach seems more likely. However, an amplicon-based study can make use of sequences deposited to reference databases, such as *RPB1*, if it becomes widely applied to identifying Mortierellaceae isolates, which could then capture much higher intraspecific diversity due to a higher sampling capacity than a genome sequencing effort.

Continued geographic sampling efforts are still needed to fully characterize Mortierellaceae species diversity and distribution. Based on available culture collections, Africa, Asia, and South America are particularly undersampled. While the Mortierellaceae phylogeny and taxonomy improved by our study, these are likely to change as we accumulate ecological and sequence data from these fungi. Eventually, this data will allow for delineation of ecological functions within each group, inference about the ecological function of new species or isolates classified within those groups, and study of how such traits and ecological adaptations evolved. As geographic sampling continues, one of the most valuable future contributions to our understanding of Mortierellaceae would be a curated database of reference sequences for selected phylogenetic markers with updated taxonomy. This would aid in accurately identifying new isolates and placing novel species within genera. Secondly, this consolidated record of the geographic and environmental origin of isolates worldwide would allow us to establish the range and ecologies of these species.

***Mortierella elongata - Arabidopsis thaliana* symbiosis**

In Chapter 3, I demonstrated that *Mortierella elongata* promotes the aerial growth and seed production of *Arabidopsis thaliana*. I found that neither BRE or MRE have a significant impact on the parameters of plant performance that I measured. I used RNA sequencing to identify genes that were differentially regulated in fungal treatments as compared to the uninoculated control. I identified differentially regulated genes involved in plant defense, hormone signaling, root development, abiotic stress, and metabolism.

Many studies have explored the impact of a variety of Mortierellaceae species in different environmental and experimental conditions. Given the variation in the Arabidopsis-*M. elongata* association under different conditions (Appendix A), care should be taken to understand the environmental context of each study and how that might impact the association. For instance, the potting mix study methodology created a stress condition that confounded the intended

hypothesis testing. While the grain spawn substrate at issue is not necessary for fast-growing strains like *M. elongata*, many Mortierellaceae strains cannot survive blending for direct inoculation, as is often used for soil or potting mix-based experiments. We will need to optimize these protocols further for standardizing across studies and to ensure that the intended hypotheses are actually being tested and conclusions are translatable.

The role of Mortierellaceae bacterial endosymbionts in the plant-fungal symbiosis was previously unexplored. However, just because BRE and MRE did not have an impact on the plant-fungal symbiosis in our experimental system and timepoints does not preclude their having an impact on *M. elongata* in other systems. It is well established that both endobacteria strongly alter the fungal growth and metabolism (Uehling et al, 2017). The *M. elongata* genome also seems to have unusually few secondary metabolite synthesis genes, some of which are present in the BRE genome (Uehling et al, 2017). It is possible that endobacteria supply the fungus with defense and signaling compounds that are relevant in other environments and interactions.

In this study, we measured plant growth and productivity at early and late life stages after a stable symbiosis had been established. The mechanism of maintaining symbiosis may be very different from that required to initiate and establish symbiosis, as demonstrated by the shift in JA levels throughout the Arabidopsis-*M. hyalina* symbiosis or transient stress responses in Arabidopsis during *S. indica* infection (Johnson et al. 2019; Meents et al. 2019; Vahabi et al. 2015). Future research may illuminate mechanisms of extremely early stages of interactions between *M. elongata* and plants, using protocols similar to those described in Meents et al. (2019).

Our preliminary agar experiments were extremely discouraging, in that Arabidopsis colonized by any Mortierellaceae was visibly more stressed and senescent than uninoculated plants. These experiments were conducted on Murashige and Skoog medium with sucrose, rather than PNM, (details in Appendix A) and with a different Arabidopsis Col-0 lineage. At the time, we also inoculated at the base of the plate and let the roots and hyphae grow together, giving plants and fungi much more time to communicate prior to making contact and also increasing the age of the

plant at the time of contact. A number of factors could be responsible for the reversal of the positive plant-fungal association we observed in the experiments described in Chapter 3. It would be informative to determine whether the medium, timing and mechanism of inoculation, or genetic drift in the plant genotype had such a strong impact on the plant-fungal association. I suspect that the medium may have played the biggest role, as PNM has no carbon source for the fungus, whereas MS+sucrose has abundant carbon. I observed significantly more abundant mycelial growth on MS plates, making the fungi less dependent on the plant for carbon and potentially increasing the concentrations of metabolic by-products that might have been toxic to the plants.

Both our preliminary and final results strongly indicate that *M. elongata* affects Arabidopsis root architecture and development. Scanning and image analysis during early stages of symbiosis might be suitable for tracking root branching and growth before roots begin to overlap and grow along the edges of the plate where they are no longer visible. Since ethylene seems to be involved in this process, it may be important to test whether light is converting fungal production of metabolic by-products to ethylene, or use phytagel to ensure that fungi are not degrading agarose to ethylene, which would not take place in a natural system (Splivallo et al. 2009; Chagué, 2010).

We were unable to analyze the fungal transcriptome due to extremely low read abundance. It might be possible to use a microbiome enrichment kit or other technique to isolate or increase the proportion of fungal RNA. This could allow co-expression network analysis and significantly improve our understanding of the fungal response to the plant, rather than just the plant response to the fungus. Once candidate fungal symbiotic genes are identified, completing and implementing the *M. elongata* transformation system discussed in Appendix B will provide a means for manipulating those genes and testing hypotheses.

The transcriptomic study indicated that both auxin and ethylene were regulated in the plants colonized by *M. elongata*. Biosynthesis of these phytohormones was downregulated, while response pathways were upregulated. I hypothesize that this could be due to fungal production of auxin and ethylene. An obvious next step is to directly quantify the concentrations of auxin and

ethylene in fungal hyphae in pure culture, in uncolonized plant roots, and in colonized roots. It would also be valuable to inoculate auxin- and ethylene-insensitive mutants with *M. elongata* to test whether the plant-fungal symbiosis is affected.

It would be highly informative to conduct shared-media assays that test whether direct contact is required for this symbiosis, or simply an exchange of signals and metabolites. A spent-medium assay could also be used to test whether constitutively produced metabolites from one organism trigger a response in the other to initiate interaction.

Conclusions

Mortierellaceae-plant associations have been described for over 100 years, but the mechanism and potential applications of these associations were not a concerted research focus until the last 5 years. Now the Mortierellaceae are emerging as an unusually tractable research system. The *Mortierella* species for which plant benefits have been described are distributed across several phylogenetic clades. It is valuable to understand the mechanism of interaction in each of these representative species to determine whether the Mortierellaceae have a conserved mechanism of plant association or how each functional group interacts with plants and how those mechanisms compare to those of the Glomeromycotina and Endogonales.

While Arabidopsis is still the quintessential model plant, it is far from representative of most agriculturally and industrially relevant plant species. Fortunately, interaction studies with Mortierellaceae have been conducted in multiple plant species. Conducting these experiments in a panel of model species, such as *Brachypodium*, tomato, and *Poplar* may allow for identifying a very narrow set of common features in their associations with Mortierellaceae.

I think that Mortierellaceae species have the potential to become agriculturally important for biocontrol of pathogens, helping to orchestrate the plant rhizobiome community, and solubilizing nutrients for plants. While these fungi are naturally occuring, Li et al. (2018) and Liao et al. (2019) showed increased plant benefit from increasing their abundance by supplemental inoculation.

Unless Mortierellaceae can be optimized for seed coating, which may be too early a life stage for such a heavy application, or blending and slurry-based application, it may be more successful to identify mechanisms and conditions by which plants recruit Mortierellaceae and optimize the plant side of the symbiosis.

However, on the fungal side of the equation, very little is known about intraspecific variation in the plant associations reported in each study. It would be helpful to characterize symbiotic associations in a panel of isolates from diverse geographic and environmental origins before extrapolating findings across a species. Such precautions will likely identify particularly strong and weak associations. Side-by-side comparisons of each end of a spectrum could shed light on the mechanism of association quite quickly. In addition, species and strains adapted to specific environmental conditions might be superior at protecting plants from biotic or abiotic stresses specific to those environments, such as heat, cold, salt, drought, water-logging, or heavy metals. The concept is based on the idea that symbionts can confer habitat-specific adaptations to their hosts. Most of the Mortierellaceae-plant interaction studies have been conducted in neutral conditions, so additional research into seemingly neutral or even weakly negative associations might identify strains better suited to specific stresses.

The current microbiome paradigm predicts that rhizosphere species work cooperatively to benefit themselves, and consequently their host plant. While there will always be limitations to the direct applicability of bi- or tripartite interaction studies, understanding the impact of each member in isolation before we can begin to recognize synergies and competitions in more complex systems. Moreover, it is possible that the endobacteria regulate how their host fungi interact with other members of the rhizosphere.

APPENDICES

APPENDIX A: PLANT-FUNGAL EXPERIMENTS

Preliminary agar experiments

For all of these experiments, I used sterilized, stratified, Arabidopsis seeds germinated as described in Chapter 3.

Preliminary Mortierellaceae species panel

Four day old seedlings were transplanted from germination plates to half-strength Murashige & Skoog medium (0.5xMS+suc). This medium was prepared by mixing 2.2 g/L Murashige and Skoog medium (Sigma Aldrich), 10 g/L sucrose, and 10 g/L agar (Sigma, product# A1296), 1 L water and the pH adjusted to 5.7 with 1 N KOH before autoclaving, cooling, and pouring into 100mm^2 square plates (with grid). We used 6 seedlings per plate, 3 plates per treatment. After transplanting, seedlings were allowed to grow on the 0.5xMS+suc plates for 7 days, then were inoculated 1cm from the bottom of the plate by cutting out two 1 cm x 0.5 cm rectangles out of the agar and filling with the inoculating agar block. Fungal strains were maintained on MEA or PDA and inoculated onto 0.5xMS+suc several days to a week in advance of use to inoculate plant interaction plates. The strains used were *M. selenospora* 1228cu and 1228wt, *Benniella erionia* (=*Mortierella sp. nov.*) GBAus27Bwt and GBAus27Bcu, *M. elongata* NVP64cu, NVP64wt NVP80cu, and NVP80wt.

We noted that after 3 days of interaction, plants were visibly smaller with purple stems and some yellowed, senescing leaves when inoculated with fungi. Also, as Arabidopsis root tips and Mortierellaceae hyphae approached each other, root growth generally slowed and even stopped, with root tips often curling up away from the media, and lateral branching increased compared to the uninoculated plants (**Fig A.1**).

Fungal Exudate Experiment

I transplanted 4 day old seedlings from germination plates to 05.X+suc plates, 6 plants per plate, 3 plates per treatment. Three days later, I inoculated the 7 day old seedlings. I had two

controls, a “true negative” control without any fungi, as described above, and an “empty moat” control without any fungi and a 0.5 cm “moat” in the agar about 2 cm from the bottom of the plate. The three fungal strains were NVP80wt, NVP64wt, and NVP64cu. Each fungal strain was previously cultured for three days on a separate 0.5xMS+suc plate. Fungal exudate blocks were cut along the periphery of the hyphal growth, approximately 0.5 cm x 2 cm. Fungal exudate blocks were inserted into moats cut into the agar as described for the “empty moat” control. Finally, fungal mycelium was inoculated onto plates with empty moats. In theory, the empty moats would prevent the diffusion of fungal exudates in advance of the hyphae and the fungal exudate blocks would only diffuse the fungal exudates without the roots ever contacting fungi.

First, I observed no fungal growth from the fungal exudate blocks, confirming that no hyphae were accidentally transferred. I did not observe any impact on root growth in the fungal exudate treatments (**Fig A.2**). I did not observe impact on root growth in the inoculated plates until the roots and/or the hyphae had bridged the moat and made contact, which occurred within 3-4 days of inoculation. The plants were still smaller and visibly distressed as compared to the controls.

Pilot study on PNM

This pilot study was the first implementation of the PNM-based experimental methods described in Chapter 3. The methods were as described, with two exceptions: 1) the uninoculated control, NVP80wt, and NVP80cu were cultured on KM, but NVP64wt and NVP64cu were cultured on MEA; 2) plants were harvested at 14 DPI, instead of 12 DPI. Data were analyzed by linear modeling of aerial dry weight as a function of treatment and starting seedling root length, with plants grouped by plate to account for subsampling and plates treated as a random effect.

I found that plants inoculated with NVP80cu and NVP80wt treatments had higher aerial dry biomass than the uninoculated control, whereas plants grown with NVP64cu and NVP64wt were not significantly different from the control (**Fig A.3**). I also observed that some of the plants had begun to bolt. This experiment demonstrated that at least NVP80 strains promoted plant growth and that NVP64 strains may be affected by BRE. This data prompted the bolting time and media

panel experiments described in Chapter 3.

Preliminary *Mortierella* species panel in potting mix

Materials and methods were exactly as described in Chapter 3 for the potting mix experiment. Due to space constraints, the full panel of fungal strains was split into two batches. The “Batch 1” strains were *M. elongata* NVP64cu, *Mortierella* sp. nov. (*Benniella erionia*) GBAus27b, *M. alpina* GBAus31, *M. humilis* PMI1414cu, *M. minutissima* AD051cu, *M. selenospora* KOD1228cu, and the no millet control. The “Batch 2” strains were *M. paraensis* KOD1235, *Dissophora ornata* KOD1234, *M. echinosphaera* KOD1233, *M. cystojenkinii* KOD1230, *M. strangulata* KOD1227, *M. gamsii* AM1032, *M. hyalina* AM1038, and the uninoculated millet control.

I expected that combining these datasets with the dataset presented in Chapter 3 would allow for normalizing between batches, since the Chapter 3 dataset had both controls and NVP64cu. However, this was an invalid interpretation of block design and the lack of overlapping controls or other treatments in every single batch prevented controlling for batch effects. Within Batch 1, I found that Arabidopsis aerial dry weight was significantly smaller in all 5 fungal treamtents as compared to the no millet control (**Fig. A.4a**). Within Batch 2, I found that Arabidopsis aerial dry weight was significantly larger in all fungal treatments except *Dissophora ornata* KOD1234 as compared to the uninoculated millet control (**Fig. A.4b**).

A critical analysis of the potting mix experiment methodology

This study began with potting mix-based experiments to maintain a relatively realistic experimental system representative of the real-world environments in which *M. elongata* and plants naturally interact. However, I encountered several significant issues and challenges with the potting mix system. First, I discovered that not only does the uninoculated spawn invite colonization by environmental contaminants, but the spawn itself has a strong, consistent negative impact on plants. Preliminary studies of *Mortierella* interacting with millet plants using

millet-based spawn suggest that some of this effect is probably due to allelopathic compounds in the grains, as millet plants are much less affected by a millet-based spawn than Arabidopsis (data not shown). For our continued experiments in Arabidopsis, I increased the proportion of perlite in the spawn from the 2:1:1 perlite:barley:millet used here to 18:1:1 perlite:barley:millet. Other studies have relied on comparisons between Uninoculated spawn and Inoculated spawn treatments, neglecting to include a NoSpawn control and potentially biasing their results toward stress mitigation and not neutral environment plant growth promotion.

Second, the potting mix also needed to be autoclaved thoroughly to ensure sterility and isolation of the experiments from contaminants. Unfortunately, autoclaved potting media accumulates unknown by-products that are toxic to plants (Kremer et al. 2018). The potting mix had to be rinsed through with large volumes of water prior to use. However, at this experimental scale, each treatment required several liters of water to thoroughly rinse the potting mix, the containment and draining of which was also a technical and logistical challenge.

Another challenge of the potting mix-based experiments was deciding at what point to conclude the experiment, since the highly stressed uninoculated spawn control plants matured much sooner than the other treatments. This meant that life stage might be an incompletely controlled factor in the biomass and seed production data. However, the difficulty of handling mature Arabidopsis without significant loss of seeds and siliques necessitated harvesting plants before full maturity and maximal seed production.

Having struggled with the negative impacts of spawn and difficulty of preparing sterile potting mix and seedlings in the potting mix experiments, I decided to switch to agar plates, as these afford superior control of the plant environment, straightforward controls, increased replication, and improved access to plant roots for qPCR, RNA-seq, and visual inspection of architecture and fungal growth. The agar experiments described in Chapter 3 were harvested at a much earlier lifestage, but still showed a very similar trend in plant aerial biomass between uninoculated and fungal treatments. The dry weight of the fully-grown plants from the potting mix experiments

include the rosette and inflorescences, both of which might have been independently affected by the fungal treatments. The bolting trial (Ch 3) demonstrated that the age at which plants first began to bolt was unaffected, but additional work should be done to assess the inflorescence development, architecture, flowering, and seed production at a level of detail that was not captured by these experiments, since I looked at plants either well before or after plants had completed these processes.

The potting mix experiment was necessary and technically sufficient to collect data about seed production, though I should have increased the sample size from which I collected seed, instead of harvesting those plants to assay dry biomass. The agar system was more suited to assay aerial growth and root gene expression. Now that *M. elongata* has been shown to impact plant growth, more extensive experiments can be justified to refine our understanding of this plant-fungal interaction. An improved potting mix system, with a grain-free inoculation protocol, would be ideal to non-destructively track plant growth over time and construct a more detailed description of how *M. elongata* affects plant growth and development. The agar system is well suited for high-throughput assays of plant and fungal knock-out mutants to further isolate important genes and pathways involved in this symbiosis.

Figures

Figure A.1 – **23 day old Arabidopsis plants on 0.5xMS+suc, 12 DPI**
Panel a) uninoculated control, b) *Mortierella selenospora* 1228wt, or c) *M. elongata* NVP64wt. Black marks tracked daily root tip growth.

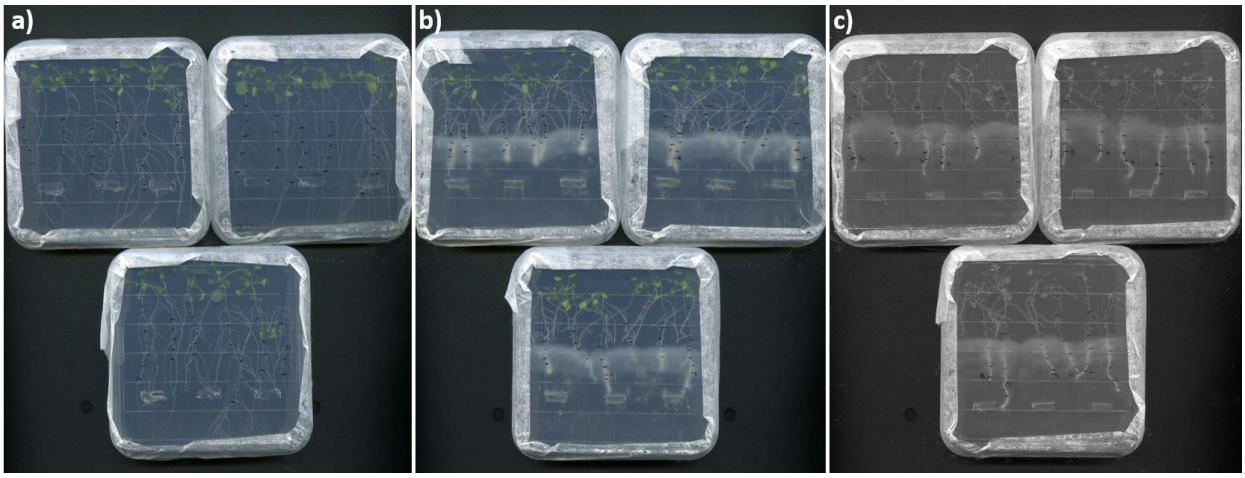


Figure A.2 – **Plates from the Fungal Exudate pilot study**

These plants are 21 days old, at 10 DPI. Treatments are a) “Empyt moat” control, b) “negative control”, c) , and d) “empty moat” NVP64cu.

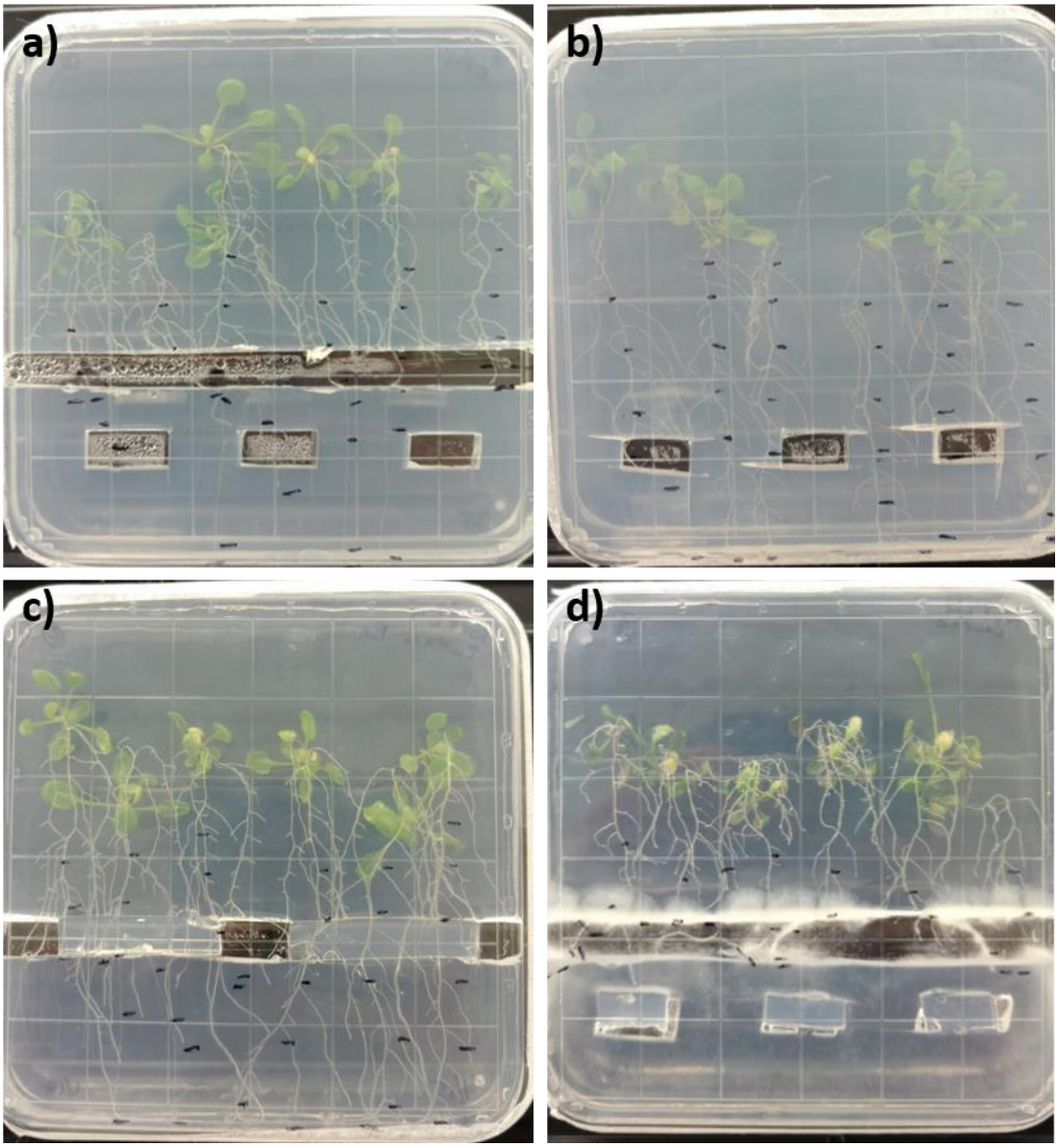


Figure A.3 - **Estimated marginal mean of Arabidopsis aerial dry weight (agar pilot study)**

The degrees of freedom for each comparison were approximated using the kenward-roger method and the *p*-values adjusted for multiple comparisons using the Tukey method for comparing a family of 5 estimates. Letters indicate significantly different groups with an alpha value of 0.05.

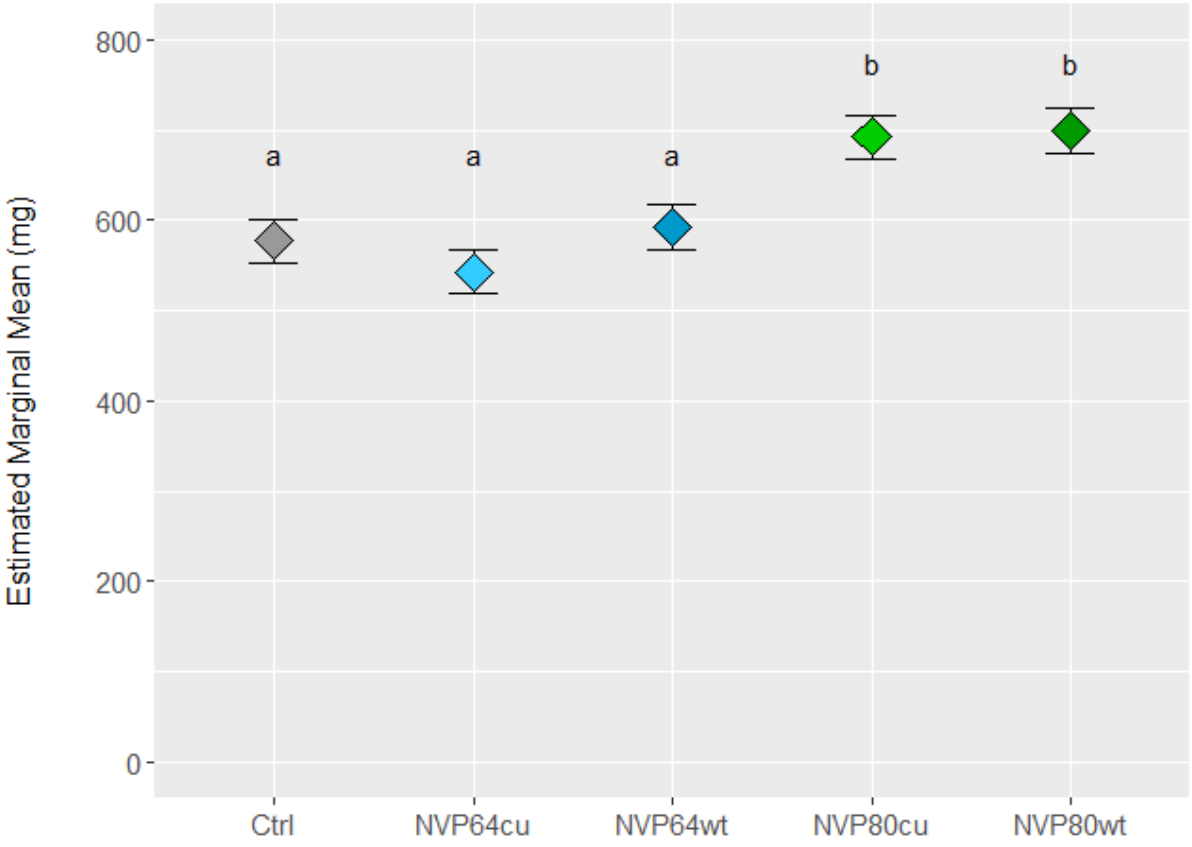
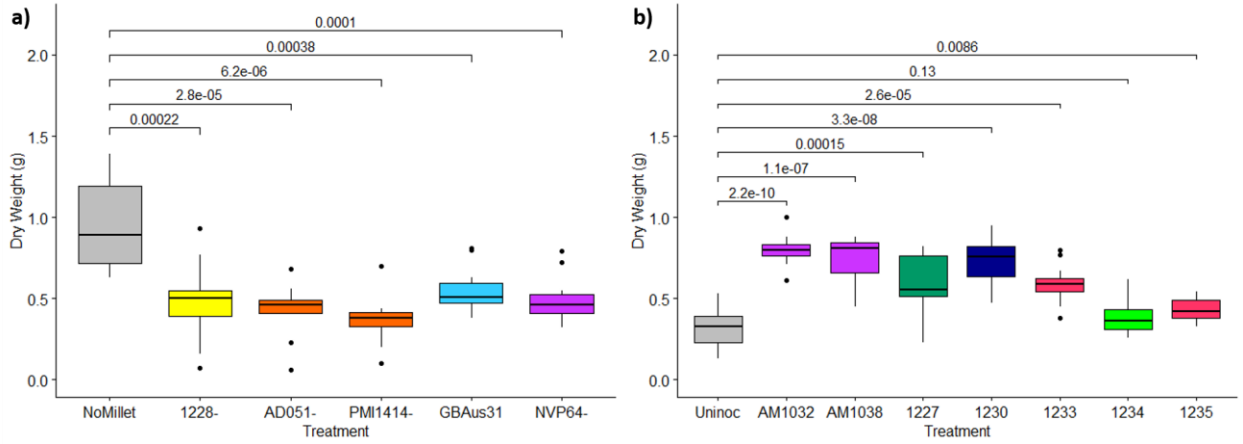


Figure A.4 – Aerial dry biomass of Arabidopsis grown in sterile potting mix (species panel)

In each treatment, the potting mix was amended with: nothing (NoMillet), sterile millet mix (Uninoculated), or millet mix inoculated one of twelve fungal strains (*M. elongata* NVP64cu, *Mortierella* sp. nov. (*Benniella erionia*) GBAus27b, *M. alpina* GBAus31, *M. humilis* PMI1414cu, *M. minutissima* AD051cu, *M. selenospora* KOD1228cu, *M. paraensis* KOD1235, *Dissophora ornata* KOD1234, *M. echinosphaera* KOD1233, *M. cystojenkinii* KOD1230, *M. strangulata* KOD1227, *M. gamsii* AM1032, *M. hyalina* AM1038). Colors correspond to the revised taxonomy proposed in Chapter 2, horizontal bars and numbers indicate pairwise t- tests with alternative hypotheses defined as a) NoMillit being “greater than”, or b) Uninoc is “less than”, each fungal treatment and the resulting p-value.



APPENDIX B: *MORTIERELLA ELONGATA* TRANSFORMATION SYSTEM

Introduction

Transformation systems are used to manipulate genetic material, whether to add, modify, or delete genes or alter the expression of a gene by interfering with the mRNA post-translation. There are four basic approaches to transforming filamentous fungi: protoplasting, *Agrobacterium*-mediated transformation, electroporation, and nuclear bombardment. Not all approaches are suitable to all fungi or applications. In particular, there are several challenges to developing a transformation system in zygomyceteous fungi, including *M. elongata*. First, the fungal hyphae are coenocytic (lacking regular septae), which precludes traditional protoplasting techniques that involve digesting the cell wall away from fungal cells, since the resulting protoplasts would be too large and fragile to survive the protoplast collection and downstream processes. In addition, each protoplast would have many copies of the nucleus. It is extremely difficult, if not impossible, to transform every nucleus with the desired mutation. Another approach is to collect and transform spores, directly or as they germinate. Of the three spore forms produced by *M. elongata*, sporangiospores are the most suitable for transformation, as they have the smallest number of nuclei, thinner cell walls, and they are typically more abundantly produced. However, many strains of *M. elongata* do not sporulate very aggressively and the spores are very small (about 6 µm x 12 µm) and unpigmented. This makes it difficult to confirm collection of spores.

The only extant transformation system in Mortierellaceae is in *M. alpina*. There was one report of successful protoplasting and PEG-mediated transformation with a construct confering HygB resistance under the control of the His4 promoter and relying on random chromosomal integration in the ribosomal region (Mackenzie et al, 2000). The more common approach is to transform germinating sporangiospores, by nuclear bombardment or *Agrobacterium tumefaciens*-mediated transformation (Takeno et al. 2004b; Ando et al. 2009a; Ando et al. 2009b).

Selection for transformants is usually accomplished by working in a uracil auxotrophic strain

or fungicide selection (Takeno et al. 2004a; Ando et al, 2009a; Ando et al. 2009b). The most effective and affordable fungicide for most Mortierellaceae is carboxin, which targets succinate dehydrogenase B (Laleve et al. 2014; Nyilasi et al. 2015). Resistance of *sdhB* to carboxin can be accomplished by a single nucleotide substitution in one of three possible locations, which all result in amino acid substitutions (Laleve et al. 2014). The most effective of these three mutations is H272L in *Botrytis cinerea*, which corresponds to H243L in *M. alpina* and H244L in *M. elongata* (Laleve et al. 2014). Unfortunately, since resistance is so easily conferred, it also arises spontaneously and accounts for about 10% of colonies that appear to be transformants when carboxin resistance is the only selective marker (Ando et al. 2009a). The concentration of carboxin required for selection varies considerably between fungal strains and even between strains of the same species (Nyilasi et al. 2014).

Most constructs for transformation of *M. alpina* used the Histone 4.1 promoter, but a screen of a number of *M. alpina* gene promoters yielded a number of other options, the chief among them being the promoter for ATP binding protein *SSA2*, particularly a truncated version with only the last 400bp (Okuda et al. 2014).

Transformation of *M. alpina* has focused on increasing the production of eicosapentaenoic acid (EPA) by adding additional copies of the native biosynthesis gene w3-desaturase (Ando et al. 2009b). However, our interest in a transformation system in *M. elongata* is much broader, including GFP for microscopy and gene manipulation to test hypotheses regarding the genetic basis of the *Mortierella*-plant symbiosis. GFP expression is the simplest starting point, since a construct for eGFP compatible with fungal codon usage is already available and does not require cloning native genes. Also, use of *Agrobacterium*-mediated transformation is a more approachable system than nuclear bombardment, since it does not require a gene gun. Compatibility between the fungus, the Agrobacterium strain, and the plasmid is vital to the success of a transformation system, necessitating careful design of each component.

Methods

Media Recipes

The sporulation medium Czapek-Dox Agar (**CZA**) was prepared by dissolving 2.0 g/L $NaNO_3$, 1.00 g/L K_2HPO_4, 0.50 g/L KCl, 0.50 g/L $MgSO_4*7H_2O$, 0.01 g/L $FeSO_4*7H_2O$, and 30.0 g/L Sucrose in 1 L Water and adjusting the pH to 6.0 with ~3 dozen drops 3.7% HCl, adding 20.0 g/L BactoAgar, and autoclaving. S.O.C. Medium (**SOC**) was prepared with 20 g/L tryptone, 5 g/L yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM MgSO4, and 20 mM glucose.

Protoplasting

NVP64cu was cultured in 400mL PDB for 3 days at room temperature, shaking at 120rpm. The mycelium was collected on a sterile Whatman filter by vacuum filtration, washed with sterile water, and transferred to a sterile 50mL Falcon tube. The mycelium was suspended in 40mL 0.6M KCl, supplemented with 1g Glucanex (Sigma), and incubated at room temperature, shaking at 75rpm for 4 hours.

Sporangiospore Collection

Fungal strains were transferred to CZA plates and cultured at room temperature for 2 weeks, at which point sporangiophores were visible in mycelium near the agar surface. Plates were washed with 3mL sterile 0.5% Tween-20 and the agar surface scraped with sterile microspatulas to release spores and mycelium. The spore suspension was filtered through a double layer of sterile Nitex membrane (100um pore size).

Fungicide resistance panels

To test the resistance of *M. elongata* to potential selective fungicides, 50mL PDB was supplemented with 25, 50, 100, 200, and 300ug/mL nourseothricin or 6, 240, 900, 1710, and 3000 ug/mL hygromycin. Flasks were inoculated with small plugs of *M. elongata* NVP64WT previously cultured on solid medium. Flasks were observed daily for growth.

To assess strain resistance to a wide range of carboxin levels, I collected sporangiospores from *M. alpina* GBAus31, NVP17b, and NVP153 and *M. elongata* NVP5, NVP64cu, NVP80cu,

and AG77- as described above and plated them on PDA and PDA supplemented with 1mg/mL hygromycin and 67, 333, 1000, and 1500 ug/mL carboxin. The PDA plate served as a positive control for spores in the spore suspension. Plates were monitored daily for signs of spore germination and growth.

Amplifying *Mortierella elongata* genes & promoters

To amplify the *ef1a* promoter, we performed PCR of 1kb of genomic DNA 3' of *ef1a* from *M. elongata* using primers designed from the *M. elongata* AG77 genome, primers EF1Ap_F and EF1Ap_R, and HF Phusion PCR reagents (**Table B.1**). The HygB gene was amplified using plasmid template DNA from the Trail lab, primers Hyg5 and Hyg3, and HF Phusion PCR reagents. The PCR products were cleaned with the WizSV Gel + PCR Cleanup System (Promega).

Plasmid & Construct Design

20p_CBX_TrpCt has 20bp of the pEF1a sequence, *sdhB* H244L, and TrpCt. The 20bp is to allow for overlap extension PCR to attach the pEF1a promoter. The H244L site was located by aligning the protein sequences of *sdhB* from *B. cinerea* BQ-3 (KR866382.1), *M. alpina* 1S-4 (AB373636.1), and *M. elongata* AG77, which was identified using a BLAST search of the *M. alpina* sequence in the MycoCosm genome portal (**Fig. B.1**). pSSA2_CBX_TrpCt_15ApeI has the truncated *SSA2* promoter developed by Okuda et al. (2014) and the same CBX gene and TrpC terminator used in 20p_CBX_TrpCt, along with 15bp overlap with the pRFHUE-eGFP plasmid as digested with AfeI for integration with InFusion. Constructs were designed in SnapGene. Primers and synthetic constructs were ordered from IDT.

Cloning & Overlap Extension PCR

To attach pEF1a to 20p_CBX_TrpCt, we performed overlap extension PCR. Since this protocol relies on overlap between the two components, we first used an extended PCR primer to add 13 bp of the CBX 5' sequence to the 3' end of pEF1a in an HF Phusion PCR reaction (primers EF1Ap_F and CBX_pEF1a_R) and cleaned the PCR product with the WizSV cleanup kit.

Overlap extension PCR is a two step reaction, the first step uses each of the two overlapping fragments as a PCR primer for synthesizing the other fragment and generating full-length templates for the second step which has traditional PCR primers for each end of the full construct. We used HF Phusion reactions with recommended reaction conditions, annealing temperatures of 66, 63, 60, 55, and 50 in a gradient PCR for the first step and 56 for the second step. There were 10 PCR cycles for the first step and 30 cycles for the second step. In the first step, it is important to use equivalent concentrations (75-100 ng) of each component to be joined.

Propagation of pDS23_eGFP

We received a streak plate of *E. coli* pDS23_eGFP from the Trail lab. We picked a colony to inoculate 5mL of LB liquid medium supplemented with 100 ug/mL ampicillin and incubated at 37°C overnight under agitation. A glycerol stock of *E. coli* pDS23_eGFP was prepared by mixing equal volumes of the overnight culture with autocleved 50% glycerol. The pDS23_eGFP plasmid was extracted using Zyppy Plasmid Miniprep Kit (Zymo) and stored at -20°C.

We chemically transformed *E. cloni* cells generously provided by the Hamberger lab with the extracted pDS23_eGFP plasmid. The 20 µl *E. cloni* cell aliquot was thawed on ice for 5 minutes, then 1.5 µl of 10 ng/µL plasmid DNA was added. The reaction was incubated on ice for 20-30 minutes, heat shocked at 42°C for 30 seconds, and returned to the ice. Next, 250 µl SOC was added and the suspension shaken at 37°C for 1 hour, then plated on LB agar medium supplemented with 100 ug/mL ampicillin and incubated overnight at 37°C.

Preparation of Electrocompetent Agrobacterium

Agrobacterium AGL1 was streaked onto solid LB medium supplemented with 20ug/mL rifampicin, and 75ug/mL carbenicillin (hereafter LB-Rif/Carb) and incubated at 28 °C for 1–2 days until colonies are visible. I picked a colony to inoculate 5 mL of liquid LB-Rif/Carb and incubated overnight at 28 °C, shaking at 200 rpm. I used the overnight culture to inoculate 100 mL of liquid LB-Rif/Carb and incubated for 15–18 h at 28 °C and shaking at 170 rpm until the OD600 reached 0.5–0.8. I aliquoted the culture into 50mL Falcon tubes, cooled it to 4 °C (about 30 min),

centrifuged for 10 min at 3,000*g at 4°C, and discarded the supernatant. The pellets were resuspended in 5 mL of ice-cold sterile water and adjusted the volumes to 50 mL with ice-cold water, then repeated the centrifuge and washing step twice more. The pellets were resuspended in 100 mL of 10 % (v/v) ice-cold glycerol solution, centrifuged for 10 min at 3,000 × g at 4°C, and the supernatant discarded. The pellets were resuspended in 50 mL of 10 % (v/v) ice-cold glycerol solution, combined into one Falcon tube, and centrifuge for 10 min at 3,000 × g at 4°C. The supernatant was discarded and the pellet resuspended in 1 mL of 10 % (v/v) glycerol and portioned into 50 µL aliquots in 1.5mL tubes, flash frozen in liquid nitrogen, and stored at −80°C.

Results & Discussion

While some labs have reported successfully protoplasting Mortierellaceae hyphae, my attempts were unsuccessful. Therefore, I decided to use sporangiospores, since protoplasting coenocytic hyphae seemed an unnecessary challenge in light of the abundant spore-based protocols for *M. alpina*. To this end, we performed several fungicide panels. Several Mortierellaceae species have been tested for susceptibility to nourseothricin, hygromycin, and carboxin, but *M. elongata* was not included in that study (Nyilasi et al. 2015). Our first choice of plasmid, pDS23_eGFP, conferred resistance to nourseothricin. We tested *M. elongata* NVP64wt susceptibility to 0-300ug/mL nourseothricin, but observed no difference in fungal growth between any flasks, indicating no susceptibility to nourseothricin in NVP64wt. We then focused on hygromycin, as it is a frequently used marker in ATMT of fungi. While I did observe impaired growth at 1700ug/mL, all flasks eventually grew after 5-7 days. Finally, I tested a panel of *M. elongata* and *M. alpina* strains for spore germination and growth on solid medium with 1mg/mL hygromycin in combination with a range of carboxin concentrations. All strains grew abundantly on the negative control plates. For one week, none of the strains grew on any of the fungicide treated media. Eventually, *M. elongata* NVP5 grew a single colony on a 66 µg/mL carboxin plate and *M. alpina* NVP17b grew a single colony on a 300 µg/mL carboxin plate. I found that not only

was the lowest concentration of carboxin (66 µg/mL) effective with 1mg/mL hygromycin, but higher carboxin concentrations crystallized too aggressively in the medium, liquid or solid, to be of practical use in a transformation system. This combination is anticipated to function in the transformation system by screening most all spores with the carboxin and the hygromycin delaying the growth of spontaneous mutants. Therefore, selecting positive transformants within 1-3 days should not suffer from the 10% false positive rate observed in the *M. alpina* system and requires lower concentrations of both fungicides than would otherwise be required for selection for most Mortierellaceae species and strains. This allows much more flexibility in selection of the background fungal strain by criteria other than highest fungicide susceptibility.

The pDS23_eGFP plasmid is used for ATMT in *Fusarium graminearum* to express eGFP and confers resistance to nourseothricin (nat). Since *M. elongata* was resistant to nourseothricin, I planned to use restriction enzymes EcoRI and NruI to cut out the *nat* gene and replace it with a construct carrying HygB and CBX to confer hygromycin and carboxin resistance, respectively, the latter under the control of the native *M. elongata* EF1a promoter (**Fig. B.2**). I also received the HygB resistance gene from Trail lab, which I amplified from a second plasmid commonly used in their protocols.

I did successfully amplify and sequence the native *M. elongata* EF1a promoter (termed pEF1a), which was assumed to exist in the 1kb genomic DNA sequence 5' of the EF1a gene. The construct was cloned into pJet and we used PCR to add 13bp of CBX construct to increase overlap between the components (pEF1a_20CBX).

Since the *sdhB* genomic sequence includes introns that significantly and unnecessarily increase the insert length, I designed and ordered a synthetic construct from IDT with the last 3' 20bp of pEF1a, the *M. elongata sdhB* coding sequence with the H244L point mutation, and TrpCt (20pEF1a_CBX_TrpCt). I repeatedly tried to perform overlap extension PCR to attach pEF1a_20CBX to this construct. Gel electrophoresis of the PCR products only ever showed the 1kb pEF1a or 1.7kb CBX fragments, never the 2.6kb joined fragment.

Finally, I intended to digest the pDS23_eGFP plasmid and attempt InFusion with the plasmid, pEF1a, and 20pEF1a_CBX_TrpCt. However, the glycerol stock of *E. coli* pDS23_eGFP could not be revived and I did not get colonies from transforming *E. cloni* with pDS23_eGFP extracted from the original *E. coli* pDS23_eGFP overnight culture.

In light of these challenges, I attempted to procure the plasmid and Agrobacterium C58C1 used in *M. alpina* (Ando et al. 2009a; Ando et al. 2009b). Personal communication with the corresponding authors of those manuscripts was not fruitful. Nor were the Agrobacterium strain nor the plasmid available from a repository. Therefore, I identified *A. tumefaciens* AGL1 as an appropriate strain for our purposes, since it has been used to transform filamentous fungi and was available through our collaboration with Dr. Benning (Wang et al. 2020). Specifically, *A. tumefaciens* AGL1 was used to transform *Aspergillus carbonarius* with the plasmid pRFHUE-eGFP, which confers expression of GFP and resistance to Hygromycin (**Fig. B.3**; Crespo-Sempere et al. 2011). Therefore, the only changes required for use in transforming *M. elongata* were to add the carboxin resistance gene. Of the restriction enzymes with only one cut site (unique cutters) in pRFHUE-eGFP, AfeI cuts at a very convenient location in the plasmid and is available through our collaboration with Dr. Hamberger. To add carboxin resistance to pRFHUE-eGFP, I designed the cassette pSSA2_CBX_TrpCt_15ApeI. The CBX gene is the *SdhB* gene sequence reported from *M. alpina*, without introns, with the H244L mutation as appropriate for *M. elongata*. It is regulated by the *SSA2* promoter identified by Okuda et al. (2014) and terminated by the classic *Aspergillus nidulans TrpC* terminator sequence also used in the *M. alpina* transformation systems. Finally, it includes the 5' end 15bp overlap with the plasmid vector as it would be cut by AfeI, which is required for InFusion cloning and controls the direction of integration into the plasmid (**Fig. B.4**).

Future Directions

1. Perform the InFusion protocol in collaboration with Dr. Hamberger using primers InFusion-FOR and InFusion-REV.
2. Isolate the plasmid and screen for successful integration using PCR primers pRFHUE-CBXscrF and pRFHUE-CBXscrR (**Table B.1**). If the CBX cassette was integrated, the PCR product should be ~2600 bp. The PCR product can be sequenced to ensure correct orientation and screen for mistakes in the sequence using two PCR primer pairs pRFHUE-CBXseqF1/pRFHUE-CBXseqR1 (1151bp product) and pRFHUE-CBXseqF2/ pRFHUE-CBXseqR2 (1277bp product), since the full insert is 2100bp and too long to Sanger sequence fully in one reaction.

    a. Future use of this vector for *M. elongata* transformation will likely require substitution of the eGFP gene with another gene of interest. Assuming use of the same promoter and terminator sequences, the gene can be replaced using restriction sites TspMI, XmaI, or SmaI and KpnI or FspI, the latter two of which are available through our collaboration with Dr. Hamberger.
3. Attempt and optimize the method described by Sakuradani et al. (2015) to generate & collect a large number of *M. elongata* sporangiospores.

    b. Once a supply of spores has been collected, a small subset should be used to stain with DAPI to determine the number of nuclei in *M. elongata* sporangiospores. If the spores are uninucleate, then most traditional targeted gene mutations and deletions are available to future research efforts. Multinucleate spores are a considerably more complex challenge.
4. Transform *A. tumefasciens* AGL1 with pRFHUE-eGFP-CBX. I used the Hamberger lab protocol described above to generate electrocompetent Agrobacterium AGL1 compatible with downstream protocols, but these cells have not been tested.
5. Use the freshly transformed AGL1 to transform the *M. elongata* sporangiospores.

Transformation Protocol

Media Recipes

Czapek-Dox Agar (**CZA**): 2.0g $NaNO_3$, 1.00g K_2HPO_4, 0.50g KCl, 0.50g $MgSO_4*7H_2O$, 0.01g $FeSO_4*7H_2O$, 30.0g Sucrose, 1L Water, pH 6.0 with ~3 dozen drops 3.7% HCl, 20.0g BactoAgar, and autoclave.

Minimal Medium Broth (**MMB**): 10mM K_2HPO_4, 10mM KH_2PO_4, 2.5mM NaCl, 2mM $MgSO_4*7H_2O$, 0.7mM $CaCl_2$, 9uM $FeSO_4*7H_2O$, 4mM $(NH_4)_2SO_4$, 10mM Glucose, 1L Water, pH 7.0, and autoclave.

Potato Dextrose Agar (**PDA**): 12g Potato Dextrose Broth, 5g Yeast Extract, 1L Water, 15g BactoAgar, and autoclave.

Transformant Selection Agar (**TSA**): 12g Potato Dextrose Broth, 5g Yeast Extract, 1L Water, 15g BactoAgar, autoclave, cool, and supplement with 200g/mL Cefotaxime, 1mg/mL Hygromycin, 66ug/mL Carboxin, and 0.3g Nile Blue A.

Induction Medium Broth (**IMB**): 10mM K2HPO4, 10mM KH2PO4, 2.5mM NaCl, 2mM MgSO4-7H2O, 0.7mM CaCl2, 9uM FeSO4-7H2O, 4mM (NH4)2SO4, 10mM Glucose, 0.5%w/v Glycerol, 39.2mg Acetosyringone, 40mM MES, 1L Water, pH 5.3, and autoclave.

Co-cultivation Agar (**CCA**): 10mM K2HPO4, 10mM KH2PO4, 2.5mM NaCl, 2mM MgSO4-7H2O, 0.7mM CaCl2, 9uM FeSO4-7H2O, 4mM (NH4)2SO4, 5mM Glucose, 0.5%w/v Glycerol, 39.2mg Acetosyringone, 40mM MES, 1L Water, pH 5.3, 15g BactoAgar, and autoclave.

Transformation of Agrobacterium Cells (adapted from Hamberger lab protocol)

Mix 50 ng of expression vector with 50 µL of electrocompetent Agrobacterium AGL1 cells thawed on ice. Transfer the mixture to a pre-cooled 2 mm electroporation cuvette and electroporate using a Gene Pulser (Capacity 25 µF; 2.5 kV; 400 Ω). Let the transformed bacteria recover in 450 µL of Luria-Bertani (LB) broth medium for 2-4 h at 28°C, shaking at 170 rpm. Spread 50-100 µL on agar plates of LB supplemented with 50 µg/mL Kanamycin, 20 µg/mL

Rifampicin, and 75 µg/mL Carbenicillin (hereafter LB-K/R/C). Seal the petri dish with parafilm and incubate at 28°C. Colonies should appear after 48 h.

NOTE: The plate can be stored at 4 °C. Freshly transformed Agrobacterium is best for transient expression; however, Agrobacterium can be kept on plates for a few weeks, without losing the ability to transform *Nicotiana benthamiana*.

Generating & Harvesting *Mortierella elongata* spores

Inoculate *M. elongata* on fresh PDA and cultivate for at least 5 days to generate fresh mycelium for subsequent inoculations. Pour 150 mL of CZA into a tissue culture flask. Inoculate in six places with the fresh culture. Cultivate at room temperature for at least 14 days and another 14 days at 4°C to induce sporulation. The culture flask can be kept as a spore stock at 4°C for up to 3 years.

Pour 30 mL sterilized 0.05% Tween 80 into the tissue culture flask and scrape mycelium off the agar surface using a cleaning brush. Filter this suspension through a 125 mL capacity Buchner funnel fitted with Miracloth and then a 60 mm glass disc (rough porosity grade) into a 50mL Falcon tube. Rinse the agar surface of the tissue culture flask with 30 mL sterilized 0.05% Tween 80 twice more and filter through the same Buchner funnel each time into fresh 50 mL Falcon tubes. Combine into two Falcon tubes and centrifuge at 8,000*g for 10 min and discard the supernatants. Wash each spore pellets with 25 mL sterilized water with gentle shaking, combine into one Falcon tube, centrifuge at 8,000*g for 10min and discard the supernatant. Add sterilized water to adjust the spore concentration to approximately 10^8 spores/mL, determined with a Burker-Turk counting chamber.

ATMT of *Mortierella elongata* sporangiospores

Using a plate of transformed Agrobacterium, do colony PCR on 2-3 colonies to confirm the vector is present. Use a single colony to inoculate a 5mL starter culture of MMB-Kan/Rif/Carb and grow for 48hr at 28°C shaking at 170 rpm. Centrifuge Agrobacterium cells at 5,800*g, resuspend in IMB, re-centrifuge, and discard supernatant. Resuspend the pellet in fresh IMB to an OD600 =

0.15 and grow at 28°C, shaking at 200rpm, until OD600 = 0.5-1.5 (8-12 hours).

Mix equal volumes of Agrobacterium cells and the *M. elongata* sporangiospore suspension (concentration 10^8 spores per mL) and plate 100 µl of the mixture on nitrocellulose membranes (0.45um pore) on CCA (at least 9+ plates). After 24, 48, 72, and 96 hrs at 23°C, transfer the membranes to TSA. Transfer hyphae from growing colonies (should be blue from taking up the Nile blue in the TSA medium) to fresh PDA-Hyg/Cbx (may take 3-5 days) and observe for continued growth.

Figures & Tables

Figure B.1 - Alignment of the *sdhB* amino acid sequences of *M. elongata* AG77, *M. alpina* 1S-4, and *B. cinerea* BQ-3

The yellow highlight indicates the conserved histidine residue at which site directed mutagenesis to a leucine confers resistance to carboxin.

```
>AG77              ----MSFSLAKQSVLGLTRPVLK----------YSVPSAPV--------AIARTFATEAPA
>M.alpina          ----MSLSIAKQSALGLSRSI-K----------YSIPSAPV--------AIARSFATEAPA
>Botrytis_cinerea  MAALRTGARSARAIFAASRPAFRTQMRTMASVDSSVPESPTVSPSRPVESASKTSTVKEPA

KKT----KTFQIYRWNPDQPAEKPKLQSYEVDMNNCGPMVLDALIKIKNEIDPTLTFRRSCREGICGSCAMNIGGSNTLA
KKT----KTFQIYRWNPDQPAEKPKLQSYEVDMNNCGPMVLDALIKIKNEIDPTLTFRRSCREGICGSCAMNIGGSNTLA
ADSESLIKTFNIYRWNPDEPTSKPRMQSYTLDLNKTGPMMLDALIRIKNEVDPTLTFRRSCREGICGSCAMNIDGVNTLA

CICKIEVDSK-PTKIYPLPHTYVIKDLVPDLTEFYAQYKSIEPFLKQKTPEPE-RENLQTIEDRKKLDGLYECILCACCS
CICKIEVDNK-PTKIYPLPHTYVVKDLIPDLTQFYAQYKSIEPFLKQKTPEPE-RENLQTIEDRKKLDGLYECILCACCS
CLCRIPRDAKHETKIYPLPHTYVVKDIVPDLTQFYKQYKSIKPYLQHTDPAPEGKEYLQSKEDRKKLDGLYECILCACCS

TSCPSYWWNSDQYLGPAVLMQAYRWMIDSRDQFGPERRQALQNPFSLYRCHTIMNCAKTCPKGLNPGLAIAQIKKEMALE
TSCPSYWWNSDQYLGPAVLMQAYRWMIDSRDQFGPERRQALQNPFSLYRCHTIMNCAKTCPKGLNPGLAIAQIKKTMALE
TSCPSYWWNSEEYLGPAILLQSYRWLADSRDQKKEERKAALDNSMSLYRCHTILNCSRTCPKGLNPGLAIAEIKKEMAF-
```

Figure B.2 - **A map of plasmid pDS23_eGFP_CBX-HygB**

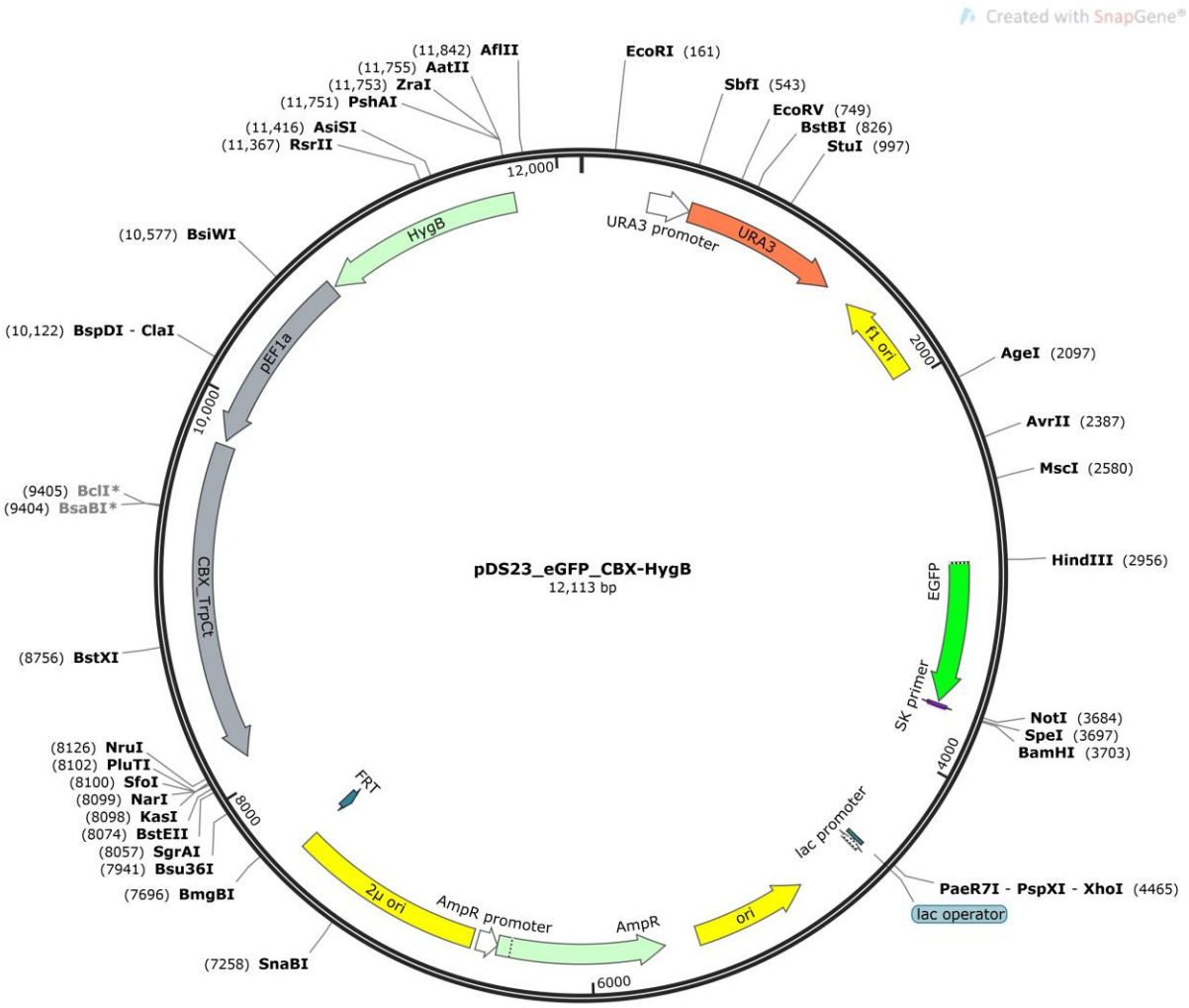


Figure B.3 - A map of plasmid pRFHUE-eGFP

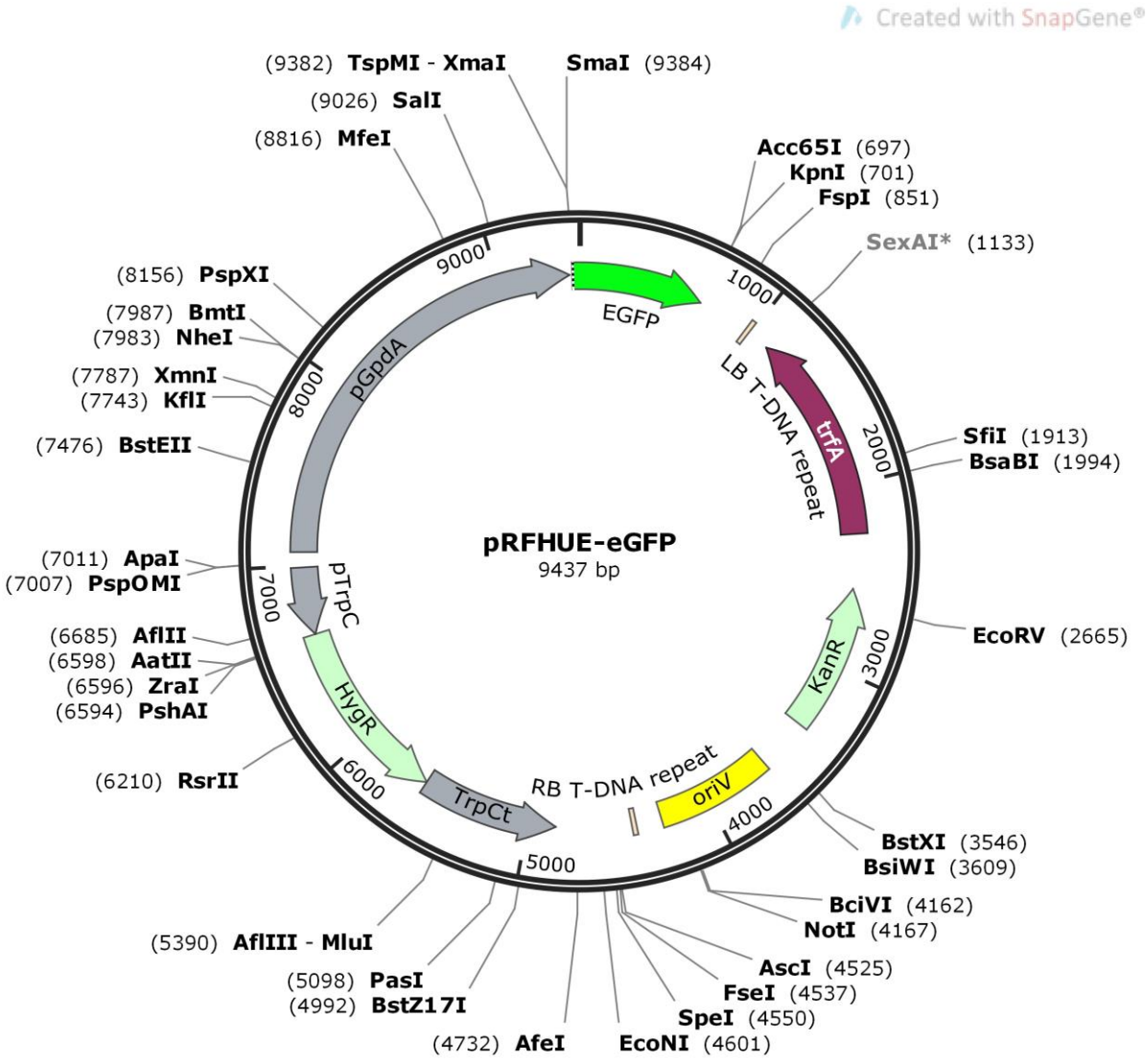


Figure B.4 - A map of plasmid pRFHUE-eGFP_CBX

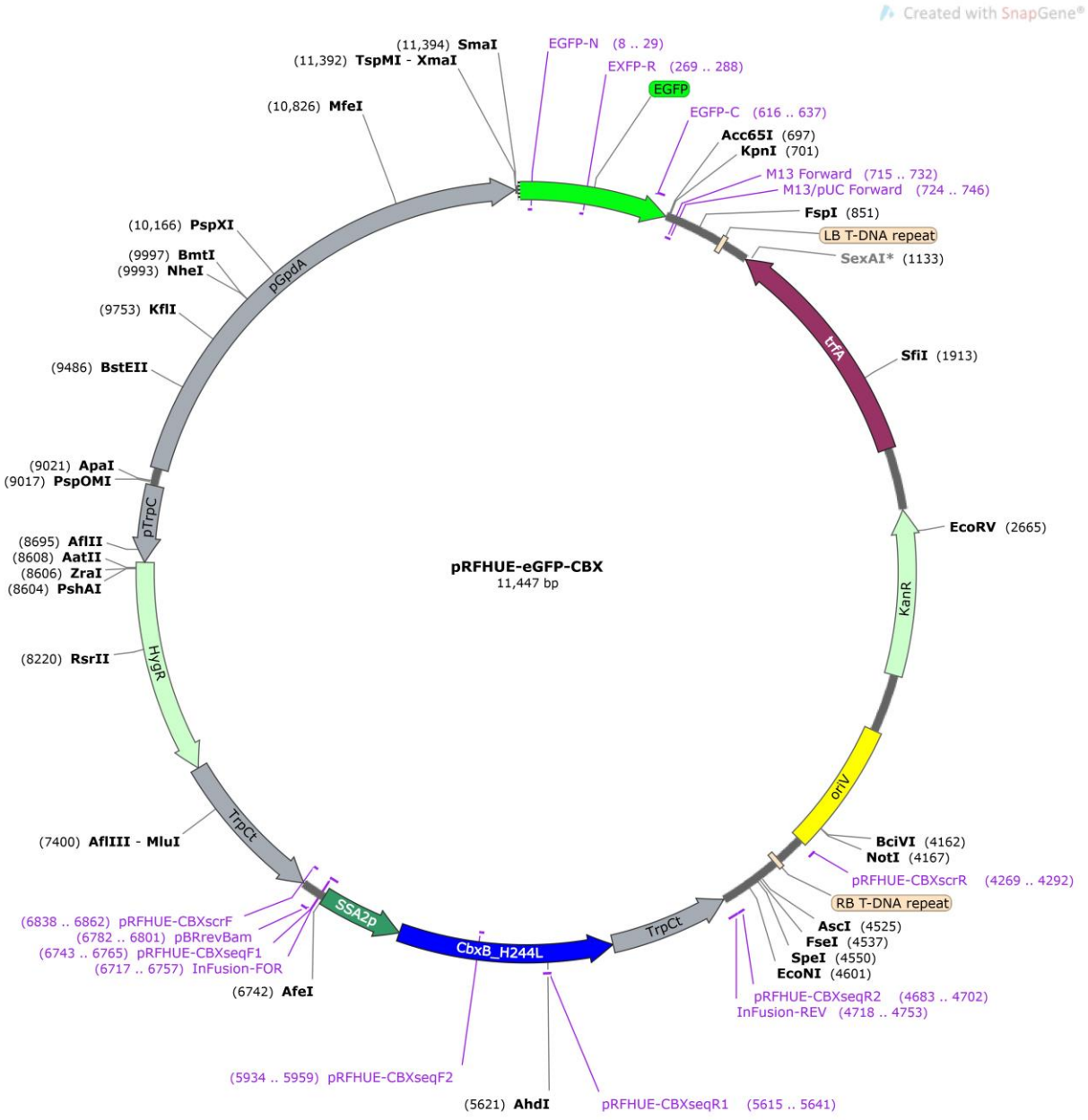


Table B.1 - **Primers for constructing and screening pRFHUE_eGFP_CBX**

Primer Name	Sequence (5' to 3')
Hyg5	GCTTGGCTGGAGCTAGTGGAG
Hyg3	CGGTCGGCATCTACTCTATTCCTT
TrpCt_R	CCGAGTGGAGATGTGGAGTGG
pRFHUE-CBXscrF	ATCCTCTACGCCGGACGCATCGTGG
pRFHUE-CBXscrR	GTCGGAAAGGCGCTCGGTCTTGCC
pRFHUE-CBXseqF1	CTCGCCACTTCGGGCTCATGAGC
pRFHUE-CBXseqR1	AGACGGCAGGTCCGAGGTATTGATCCG
pRFHUE-CBXseqF2	GTGAGGGTATCTGCGGTTCCTGCGCC
pRFHUE-CBXseqR2	AACAGTCCCCCGGCCACGGG
InFusion-FOR	TTCGGGCTCATGAGCGCTGCTATGCGAACGGTTCATTTTGC
InFusion-REV	CACGCCGAAACAAGCCCGAGTGGAGATGTGGAGTGG
EF1Ap_F	CTAGGTTCTTGTTTCTACGATTTTGG
EF1Ap_R	GCTAGAAAGTGGTGATAAATGTACAG
HygF	GACAGTTCTGGTTAGCCGTCAC
HygR	GTCGACGACAACTACCATCGATC
pDS23_A	GGGCGAACTCCGTCGCGACCGAGTGGAGATGTGGAGTGG
pDS23_B	GACCATGATTACGAATTCGCTTGGCTGGAGCTAGTGGAG
CBX_pEF1a_R	GTGTTTGTCCGGTGCTAGAAAGTGGTGATAAATGTACAG

APPENDIX C: *MORTIERELLA ELONGATA* MATING SYSTEM

Introduction

Mating systems in fungi regulate the process of sexual reproduction, which can impact the abundance, resilience, and evolution of those fungi (Burnett, 1956). Sexual reproduction is either heterothallic, where strains are required to out-cross with a compatible partner to mate, or homothallic, where a single strain possesses both mating types and is able to complete the sexual process without another individual. Mortierellaceae sexual spores are called zygospores, the morphology of which are highly variable between species. There are both heterothallic and homothallic species in this lineage, but no notable phylogenetic pattern between species having each mating strategy (Kuhlman, 1972). Heterothallic mating can be an advantageous evolutionary strategy in that it prevents self-crossing and increases genetic diversity, but the requirement for meeting a compatible partner in favorable environmental conditions could be a considerable disadvantage. Homothallic mating also results in nuclear recombination and alleviates the requirement for meeting a compatible partner (Olive, 1963). High asexual spore production can largely mitigate the disadvantages of both mating systems, leading some fungi to lose sexual reproduction altogether (Olive, 1963). Although mating and sex-regulating genes are known for the sub-phylum Mucoromycotina, these genes have not been identified yet in Mortierellaceae (Lee & Heitman, 2014).

In the Mucorales, mating is regulated by trisporic acid, which is collaboratively by the (+) and (-) strains. Both strains produce synthesized B-carotene, which (+) and (-) strains metabolize to trisporic acid precursor molecules 4-dihydrotrisporic acid and trisporol, respectively (Gooday & Carlile, 1997). Each (+)/(-) mating strain has the enzyme necessary to synthesize trisporic acid from the precursor produced by the other (-)/(+) partner (Gooday & Carlile, 1997; Lee & Heitman, 2014). It is suspected that, similar to the Mucorales, the Mortierellaceae use trisporids to regulate mating (Schimek et al. 2003).

It is also unknown what environmental conditions regulate mating, only that some media support/induce mating better than others, especially low-nutrient media having complex compounds; optimal mating media seems to vary between species (Gams et a., 1972; Kuhlman, 1972). In addition, endobacteria are known to regulate mating in other systems, such as *Rhizopus microsporus* (Mucoromycotina), in which strains cured of their endobacteria can no longer form asexual or sexual spores (Mondo et al. 2017). Prior to this research, it was unknown whether either MRE or BRE affect Mortierellaeceae mating and whether either is transmitted in mating.

Mating in *M. elongata* is heterothallic and substrate conditions have been optimized to some degree (Gams et al. 1972; Kuhlman, 1972). Since the Bonito lab has a library of *M. elongata* isolates, with and without MRE and BRE, I set out to establish mating assays to address research questions regarding the genetic basis, chemical signals, and impact of endobacteria on *M. elongata* mating.

Methods

Media Recipes

Hay agar has been shown to stimulate the highest zygospore production in *Mortierella elongata* compared to a number of other media recipes (Gams et al. 1972; Kuhlman, 1972). We chose two different hay agar media types for our mating assays. We used grass hay produced for animal consumption, obtained from an equine medical center, which contained a few forbs, especially clover.

Hay agar 1 (HAY1) was made by autoclaving 50g of hay in 1L of water on a 25min sterilization cycle and filtered with a Cat1 Whatman filter. The volume was adjusted to 1L with MilliQ water, the pH was adjusted to ~6 with K_2HPO_4, and 10g Difco BactoAgar added before autoclaving again for 25min sterilization. Plates were poured with about 20mL per plate.

Hay agar 2 (HAY2) was made by autoclaving 12.5 g of hay in 900 mL of water in a 2L Erlenmeyer flask for 45 min on liquid cycle. It was filtered through a coffee filter in a metal strainer,

the volume of filtrate measured, supplemented with BactoAgar to achieve a final strength of 1%, and the medium autoclaved for 24 min on liquid cycle. Plates were poured very thin, about 15mL per plate.

Mating & Microscopy

I placed 1cm^2 blocks of each strain on the HAY agar, 1cm apart and near the center of the plate (**Fig. 1**). I wrapped plates with extra parafilm or saran wrap to prevent desiccation and incubated the plates for 4-6 weeks. I observed the plates weekly, monitoring plate macromorphology and examining the zone of interaction between strains through the bottom of the agar plate at 40x and 100x magnification on a light microscope. Signs of mating were marked on the plate with sharpie for continued observation. Mating structures were visualized more closely by excising them from the agar and mounting on a glass slide and examination at 100x-400x magnification.

Results & Discussion

Mating

Our first round of mating plates used HAY1 and strains PMI86, NVP64wt, NVP64cu, NVP5. We observed zygospores after 6 weeks between NVP64cu*PMI86 and NVP64cu*NVP5 (**Fig. 2**; **Table C.1**). Significantly more zygospores were produced in the NVP64cu*PMI86 pairing. We designated NVP64cu to be type 'A' and its partners type 'B', since there are already '+' and '-' type strains designated, and selected PMI86 and NVP64cu to be tester strains. We will try to obtain these reference isolates in order to determine the +/- designation for our types A/B.

In the second mating panel, I used HAY2 and paired 15 strains of interest with our tester strains, PMI86 and NVP64cu, and re-paired both NVP64wt and NVP64cu with PMI86, the former to re-check for mating in the wild-type strain and the second as a positive control. In the second panel, I observed zygospores in matings between NVP64cu and GBAus25, NVP4, AD073c, and JL63. Interestingly, I never observed zygospores or mating macromorphology in any pairing that

involved either NVP80wt (which has MRE) or NVP80cu.

Kulhman (1972) described *M. elongata* as having both homothallic and heterothallic isolates and indicated that one of his homothallic isolates increased zygospore production when co-cultured with a heterothallic strain. However, none of his isolates were compatible with those shared by Gams, who had found them very fertile (Gams et al. 1972; Kuhlman, 1972). It is likely that *M. elongata* is a species complex, so perhaps there are both heterothallic and homothallic species in the complex and this could eventually be used as a diagnostic feature. Alternatively, the asexual spore and mycelial charactersistics of *M. elongata* are indistinguishable from those of a homothallic species, which may have since been distinguished by DNA sequencing. Finally, it is possible that homothallic species share the mating signals secreted by heterothallic species (Schimek et al. 2009; Lee & Heitman., 2014).

Morphology

In all successful matings, I observed both zygospores and suspensor cells, generally along the zone of interaction and not to one strain's "side" of the plate (**Fig. 1a-b**). Zygospore formation was usually not observed closer to the center of the plate and to proceed outward as the edges of each colony continued to meet. Instead, zygospores usually appeared first about an inch away from the inoculating agar blocks. In some cases, as plates aged, I repeatedly observed the same suspensor cells in various stages of cellular content transfer between suspensor cells where it seemed like transfer and zygospore development had frozen. In most successful matings, I observed development of hyphal clusters, that looked like hyphal knot-like aggregations, under the agar surface along the zone of interaction (**Fig. 1a-b**; **Fig. 2a-b**). These did not occur in all successful matings, nor do they strictly correlate with the location of zygospores being produced. However, I did observe them only in successful matings and zygospores do seem to cluster in small "nests" (**Fig. 2a-b**).

Most mating plates were not successful, in that I never observed zygospores. However, the scale of mating plates vs. the scale of zygospores makes it impossible to definitively say that no

zygospores existed on the plates we classified as negative. In most all putatively unsuccessful pairings, at least one of the strains produced abundant aerial hyphae on their side of the plate (**Fig. 1c**). This phenomenon was not observed in successful matings. However, it is possible that there were indeed zygospores present in the “unsuccessful” pairings that could not be observed due to the thick mycelial mat obscuring the passage of light through the agar.

Effect of Endobacteria

All matings with wild-type strains, those having endobacteria, morphologically resembled unsuccessful pairings (**Fig. 1c**). Moreover, no zygospores were ever observed in pairings with wild-type strains, though we cannot conclusively say that no zygospores were produced.

HAY+cellophane

Our collaborator Dr. Jessie Uehling at Oregon State University expressed interest in performing RNAseq of mating pairs to identify genes that might regulate mating in *M. elongata*. Cellophane is used to provide a smooth, firm surface to which mycelium cannot adhere, though they can still obtain nutrients from the agar underneath. This greatly increases the ease of collecting fungal mycelium without contaminating agar, the compounds of which often interfere with DNA and RNA extraction and analysis. However, cellophane does generally decrease fungal growth and there were concerns that mating would not be able to take place. To that end, I mated NVP64cu*PMI86 on HAY1 with a layer of sterile cellophane between the mycelium and the agar. Fortunately, I did observe mating on HAY1+cellophane, though the abundance of zygospores was reduced compared to regular HAY1 agar.

Future Directions

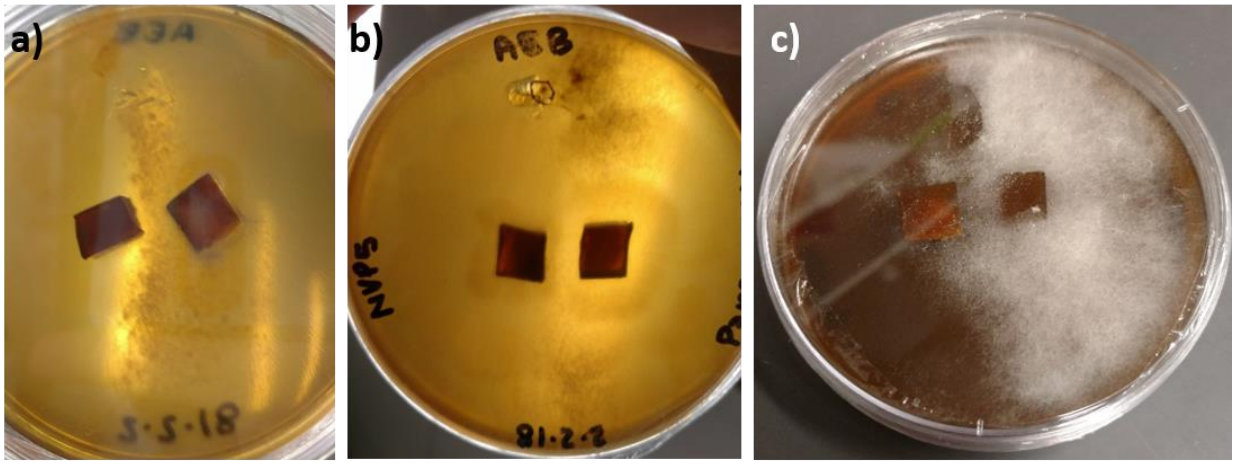
With successful mating, we can continue to screen *M. elongata* isolates for A/B mating type and eventually use the reference isolates to determine the +/- mating type. Using cellophane and close observation, the transcriptome of mating fungi may be obtained. Metabolomics of the media from mating versus single isolates may reveal candidate signals being exchanged during mating. Between these two approaches and careful genome comparison of the two mating types may be

sufficient to identify the genetic and chemical basis for mating compatibility, at which point strains could be classified as +/- with a PCR screen. It would also be extremely interesting to compare the transcriptomes and metabolomes of PMI86*NVP64cu and PMI86*NVP64wt to identify the mechanism by which BRE are putatively interfering with mating.

Figures & Tables

Figure C.1 - Macromorphology of *Mortierella elongata* mating on HAY1

Panels a-b) show compatible mating of *Mortierella elongata* strains and panel c) shows an incompatible mating.



PMI86 NVP64cu NVP5 NVP64cu NVP5 NVP64wt

Figure C.2 - Micromorphology of *M. elongata* zygospores

Zygospores were produced by mating NVP64cu*PMI86 on HAY1, viewed at a) 40x, b) 100x, c) 400x, and d) 1000x magnification under a light microscope. White arrows indicate zygospores in lower magnification images.

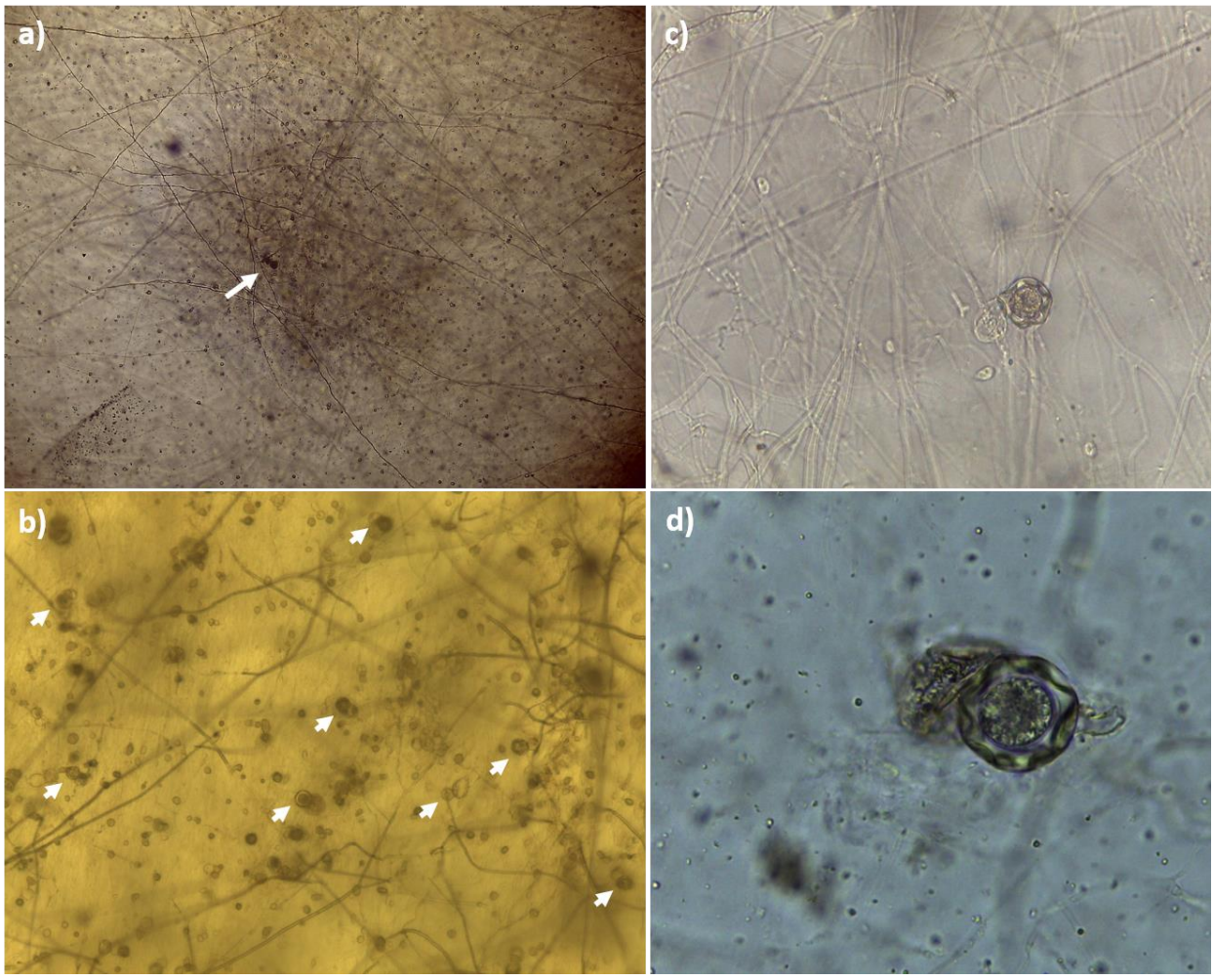


Table C.1 – ***Mortierella elongata*** **mating strains**

The strains tested for mating, the mating type when zygospores were observed, and the type of endobacteria present, where applicable.

Strain	Mating Type	Endobacteria
NVP5	B	
NVP5	B	
PMI86	B	
NVP64cu	A	
NVP64wt		BRE
NVP80cu		
NVP80wt		MRE
GBAus25	B	
NVP4	B	
AD073c	B	
GBAus38		
KOD979		
AG77-		
AD022wt		
AD022cu		BRE
AD073cu		
AD073wt		MRE
JL11		
JL51		
JL63	B	

APPENDIX D: SUPPLEMENTARY METHODS

Fast DNA Extraction

Extraction Solution (ES): 5mL 1M Tris stock (pH 9), 0.93g KCl, 0.19g Na2-EDTA, 50mL dH2O, titrated to pH 9.5-10.0 with 1M NaOH, sterilized with 0.2 µm filter, and aliquoted into 2mL Eppendorf tubes.

1. 3% BSA: 1.5g BSA in 50mL dH2O.
2. Pipette 20 µl ES into 8-strip tubes.
3. Place 10-20 mg of tissue sample into 8-strip tube, submerging in the ES and crushing/grinding if possible. Do not overload ES, err on the side of too little tissue to avoid high concentrations of PCR inhibitors.
4. Incubate at room temperature for 10+ minutes, then for 10 minutes at 95C in a thermal cycler.
5. Add 40 µl 3% BSA so that the final ES:BSA ratio is 1:2 (1:3 and 1:4 can also work well).
6. Samples are now ready for PCR. Use 1-2 µl for PCR and store DNA extractions in a freezer.

Seed counting by automated image analysis in ImageJ

Preliminary manual image analysis to determine parameters:

1. Open sample image in ImageJ
2. Select Working Area, i.e. the area to analyze for that sample
3. Edit>clear outside
4. Image>Adjust>Threshold (unselect dark background, we used 1.25%)
5. Analyze> Analyze Particles
6. Summary, Outlines, Min = 10??, Max = 1000
7. Show Results

Recording Macros for Batch Analysis

8. Start with an open image file, go to Plugins>Macros>Record
9. Take the image analysis steps determined in preliminary manual analysis
10. Hit “Create” and save in the ImageJ Macros folder

Using Macros for Batch Analysis

11. Process>Batch>Macro
12. Input & Output must be in SEPARATE FOLDERS to avoid overwriting input images
13. Open+ Point to macro in ImageJ Macro Folder
14. Process

In the case of the seed sheets analyzed in this study, there were four Working Areas in each raw image, one for each sample. This necessitated selecting each area in the raw image and exporting it as a separate input image to enable batch processing.

CTAB-based DNA extraction protocol

Fungal mycelium was placed into 450 µL of 2x CTAB buffer (100 mM Tris-HCl 8.0 pH, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, 4% PVP MW=10,000) and homogenized with a tube pestle. Next, 450 µl of 24:1 chloroform:isoamyl alcohol was added. The tubes were shaken briefly by hand, then centrifuged at 18,213 g for 8 min. The supernatant was removed and placed into a new tube. Chilled 2-propanol was added to the supernatant at 0.6 times its volume. The tubes were inverted about 20 times, then placed at -80°C for 8 minutes. The tubes were immediately placed in a pre-cooled 4°C centrifuge and centrifuged at 18,213 g for 15 minutes to pellet genomic DNA. The supernatant was discarded and the pellet washed with 800 µl of chilled 80% ethanol, then centrifuged at room temperature for 90s. This rinse was repeated exactly. All ethanol was aspirated from the pellet and the pellet vacuum dried for 2 minutes. The pellet was resuspended in 55 µl of nuclease-free water. DNA concentration was quantified by absorbance using the Denovix DS-11 FXl.

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