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Divergent evolution of early terrestrial fungi reveals the evolution of Mucormycosis pathogenicity factors

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ABSTRACT

Fungi have evolved over millions of years and their species diversity is predicted to be the second largest on the earth. Fungi have cross-kingdom interactions with many organisms which have mutually shaped their evolutionary trajectories. Zygomycete fungi hold a pivotal position in the fungal tree of life and provide important perspectives on the early evolution of fungi from aquatic to terrestrial environments. Phylogenomic analyses have found that zygomycete fungi diversified into two separate clades, the Mucoromycota which are frequently associated with plants and Zoopagomycota that are commonly animal-associated fungi. Genetic elements that contributed to the fitness and divergence of these lineages may have been shaped by the varied interactions these fungi have had with plants, animals, bacteria and other microbes. To investigate this, we performed comparative genomic analyses of the two clades in the context of Kingdom Fungi, benefiting from our generation of a new collection of zygomycete genomes. We identified lineage-specific genomic content which may contribute to the disparate biology observed in these zygomycetes. Our findings include the discovery of undescribed diversity in CotH, a Mucormycosis pathogenicity factor, which was found in a broad set of zygomycetes. Reconciliation analysis identified multiple duplication events and an expansion of CotH copies throughout Mucoromycotina, Mortierellomycotina, Neocallimastigomycota, and *Basidiobolus* lineages. A kingdom-level phylogenomic analysis also identified new evolutionary relationships within the sub-phyla of Mucoromycota and Zoopagomycota.

KEYWORDS

Comparative genomics, CotH, Evolution, Fungi, Phylogenomics, Zygomycetes

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INTRODUCTION

Fungi play diverse ecological roles and interact with various organisms in both terrestrial and aquatic environments (James, Kauff, et al. 2006; Stajich et al. 2009; Spatafora et al. 2017; Fisher et al. 2020). Since their divergence from a common ancestor with animals over 1 billion years ago, fungi have evolved complex relationships with other organisms, including animals, bacteria, plants, protists, and other fungi (Currie et al. 2003; Frey-Klett et al. 2011; Parfrey et al. 2011; Gruninger et al. 2014; Uehling et al. 2017; Wang et al. 2018; Chambouvet et al. 2019; Malar et al. 2021). As a distinct eukaryotic kingdom, Fungi are characterized by chitinous cell walls and osmotrophic feeding style, although neither of these characters is diagnostic for the kingdom (Richards et al. 2017; James et al. 2020). The versatile enzymes secreted by fungi facilitate their success in utilization of diverse polysaccharides and are key members of ecosystems supporting nutrient cycling processes (Hori et al. 2013; Chang et al. 2015; Solomon et al. 2016; Richards and Talbot 2018; Chang et al. 2022). Zygomycete fungi are a historically enigmatic group as their diversity and phylogenetic placement on the fungal tree of life remained somewhat cryptic based on morphological characters alone. The lineages emergence coincide with major transition of fungi from aquatic environment to terrestrial ecologies, which was characterized by the evolutionary loss of the flagellum (James, Letcher, et al. 2006; James, Kauff, et al. 2006; Chang et al. 2021). The zygomycete fungi are recognized by their gametangial conjugation, production of zygospore, and coenocytic aseptate or septate hyphae (White et al. 2006; Hibbett et al. 2007; Spatafora et al. 2017; Naranjo-Ortiz and Gabaldón 2020). Nevertheless, zygospore structures have not been observed for most members of zygomycete fungi due to their cryptic sexual stage or lack of appropriate culture approaches. Zygomycete

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fungi were found to be paraphyletic based on genome-scale evidence, as a result, two new phyla (Mucoromycota and Zoopagomycota) were established to accommodate the current members (Spatafora et al. 2016). However, incomplete sampling of zygomycete lineages has made resolution of the origin of terrestrial fungi difficult to resolve with standard phylogenetic approaches (Chang et al. 2021; Li et al. 2021).

Mycological and fungal cell biology research has been historically biased in favor of members of the Dikarya. Several established research model organisms have advanced fields of cell biology including the brewer's yeast *Saccharomyces cerevisiae*, the fission yeast *Schizosaccharomyces pombe*, the red bread mold *Neurospora crassa*, and the filamentous mold *Aspergillus nidulans.* These model organisms contributed to an expansion in the understanding of eukaryotes. Fungi were among the some of the first sequenced eukaryotic genomes (Goffeau et al. 1996; Wood et al. 2002; Galagan et al. 2003; Galagan et al. 2005). However, genomic research on zygomycete fungi had to wait for the first Mucoromycotina genome to be sequenced in 2009 (Ma et al. 2009). The majority of our existing knowledge of zygomycetes has come from studies of arbuscular mycorrhizae (Glomeromycotina) or saprophytes classified in Mucoromycota, such as the black bread mold *Rhizopus stolonifer*. Studies on the other zygomycetes phylum, Zoopagomycota, are still rare, and the biodiversity of Zoopagomycota fungi is likely greatly underestimated and the research progress is largely hindered by the lack of axenic cultures. Culture independent studies have identified multiple zygomycetes as amplicon-based operational taxonomic units (OTUs) in unexplored ecological sites (Metcalf et al. 2016; Picard 2017; Pombubpa et al. 2020; Reynolds et al. 2021) and many "unknown" fungal

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OTUs will likely to be identified with the help of increasing fungal genomes, especially more representatives in the sparsely sequenced zygomycete lineages.

To fill this gap, our recent emphasis on sequencing zygomycete genomes through the ZyGoLife project (Spatafora et al. 2016; https://zygolife.org) have produced over a hundred genomes. The output has become the largest collection of genomic information for this fungal clade. Various techniques were also developed and employed to obtain genome sequences of the uncultured zygomycete species. The breakthroughs include the single-cell genomics as well as fungus-host co-culture techniques (Ahrendt et al. 2018) and sequencing of metagenomes of sporocarps (Chang et al. 2019). Progress on genomics and related multi-omics have greatly expanded our knowledge on zygomycetes. This includes the identification of a mosquito-like polyubiquitin gene in a zygomycete fungus inhabiting the gut of mosquitoes (*Zancudomyces culisetae*, Zoopagomycota) (Wang et al. 2016), the discovery of a photosynthetic mycelium using algal symbionts (*Linnemannia elongata*, Mortierellomycotina) (Du et al. 2019; Vandepol et al. 2020), the isolation of cicada behavior modifying alkaloids from *Massospora* (Entomophthoromycotina) (Boyce et al. 2019), and the expansion of secondary metabolite genes of amphibian gut fungi (*Basidiobolus*, Entomophthoromycotina) via Horizontal Gene Transfer from bacteria co-existing in the gastrointestinal tract (Tabima et al. 2020). However, a conundrum remains as to the evolutionary history of the zygomycete fungi. What evolutionary processes were associated with the divergence of the ancestors of Mucoromycota and Zoopagomycota into species which primarily associate with plants and plant material or animal and fungal hosts, respectively. We hypothesize that comparisons of gene content will enable identification of genetic elements that have contributed to their success in these ecologies and

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their reproductive strategies and may be reflected in lineage-specific genes, those with expanded copy number or enrichment in specific pathways or processes that underpin adaptations to these hosts and environments. In addition, the construction of a well-resolved phylogenetic tree incorporating the expanded collection of zygomycete genomes is an important framework to consider the complex natural history and relationships among these diverse fungi. Our work has contributed to the generation of 131 recent zygomycete genomes (Table 1), which were used to investigate the evolution and cryptic genetics behind the biology of these early-diverging fungi.

The focus on these phyla is motivated by not only understanding their ecological roles and history, but also in the context of the increase in Mucormycosis, a deadly human-infectious disease, that has risen in prevalence and public attention due to high infection rates and co-morbidity during the COVID-19 pandemic (Garg et al. 2021; Revannavar et al. 2021). Mucormycosis is caused by members of Mucoromycotina, in particular many genera of the Mucorales fungi (Soare et al. 2020). We cataloged the prevalence of Mucormycosis pathogenicity factors across Mucorales genomes and profiled their evolutionary conservation among members of the Fungal Kingdom. We identified the genes for the Mucormycosis invasin factor in three Mortierellomycotina species as well (*Dissophora ornate*, *Lobosporangium transversle*, and *Mortierella* species) which all share a highly similar protein motif associated with the disease in Mucorales fungi indicating these fungi may have additional potential for mammalian infection and the more ancient nature of this factor within these fungi. Our study highlights the importance of research on zygomycetes to characterize the unique and shared molecular components of their biology that can be examined as more genome sequences

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become available. Our improved resolution phylogeny will enhance the study of the evolutionary relationships for both organismal and molecular genetics of these important fungi.

MATERIALS AND METHODS

Fungal taxa and genome sampling

In total, 181 fungal genome sequences were analyzed in this study. Nine genomes were generated in this study and 172 were obtained from GenBank or the Joint Genome Institute MycoCosm portal (Grigoriev et al. 2014; https://mycocosm.jgi.doe.gov), with 136 produced by the ongoing 1000 Fungal Genome Project (1KFG: http://1000.fungalgenomes.org/) and Zygomycetes Genealogy of Life Project (ZyGoLife: http://zygolife.org/). The dataset includes 131 zygomycetes genomes (Table 1), with 97 sampled from Mucoromycota clade and 34 from Zoopagomycota. In addition, we included 43 Dikarya genomes and seven representatives (Supplementary Table 1) from other early-diverging fungal lineages to enable kingdom-wide comparative analyses. The following nine genomes were produced for this study: *Amylomyces rouxii* NRRL 5866, *Benjaminiella poitrasii* RSA 903, *Fennellomyces* sp. ATCC 46495, *Lichtheimia hyalospora* FSU 10163, *Mucor mucedo* NRRL 3635, *Parasitella parasitica* NRRL 2501, *Radiomyces spectabilis* NRRL 2753, *Spinellus fusiger* NRRL 22323, *Piptocephalis tieghemiana* RSA 1565.

Genome sequencing and assembly

The genome sequencing of *Spinellus fusiger* NRRL 22323, *Radiomyces spectabilis* NRRL 2753, *Mucor mucedo* NRRL 3636, *Benjaminiella poitrasii* RSA 903 and *Fennellomyces* sp. ATCC

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46495, was performed from 5 ug of genomic DNA was sheared to >10kb using Covaris g-Tubes. The sheared DNA was treated with exonuclease to remove single-stranded ends and DNA damage repair mix followed by end repair and ligation of blunt adapters using SMRTbell Template Prep Kit 1.0 (Pacific Biosciences). The library was purified with AMPure PB beads. PacBio Sequencing primer was then annealed to the SMRTbell template library and Version P6 sequencing polymerase was bound to them for *S. fusiger*, *R. spectabilis* and *Fennellomyces* sp. ATCC 46495. The prepared SMRTbell template libraries were then sequenced on a Pacific Biosciences RSII sequencer using Version C4 chemistry and 1x240 sequencing movie run times. For *B. poitrasii* and *M. mucedo*, sequencing polymerase was bound to them using the Sequel Binding kit 2.1 and then the prepared SMRTbell template libraries were sequenced on a Pacific Biosystems' Sequel sequencer using v3 sequencing primer, 1M v2 SMRT cells, and Version 2.1 sequencing chemistry with 1x360 sequencing movie run times. Filtered subread data was then used to assemble all lineages using Falcon (version 0.4.2 for *S. fusiger* and *R. spectabilis*, version 1.8.8 for *M. mucedo* and *B. poitrasii*, and version 0.7.3 for *Fennellomyces* sp. ATCC 46495). *S. fusiger* and *R. spectabilis* were then further improved using finisherSC version 2.0 (Lam et al. 2015). All assemblies were then polished using either Quiver version smrtanalysis_2.3.0.140936.p5 (*S. fusiger*, *R. spectabilis* and *Fennellomyces* sp. ATCC 46495) or Arrow version SMRTLink v5.1.0.26412 (*M. mucedo* and *B. poitrasii*).

Parasitella parasitica NRRL 2501, *Piptocephalis tieghemiana* and *Lichtheimia hyalospora* were sequenced using the Illumina platform. For *P. parasitica* and *P. tieghemania*, 100 ng of DNA was sheared to 300 bp using the Covaris LE220 and size selected using SPRI beads (Beckman Coulter). The fragments were treated with end-repair, A-tailing, and ligation of Illumina

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compatible adapters (IDT, Inc) using the KAPA-Illumina library creation kit (KAPA biosystems). Additionally, a 4kb mate pair library was constructed for *P. parasitica*. For this, 5-10 ug of DNA was sheared using the Covaris g-TUBE(TM) and gel size selected for 4 kb. The sheared DNA was treated with end repair and ligated with biotinylated adapters containing loxP. The adapter ligated DNA fragments were circularized via recombination by a Cre excision reaction (NEB). The circularized DNA templates were then randomly sheared using the Covaris LE220 (Covaris). The sheared fragments were treated with end repair and A-tailing using the KAPA-Illumina library creation kit (KAPA biosystems) followed by immobilization of mate pair fragments on strepavidin beads (Invitrogen). Illumina compatible adapters (IDT, Inc) were ligated to the mate pair fragments and 8 cycles of PCR was used to enrich for the final library (KAPA Biosystems). The prepared libraries were quantified using KAPA Biosystems' next-generation sequencing library qPCR kit and run on a Roche LightCycler 480 real-time PCR instrument. The quantified libraries were then prepared for sequencing on the Illumina HiSeq sequencing platform utilizing a TruSeq paired-end cluster kit, v4. Sequencing of the flowcell was performed on the Illumina HiSeq2500 sequencer using HiSeq TruSeq SBS sequencing kits, v4, following a 2x150 indexed run recipe. Each fastq file was QC filtered for artifact/process contamination and subsequently assembled together with AllPathsLG version R49403 (Gnerre et al. 2011).

Since *P. tieghemania* is an obligate mycoparasite, it was maintained as co-culture with *Umbelopsis* sp. nov. AD052. The *P. tieghemania* contigs required further processing to separate these two assemblies. First, metagenomic scaffold sequences were binned into two groups using metabat (v2.12.1). The filtered reads were mapped to the sequences of the two bins and split into two separate datasets corresponding to each bin using bbsplit.sh in

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bbtools(ambiguous=all). The two datasets were then re-assembled separately. Scaffolds with length less than 2kb were excluded. Then, four closely related genomes were used for reference genome to classify and filter re-assembled scaffolds based on BLASTN similarity (evalue < 1e-30). One included *Piptocephalis* related genome, *Piptocephalis cylindrospora*, and the others were *Umbelopsis* related genomes, *Umbelopsis* sp. AD052, *Umbelopsis isabellina* AD026 and *Umbelopsis* sp. PMI 123. If the scaffolds were covered more by *Piptocephalis* main genome than *Umbelopsis* main genomes, it would be classified to *Piptocephalis tieghemiana*, and vice versa. The scaffolds without any similarity to the four genomes were discarded.

For *L. hyalospora*, 500 ng of DNA was sheared to 270 bp using the Covaris E210 (Covaris, Woburn, MA) and size selected using SPRI beads (Beckman Coulter, Brea, CA). The fragments were treated with end-repair, A- tailing, and ligation of Illumina adapters using the TruSeq Sample Prep Kit (Illumina, San Diego, CA), followed by quantification of libraries 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 multiplexed and the pools were then prepared for sequencing on the Illumina HiSeq sequencing platform utilizing a TruSeq paired-end cluster kit, v3, and Illumina's cBot instrument to generate a clustered flowcell for sequencing. Sequencing of the flowcell was performed on the Illumina HiSeq2000 sequencer using a TruSeq SBS sequencing kit 200 cycles, v3, following a 2x150 indexed run recipe. Genomic reads were QC filtered for artifact/process contamination and subsequently assembled with Velvet. The resulting assembly was used to create a simulated 3 Kbp insert long mate-pair library, which was then assembled together with the original Illumina library with AllPathsLG release version R42328.

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Transcriptome sequencing and assembly

For all lineages except *L. hyalospora*, Stranded cDNA libraries were generated using the Illumina Truseq Stranded RNA LT kit. mRNA was purified from 1 ug of total RNA using magnetic beads containing poly-T oligos. mRNA was fragmented and reversed transcribed using random hexamers and SSII (Invitrogen) followed by second strand synthesis. The fragmented cDNA was treated with end-pair, A-tailing, adapter ligation, and 8 cycles of PCR. For *L. hyalospora*, Plate-based RNA sample prep was performed on the PerkinElmer Sciclone NGS robotic liquid handling system using Illumina's TruSeq Stranded mRNA HT sample prep kit utilizing poly-A selection of mRNA following the protocol outlined by Illumina in their user guide: https://support.illumina.com/sequencing/sequencing_kits/truseq-stranded-mrna.html, and with the following conditions: total RNA starting material was 1 ug per sample and 8 cycles of PCR was used for library amplification. The prepared libraries were then quantified using KAPA Biosystems' next-generation sequencing library qPCR kit and run on a Roche LightCycler 480 real-time PCR instrument. The quantified libraries were then prepared for sequencing on the Illumina HiSeq sequencing platform utilizing a TruSeq paired-end cluster kit, v4. Sequencing of the flowcell was performed on the Illumina HiSeq2500 sequencer using HiSeq TruSeq SBS sequencing kits, v4, following a 2x150 indexed run recipe (2x100 for *L. hyalospora*). Filtered fastq files were used as input for de novo assembly of RNA contigs. For all lineages except *L. hyalospora* and *P. parasitica*, reads were assembled into consensus sequences using Trinity version 2.1.1. Trinity was run with the --normalize_reads (In-silico normalization routine) and --jaccard_clip (Minimizing fusion transcripts derived from gene dense genomes) options.

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For *L. hyalospora* and *P. parasitica*, Rnnotator version 2.5.6 or later was used. *P. parasitica* was further improved using eight runs of velveth (v. 1.2.07) performed in parallel, once for each hash length for the De Bruijn graph. Minimum contig length was set at 100. The read depth minimum was set to 3 reads. Redundant contigs were removed using Vmatch (v. 2.2.4) and contigs with significant overlap were further assembled using Minimus2 with a minimum overlap of 40. Contig postprocessing included splitting misassembled contigs, contig extension and polishing using the strand information of the reads. Single base errors were corrected by aligning the reads back to each contig with BWA to generate a consensus nucleotide sequence. All nine new genomes in this study were annotated using the JGI Annotation pipeline (Grigoriev et al. 2014).

Phylogenomic analyses

A set of 758 phylogenetic markers, "fungi_odb10", from the Benchmarking Universal Single-Copy Orthologs (BUSCO) v4.0.5 was employed for the kingdom-wide phylogenomic analyses (Seppey et al. 2019). We used the PHYling pipeline (DOI: 10.5281/zenodo.1257002) to extract best hit copies using hmmsearch v3.3.2 (cutoff=$1E^{-10}$) from the genes predicted in each species against the marker set. A total of 617 (out of 758) well-conserved markers were identified as the best hit from the 181 fungal genomes. A backbone tree including 80 genomes, subsampled based on BUSCO scores and phylogenetic placement (except for the outgroup *Drosophila melanogaster*), recovered 604 orthologs. All orthologs were aligned separately using hmmalign v3.3.2 to the marker profile-HMM and then concatenated into a super-alignment with partitions defined by each marker. The best phylogenomic tree was searched and

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identified using the super-alignment file and partition scheme as the input with the best-fit model option for maximum likelihood analyses implemented in IQ-TREE v.1.5.5 (Nguyen et al. 2015; Kalyaanamoorthy et al. 2017). Branch supports were evaluated using 1000 ultrafast bootstrap replicates (Hoang et al. 2017).

Identification of lineage-specific genes and Pfam domains in zygomycete fungi

All orthologous groups of the 80 genomes included in the backbone tree were identified using a comparative genomic pipeline that utilized all-vs-all BLASTp search v2.6.0 (cutoff=$1E^{-5}$) (DOI: 10.5281/zenodo.1447224) (Altschul et al. 1990). Orthagogue v1.0.3 was used to infer putative orthologs and Markov-Clustering Algorithm v14-137 (MCL, inflation value of 1.5) was utilized to generate disjoint clusters (Van Dongen 2000; Ekseth et al. 2014). Shared genome components were counted using a permissive strategy that a gene family shared by at least 10 of the 80 included taxa was retained. Zygomycetes-specific genes are the ones that only exist in zygomycete fungi (Mucoromycota and Zoopagomycota) and are absent in all other lineages. The absence-presence pattern of gene families across the Kingdom Fungi was plotted using the "aheatmap" function in R package "NMF" (Gaujoux and Seoighe 2010). Protein domains coded by the 80 taxa were examined in a similar way. Each Protein Family (Pfam) entry in the Pfam database v31.0 was searched against the predicted proteomes of all included 80 taxa (using the threshold of 1E-3 with >50% overlap percentage). The Pfam domains dominated in either Mucoromycota or Zoopagomycota were inferred by the ratios of their copy numbers in Zoopagomycota and Mucoromycota. The disproportion was visualized by plotting the binary logarithm of the ratio for each Pfam entry so that dominated Pfam domains in each phylum will

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be isolated on the edge. The figure was plotted using R package "ggplot2" (Wickham 2016). Subphylum-level distribution of each discussed Pfam domain was plotted using the "radarchart" function implemented in R package "fmsb". All lineage-specific genome content was summarized in Table 2 (with detailed Pfam names listed in Supplementary Table 2). Gene Ontology (GO) terms of Zoopagomycota "unique" genes were inferred and annotated using InterProScan v5.54 and WEGO v2.0 respectively (Jones et al. 2014; Ye et al. 2018).

Phylogenetic analysis of the spore coating protein (CotH) in fungi

A total of 846 protein sequences that contain at least one CotH domain were identified in the 80 genomes included in the backbone tree. Absent in all Dikarya species, CotH genes were largely found in zygomycetes (all included six Mortierellomycotina members, 27 Mucoromycotina taxa, and one *Basidiobolus*) and in Neocallimastigomycota (including 2 taxa). Previously classified CotH families 1-5 (CotH 1-5) from *Rhizopus oryzae* were included in our phylogenetic analyses to categorize the newly identified CotH copies. Highly similar CotH sequences (>90%) were removed using CD-HIT v4.6.4 and poor-quality ones were manually excluded from the multiple sequence alignment using MUSCLE v3.8.31 (Edgar 2004; Fu et al. 2012). We employed IQ-TREE v1.5.5 to identify the most appropriate substitutional model and to reconstruct the phylogenetic tree of all fungal CotH copies with ultrafast bootstraps (1000 replicates) (Nguyen et al. 2015; Hoang et al. 2017; Kalyaanamoorthy et al. 2017). The final input includes 754 sequences with 230 distinct patterns for CotH classification. Species-gene tree reconciliation analysis was conducted with Notung v3.0 BETA using the 80-taxa backbone tree

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as the species tree (Stolzer et al. 2012). The *--phylogenomics* command line option was used to generate a summary report of gain and loss events of CotH families in Kingdom Fungi.

RESULTS

Phylogenetic relationships and genome statistics of zygomycete fungi.

Collaborative efforts to sequence fungi have generated the 131 zygomycetes genomes presented in this study and the relationships among these species has remained an open research question. Most of the assembled zygomycete genomes were assessed to have BUSCO scores higher than 80% (Fig. 1a, Table 1). The phylogenetic analysis using all available zygomycetes genomes and 50 additional representatives from other fungal clades (Fig. 1a and Supplementary Figure 1) provided an updated tree representing the placement of these fungi in the kingdom. At the phylum level, the reconstructed phylogeny exhibits the same topology as presented in Spatafora et al. (2016). That is, Zoopagomycota forms a sister group to the clade comprising Mucoromycota and Dikarya, and the traditional zygomycete fungi (Mucoromycota and Zoopagomycota) remain paraphyletic. The increased sampling size and new set of protein-coding gene phylogenetic markers provide additional confidence in these arrangements. This is in contrast to a kingdom-wide study that also uses protein-coding genes from BUSCO datasets suggests that zygomycetes could still be monophyletic with a different sampling strategy (Li et al. 2021). It should be noted that the marker sets used in this study (fungi_odb10 with 758 markers) and Li et al. (fungi_odb9 with 290 markers) differ, as well as the strategies to extract the hits—protein searches against genome annotations in this study and BUSCO predicted gene

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models in Li et al. Regardless of whether zygomycetes are paraphyletic or monophyletic, it is not controversial that Mucoromycota and Zoopagomycota are monophyletic phyla. At the subphylum level, however, the tree topology recovered new phylogenetic relationships with consistency in both the comprehensive tree (Fig. 1a) and the backbone tree (Fig. 2a). For example, Glomeromycotina grouped with Mortierellomycotina instead of being the earliest branch within Mucoromycota (Spatafora et al. 2016). *Basidiobolus* members were not grouped within Entomophthoromycotina, instead, they were found as the earliest diverging lineage in Zoopagomycota (Figs. 1a, 2a, and Supplementary Figure 1). The present subphylum-level classification received full bootstrap supports in the comprehensive tree (Fig. 1a and Supplementary Figure 1). This tree topology is identical in the backbone tree with strong supports, only two nodes within Zoopagomycota clade receiving relatively low values (82/100, Fig. 2a).

Our results suggest that the saprobe, *Calcarisporiella thermophila,* is sister to the rest of the Mucoromycotina. Plant symbionts like *Bifiguratus*, *Endogone*, and *Jimgerdemannia* form a monophyletic clade which was placed between *C. thermophila* and Mucoromycotina (Fig. 1a). Members of saprobes, pathogens, and mycoparasites were joined in more derived groups of Mucoromycotina.

In the Kickxellomycotina clade, the mycoparasite, *Dimargaris cristalligena*, is sister to the other members. *Ramicandelaber brevisporus* follows and leads to two separate monophyletic clades composed of insect symbionts (*Capniomyces*, *Furculomyces*, *Smittium*, and *Zancudomyces*) and soil saprobes (*Coemansia*, *Kickxella*, *Linderina*, *Martnesiomyces*). Both clades (insect symbionts & soil saprobes) are on relatively long branches implying early

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divergent evolution and underexplored biodiversity (Fig. 2a and Supplementary Figure 1). Insect pathogens were grouped together on a separate lineage, Entomophthoromycotina, forming a sister clade to Kickxellomycotina (Figs. 1a, 2a). The three included *Conidiobolus* species support a paraphyletic genus with the *C. coronatus* monophyletic with *C. incongruus,* while *C. thromboides* was more closely related to *Zoophthora radicans* and *Entomophthora muscae*. Zoopagomycotina is monophyletic and sister to the joined group of Entomophthoromycotina (excluding *Basidiobolu*s) and Kickxellomycotina (Figs. 1a, 2a).

The density of genes arranged in the genome of zygomycete fungi exhibited varying patterns among subphyla which was observed in plots of gene counts against genome sizes (Fig. 1b). Most zygomycete fungi have genome sizes ranging from 20 Mb to 100 Mb and gene counts range from 5k to 20k. The Mucoromycotina fungi have relatively similar genome sizes, but gene counts vary from 6k to 21k. The soil saprobes in Kickxellomycotina and small animal associates in Zoopagomycotina have small genome sizes (10-20 Mb) and gene counts (4-8k). On the other hand, Glomeromycotina fungi tend to have large genome sizes (>100 Mb) with the most abundant gene numbers (20-30k) in all zygomycete fungi, which are among the largest fungal genome sizes sequenced to date. As an extreme case, the genome sizes of Entomophthoromycotina members exhibit the widest range and can be as large as 1.2 Gb according to the existing genome assemblies (Stajich et al, in revision), however, their gene counts (9-23k) are more modest.

Orthologous gene families and Pfam domains in zygomycete fungi

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The 80 species used for the backbone tree were examined for orthologous gene families across the Kingdom Fungi. We identified 8,208 orthologous families which had genes from at least 11 of the 80 genomes. These gene families were subjected to more focused analyses to examine the presence/absence pattern of genome contents across the Kingdom Fungi, with a special attention on the divergent evolution between Mucoromycota and Zoopagomycota (Fig. 3). The Mucoromycota members harbor 171 phylum-specific gene families that are present in at least two of the three Mucoromycota subphyla and absent in all other fungal lineages, while Zoopagomycota only have nine such gene families (Table 2). At the subphylum level there were considerably more lineage-specific gene families, ranging from 1,186 (in Zoopagomycotina) to 7,779 (in Mucoromycotina) (Table 2).

We used protein domains cataloged in the Pfam database as an additional means to catalog unique and shared content. A total of 7,616 Pfam models had at least one similar sequence in the examined 80 genomes. Mucoromycota members possess two unique Pfam domains, with the CheR (PF01739) found in all three subphyla and the C9orf72-like (PF15019) in Mucoromycotina and Mortierellomycotina, while no phylum-specific Pfam domains were identified in the Zoopagomycota. At the subphylum level, a range of unique Pfam domains were observed, with 11 to 32 in the three subphyla of Mucoromycota and 0-5 in the ones in Zoopagomycota (Table 2 and Supplementary Table 2). Interestingly, the CotH domain (PF08757), a potential invasin factor of Mucormycosis, was found in Mortierellomycotina, *Basidiobolus*, and Neocallimastigomycota genomes (Fig. 2b), but had previously only been described in the Mucoromycotina (Chibucos et al. 2016). In addition, the oxidation resistance protein domain (TLD, PF07534) has greatly expanded in copy number in the Glomeromycotina

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with up to 400 copies (Fig. 2c). Kickxellomycotina and Zoopagomycotina members lacked Biotin and Thiamin synthesis associated domain (BATS, PF06968) and mycobacterial membrane protein large transporter domain (MMPL, PF03176) (Fig. 2d and 2e). Interestingly, *Basidiobolus meristosporus* is the only Zoopagomycota member that maintains at least one copy of every examined domain (Fig. 2b-e), including CotH and MMPL that are absent in all other Zoopagomycota members.

To identify all Pfam domains that are consistent with divergent evolution between Mucoromycota and Zoopagomycota, we calculated the relative abundance of each Pfam domain in their genomes. In total, 285 Pfam domains were present at least four-fold differences (i.e., absolute value of the binary logarithm >2) between the two phyla with 243 of them featured in Mucoromycota while 42 in Zoopagomycota (Fig. 4 and Supplementary Table 3). Without consideration of non-zygomycetes lineages, we found 70 Pfam domains in Mucoromycota that are completely missing in Zoopagomycota, whereas no such Pfam domains can be identified in Zoopagomycota. Zoopagomycota is a historically understudied fungal clade with few representative genomes until our recent studies. As a result, the lack of Zoopagomycota specific Pfam domains may be an artifact of insufficient sampling before domain curation in Pfam. To overcome this possibility, we examined the orthologous gene family dataset to calculate the relative abundance of gene families to test for differences between the two phyla. This revealed 22 gene families in Zoopagomycota that were absent in all Mucoromycota members (Supplementary Figure 3). Gene Ontology analysis shows that more than 50% of these genes are involved in binding, catalytic activity, cellular process, and metabolic process (Supplementary Figure 4). Finer scales of examination suggest they are

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closely related to nitrogen compound, organic substance, and primary metabolic process (Supplementary Figure 5). This provides a compact set of genes and processes that are distinct to the Zoopagomycota which could contribute to their unique lifestyles, reproductive biology, and ecological specializations.

We found that many phylum-level distinct Pfam domains were favored unevenly in each subphylum group (Fig. 5). For example, both Pil1 (PF13805) and SUR7 (PF06687) domains are eisosome components and are involved in the process of endocytosis. They are missing entirely from the Zoopagomycota but are encoded in the genomes of all (Pil1) or a majority (SUR7, except for *Mortierella multidivaricata* and *Gigaspora rosea*) of Mucoromycota members (Figs. 4, 5a, & 5b). Interestingly, the Pil1 domain was enriched in copy number in the Mortierellomycotina (Fig. 5a), and SUR7 domain has the largest copy number in Mucoromycotina (Fig. 5b). The SMG1 domain (PF15785), a phosphatidylinositol kinase-related protein kinase, is a key regulator of growth. The Mucoromycota members maintain a single-copy SMG1 domain (except for *Cunninghamella bertholletiae* with 3 copies, and none in *Mucor circinelloides*, *Phycomyces blakesleeanus*, and *Syncephalastrum monosporum*), which is absent in Zoopagomycota species (Fig. 5c). There are 67 additional Pfam domains including DENN (PF02141), uDENN (PF03456), dDENN (PF03455), Pox_ser-thr_kin (PF05445) (Supplementary Table 3) with a similar presence/absence pattern and may be important components to better understand and characterize the Mucoromycota fungi.

In contrast, while there are no Zoopagomycota-specific Pfam domains, there are some domains that exhibit copy number variance at the subphylum level. For example, the Tyrosinase domain (PF00264) is an important enzyme that controls the production of melanin

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and parasite encapsulation, especially in insects. It is also suggested that Tyrosinase is involved in the host-microbe defensive mechanism. The Tyrosinase domains are found on average with 48 copies in the Entomophthoromycotina members but absent in nearly all Mucoromycotina (except for *Calcarisporiella thermophila* with 7 copies) and Mortierellomycotina (except for *Mortierella verticillata* with 1 copy) (Fig. 5d). Similarly, Trypsin domain (PF00089), serine protease found in the digestive system of many vertebrates, was also enriched in copy number in the Entomophthoromycotina with 80 copies on average (Fig. 5e). The domain LPMO_10 (PF03067) is found in lytic polysaccharide monooxygenases which can cleave glycosidic bonds in chitin and cellulose and is significantly enriched in Zoopagomycota (Fig. 5f). All three examples (Trypsin, Tyrosinase, and LPMO_10) are related to animal-fungus interactions in the degradation of protein, chitin, and cellulose.

Discovery of CotH in early-diverging fungi

The CotH domain as characterized in Mucorales fungi has positive correlations with the clinical pathogenesis of Mucormycosis (Chibucos et al. 2016). In our kingdom-wide study, we found additional copies of the CotH domain in a broader collection of fungi. Other than in Mucorales fungi, CotH was also found in *Basidiobolus*, Mortierellomycotina, and Neocallimastigomycota. The presence of this domain could indicate the potential of these fungi to support pathogenic interaction with animal hosts (Fig. 2b). A total of 846 CotH copies were identified in 34 zygomycetes genomes and two Neocallimastigomycota representatives (contributing 348 of the copies). Five CotH families (CotH 1-5) that were previously classified in *Rhizopus oryzae* were included in our phylogenetic analysis and helped us categorize the newly

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459 identified CotH copies (Fig. 6a). Zygomycetes CotH copies formed four distinct clades. ZyGo-A
460 clade includes CotH families 1-3 that maintain true invasin motifs and can only be coded by
461 Mucoromycotina and Mortierellomycotina members. ZyGo-B clade includes CotH families 4-5
462 and copies from Mucoromycotina. ZyGo-C clade is loosely joined with ZyGo-B (bootstrap
463 support 34/100) and includes copies from Mortierellomycotina, and *Basidiobolus*. ZyGo-D is the
464 largest clade among the four but only includes copies from Mucoromycotina. Both ZyGo-C and
465 ZyGo-D clades represent new families of CotH. Interestingly, the distantly related anaerobic gut
466 fungi (AGF, Neocallimastigomycota) can code CotH as well and form several distinct clades. In
467 total, 531 duplications and 795 losses were identified along the evolution of CotH families in
468 Kingdom Fungi. Nine nodes were associated with more than 10 duplication events (Fig. 6b). The
469 absence of CotH in the most recent common ancestor of fungi was also inferred by Notung
470 reconciliation analysis.
471

472 **DISCUSSION**

473 *Genome evolution of zygomycete fungi*

474 Zygomycetes are important members of early-diverging fungi and studying their
475 evolutionary history can help us better understand the terrestrialization of eukaryotes.
476 Zygomycetes are ubiquitous and can live as arbuscular mycorrhizae, ectomycorrhizae,
477 saprobes, or symbionts of various organisms, including animals, bacteria, plants, and fungi.
478 During the evolutionary adaptation and diversification of zygomycetes, many associated
479 organisms (hosts, symbionts, etc.) may have mutually shaped the structure and content of their
480 genomes. Mucoromycotina members have served as exemplars to investigate various

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evolutionary events at the genome-scale. For example, whole-genome duplications have been identified repeatedly in Mucoromycotina (Ma et al. 2009; Corrochano et al. 2016), which contributed to the large expansion of gene counts (5-20k) among some Mucoromycotina members (Fig. 1b). Phylogenomic analyses suggest that an early split of Mucoromycotina involved the evolution of thermophily (i.e., *Calcarisporiella thermophila*) (Figs. 1a, 2a), which is followed by various lineages containing members of ectomycorrhizae, mycoparasites, plant and animal pathogens. In addition, transposable elements (TEs) have been suggested to be rich in some Mucoromycotina taxa, including *Rhizopus oryzae* (Ma et al. 2009) and *Endogone* sp. (Chang et al. 2019). The high proportion of TEs were also evident in other lineages of zygomycete fungi, like *Gigaspora* members (Morin et al. 2019) and *Basidiobolus meristosporus* (Muszewska et al. 2017). It has been suggested that TEs may have played a role in shaping transcriptional profiles, helped fungi adapt to different ecological niches, and contributed to the current fungal biodiversity (Castanera et al. 2016; Muszewska et al. 2017). It is still unclear what roles TE might have played in the evolution of Entomophthoromycotina members that exhibit the widest span of genome sizes (25-1200 Mb) in Kingdom Fungi and what resulted in the gigantic size of *Entomophthora muscae* and *Massospora cicadina*. More samples from this and related lineages may help us reconstruct the evolutionary history for the observed genome size modification in zygomycete fungi.

Phylogenomics of zygomycetes and Basidiobolus

Zygomycete fungi hold important phylogenetic placement on the fungal tree of life. The former taxonomic unit, Zygomycota, has been recognized paraphyletic and thus been

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abandoned and replaced by Mucoromycota and Zoopagomycota to accommodate the six major lineages—Glomeromycotina, Mortierellomycotina, Mucoromycotina, Entomophthoromycotina, Kickxellomycotina, and Zoopagomycotina (James, Kauff, et al. 2006; White et al. 2006; Hibbett et al. 2007; Spatafora et al. 2016). Since the loss of flagella, the first evolutionary split of terrestrial fungi leads to Zoopagomycota and the clade of Mucoromycota and Dikarya (Chang et al. 2021). Mucoromycota is the sister clade of the subkingdom Dikarya clades (Ascomycota and Basidiomycota) (Figs. 1a & 2a), and analysis of zygomycete fungi is therefore essential for us to accurately reconstruct the evolutionary events that led to major lineages of terrestrial fungi. The arbuscular mycorrhizal fungi of Glomeromycotina with their distinct ecology formed a monophyletic clade with the soil saprobes and root endophytes of Mortierellomycotina (Figs. 1a & 2a). Mucoromycota members are mostly associated with plants or more commonly as decomposers of plant carbohydrates. Zoopagomycota members are mostly animal associated (either as commensals or pathogens) or mycoparasites. The Entomophthoromycotina clade presents several interesting patterns. For example, our phylogenomic results confirm the non-monophyly of *Conidiobolus* and encourage further work to reclassify this genus. One recent taxonomic effort based on a four-gene phylogeny proposed three new genera (*Capillidium*, *Microconidiobolus*, and *Neoconidiobolus*) to help delimitate *Conidiobolus* where *C. thromboides* was suggested in the *Neoconidiobolus* clade (Nie et al. 2020). In addition, our results suggest *Basidiobolus*, a traditional member of Entomophthoromycotina, as the earliest diverging lineage within Zoopagomycota (Figs. 1a, 2a & Supplementary Figure 1).

Basidiobolus has been characterized as a “rogue” taxon and is often found with conflicting phylogenetic placements. Using nuclear rRNA genes (18S+28S+5.8S genes),

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Basidiobolus, Olpidium brassicae (a plant pathogen), and *Schizangiella serpentis* (a snake pathogen) were grouped together and placed at the earliest diverging branch within Zoopagomycota (White et al. 2006). In a separate study using four genes (nuclear 18S and 28S rDNA, mitochondrial 16S, and RPB2), *Basidiobolus* was suggested as the earliest diverging member of Entomophthoromycotina (Gryganskyi et al. 2012). The recent genome-scale study based on 192 conserved orthologous proteins favored the *Basidiobolus* placement in Entomophthoromycotina as well (89/100 bootstrap support) (Spatafora et al. 2016). Interestingly, a recent genome-scale phylogenetic study examining the entire Kingdom Fungi found that *Basidiobolus* formed a sister clade to Mucoromycota instead of joining Zoopagomycota (Li et al. 2021). In the present study, we included the largest collection of zygomycete genomes to date and employed the newly released 758 “fungi_odb10” markers. The results suggested that *Basidiobolus* formed their own group within Zoopagomycota as the earliest diverging lineage (with 100/100 bootstrap, Supplementary Figure 1). The complexity of *Basidiobolus* is also evidenced by their enriched secondary metabolite genes, regionally duplicated genomes, and broad range of potential hosts including insects, amphibians, reptiles, and human beings (Henk and Fisher 2012; Tabima et al. 2020). This may explain the sources of phylogenetic conundrums that we have encountered in the last decades using different molecular markers. The natural history of *Basidiobolus* may not be easily resolved until an appropriate approach can be carried out to parse their complex genome composed of redundant genes from various sources, such as large-scale gene duplications or horizontal gene transfers. In addition, the kingdom-wide comparison helped discover many unique genome

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546 components in *Basidiobolus*, including the ones featured in Mucoromycota clades (e.g., CotH
547 and MMPL), which will be discussed in the following sections.
548
549 *Divergent evolution of zygomycete fungi*

550 We were able to identify different sets of gene content and Pfam domains favored by
551 each of the zygomycete phyla, which well correspond to their disparate lifestyles (Figs. 3 & 4).
552 As suggested by the presence of both Pil1 and SUR7 domains, eisosome-mediated endocytosis
553 and related active transportation are important facilitators to saprotrophic Mucoromycota
554 fungi (Walther et al. 2006). Among the 70 Mucoromycota-featured domains (Fig. 4 and
555 Supplementary Table 3), DENN, uDENN, and dDENN also serve as regulators during eukaryotic
556 membrane trafficking events (Zhang et al. 2012). This implies that Mucoromycota fungi are able
557 to transport particles via membrane trafficking domains, while Zoopagomycota fungi, as
558 animal-associated microbes, may use different mechanisms. Noteworthy, the Pox_ser-thr_kin,
559 a poxvirus serine/threonine protein kinase, specifically identified in Mucoromycota genomes
560 (Fig. 4 and Supplementary Table 3) indicates that large DNA viruses are embedded in
561 Mucoromycota genomes (Jacob et al. 2011). Mycoviruses have been extensively studied in
562 Dikarya fungi, especially for plant pathogens (Ghabrial et al. 2015; Marzano et al. 2016). The
563 existence of mycoviruses among early-diverging fungi have not been examined until recently,
564 which led to the discovery of a fungal–bacterial-viral system in the plant pathogenic *Rhizopus*
565 *microsporus* (Espino-Vázquez et al. 2020) and RNA mycoviruses in roughly one fifth laboratory
566 cultures of early diverging fungal lineages (Myers et al. 2020). Our preliminary analyses suggest
567 that Mucoromycota members contain genomic hallmarks that interact with both bacteria

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(MMPL domain, Fig. 2e) and viruses (Pox_ser-thr_kin domain, Supplementary Table 3). The "mycobacterial membrane protein large transporter" domain is well represented in all three subphyla of Mucoromycota as well as *Basidiobolus* (Fig. 2e) with the fact that fungal-bacterial interactions have been well documented in these lineages (Uehling et al. 2017; Desirò et al. 2018; Chang et al. 2019; Bonfante and Venice 2020; Tabima et al. 2020). Although the TLD domain universally presents in almost all fungal lineages (except *Wallemia ichthyophaga*), the exceptionally large number of TLD domains identified in Glomeromycotina members are unusual (Fig. 2c). It implies that TLD and related oxidation resistance proteins serve important functions to protect these arbuscular mycorrhizal fungi from reactive oxygen species (Blaise et al. 2012).

Zoopagomycota, on the other hand, do not have exclusive Pfam domains, even though many domains are highly enriched suggesting important functions. Tyrosinase is a good example. Tyrosinase domains have well-recognized functions to synthesize melanin via the amino acid L-tyrosine in melanosomes. Melanin is important to protect organisms from diverse biotic and abiotic factors, including helping microbes counteract the attacks from host immune systems, specifically by neutralizing reactive oxygen species or other harmful molecules (Cordero and Casadevall 2020). As such, it is not surprising to find that Zoopagomycota fungi, especially the insect-associated ones, maintain a large number of melanin synthetic enzymes presumably helping them evade host immune responses. Trypsin is another Pfam domain featured in Zoopagomycota (Fig. 4). Trypsin domains catalyze the hydrolysis of peptide bonds and break proteins into smaller pieces, which is extremely active in digestive systems. We discovered up to 59 copies (in *Smittium culicis*) of Trypsin domain in the insect gut-dwelling

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fungi (Harpellales, Kickxellomycotina). Interestingly, insect pathogenic species in Entomophthoromycotina were found heavily relying on hydrolases with 204 copies of Trypsin domains in *Zoophthora radicans* alone (43-138 copies in other Entomophthoromycotina members), while other zygomycete lineages maintain 0-18 copies variously (Fig. 5e). Trypsin and Trypsin-like proteases have been studied in insects and entomopathogenic fungi for decades (Paterson et al. 1993; Dubovenko et al. 2010; Lazarević and Janković-Tomanić 2015). Results suggest that the Trypsin and Trypsin-like proteins are important for nutritional uptake and pathogenic processes of insect-associated fungi, which was also suggested with the potential to help develop new agents to control pest insects (Borges-Veloso et al. 2015; Lazarević and Janković-Tomanić 2015). The abundance of Trypsin domains identified in Zoopagomycota suggests that the expansion of Trypsin across fungal tree of life have occurred more than once (e.g., Ascomycota and Zoopagomycota) (Dubovenko et al. 2010). In addition, the emergence and detailed evolutionary patterns of Trypsin and Trypsin-like proteins in Ascomycota, Zoopagomycota, and insects deserve further examination. Many polysaccharides and protein degrading enzymes were also found expanded in Zoopagomycota, such as LPMO_10, Glyco_hydro_72 (PF03198), and Peptidase_M36 (PF02128) (Fig. 4), suggesting their important functions during the interactions of Zoopagomycota fungi with small animals or other fungi. The fungalysin metallopeptidase (Peptidase_M36) and the associated fungalysin/thermolysin propeptide motif (FTP, PF07504) were both found expanded in the obligate mycoparasite *Syncephalis* (Lazarus et al. 2017). Both domains may help mycoparasites inhibit peptidases produced by the hosts, but their exact function has not been clearly known (Markaryan et al. 1996; Finn et al. 2016). Interestingly, the BATS domain involved in the biotin

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and thiamin synthesis is found absent in Kickxellomycotina and Zoopagomycotina members (Fig. 2d). Both subphyla are short for available cultures, which is especially the case for the animal associated species. The inability to synthesize biotin and thiamin may be one of the culprits for the unsuccessful culture establishment in the lab. Supplementary biotin and thiamin could be suggested for future efforts on development of new cultures in these fungal lineages.

Human infectious diseases caused by zygomycete fungi

Mucormycosis is a deadly human-infectious disease usually caused by *Rhizopus, Mucor,* and *Lichtheimia*. The current COVID-19 pandemic has triggered multiple cases of Mucormycosis in susceptible patients (Garg et al. 2021; Revannavar et al. 2021). The CotH was originally identified in bacteria as a spore-coat protein. It was later found in Mucorales fungi and identified as a potential invasin factor of the human-infectious Mucormycosis. The CotH was suggested to be directly involved in interactions between Mucorales pathogens and human endothelial cells (Chibucos et al. 2016). Our comparative genomic analyses provided a broader survey of CotH leading to discoveries of novel CotH families in Mucoromycotina strains and unexpected fungal lineages (*Basidiobolus*, Mortierellomycotina, and Neocallimastigomycota). CotH was maintained by almost every member of Mucoromycotina except the early-diverging taxa—*Calcarisporiella thermophila* and *Bifiguratus adelaidae*. Unexpectedly, all members of Mortierellomycotina were also able to code CotH domains with the same or highly similar pathogenic motif “MGQTNDGAYRDPTDNN”, which was proposed as a key factor for Mucormycosis. This implies that the included Mortierellomycotina taxa (*Dissophora ornate*, *Lobosporangium transversle*, and *Mortierella* species) may be facultative pathogens or have the

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potential to cause Mucormycosis or related human-infectious diseases if treated without caution. The results are informative to guide clinical practice as Mucormycosis may arise from many previously less documented situations, including the injuries during the natural disasters, unconscious contact, and triggered by other diseases like Novel Coronavirus Pneumonia (caused by COVID-19) (Neblett Fanfair et al. 2012; Revannavar et al. 2021). *Basidiobolus* is the only Zoopagomycota member that encodes CotH, albeit the copy number is low. On the other hand, Neocallimastigomycota members produce surprisingly high numbers of CotH domains with the largest duplication event (Fig. 6b). It is not clear why anaerobic gut fungi maintain so many CotH copies since they serve as primary plant polysaccharide degraders and do not pose any identifiable harm to their mammal hosts. Phylogenetic analyses suggest that CotH domains in fungi can be classified into at least seven major groups (ZyGo-A, B, C, D, and three AGF groups; Fig. 6a). The ZyGo-A is the only clade containing all known Mucormycosis invasin factors (i.e., CotH 2 and CotH 3) where Mortierellomycotina members are tightly clustered (Chibucos et al. 2016). The members in ZyGo-A, Mucoromycotina and Mortierellomycotina, should both have the potential to cause Mucormycosis.

There are additional emerging pathogens in Zoopagomycota. For example, members of the entomophthoralean fungi can cause infection in both insects and mammals, not only in immunocomprised patients, but also reported from immunocompetent individuals due to insect bites or other undetermined environmental contacts, especially in tropical and subtropical regions (Vilela and Mendoza 2018). *Basidiobolus* and *Conidiobolus* are two additional agents of human skin, subcutaneous, and gastrointestinal infections (Khan et al. 2001; Shaikh et al. 2016). *Basidiobolus* can be isolated from various types of environments,

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including soils or leaf litters, dung of frogs or lizards, and various insects (e.g., mosquitoes, mites, springtails) (Lyon et al. 2001; Garros et al. 2008; Manning and Callaghan 2008; Werner et al. 2012). Recently, people also found that *Basidiobolus* can infect human eyes (Tananuvat et al. 2018; Vilela and Mendoza 2018). The two CotH copies identified in *Basidiobolus* genomes may be involved in the pathogenic processes. *Conidiobolus*, however, do not maintain CotH copies, suggesting that *Conidiobolus* may take different strategies to infect mammalian hosts. Our comparative genomic analyses provided a broader view regarding the molecular mechanism of human-infectious zygomycete fungi. As the quick accumulation of genomic resources for this fungal lineage, a detailed natural history and complete pathogenic pathways should be revealed in the near future.

Our combination of phylogenomic and comparative genomic study of zygomycete fungi provided a fresh view of the phylogenetic relationships within the group. Multiple lineage-specific genome contents were revealed to help understand their cryptic ecology and relationships with other organisms in the environment. The unexpected findings of CotH in Mortierellomycotina, *Basidiobolus*, and Neocallimastigomycota proved the effectiveness of comparative genomics to predict biology of understudied organisms and the importance of fungal studies in the era of global climate change. The presented results may be applied to prevent further damage caused by the human-infectious Mucormycosis.

DATA AVAILABILITY

Assembled genomes and annotation files are available at JGI MycoCosm website. All genome accession numbers are listed in Table 1.

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ACKNOWLEDGEMENTS

This material is based upon work supported by the National Science Foundation (DEB-1441604 to JWS and DEB-1441715 to JES), The authors thank Drs. M. Catherine Aime, William J. Davies, Gunther Doehlemann, Toni Gabaldón, Timothy Y. James for permission to use genomes ahead of publication. The work (proposals 10.46936/10.25585/60001019 and 10.46936/10.25585/60001062) conducted by the U.S. Department of Energy Joint Genome Institute (https://ror.org/04xm1d337), a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy operated under Contract No. DE-AC02-05CH11231. JES is a paid consultant for Zymergen and Sincarne.

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FIGURE LEGEND:

Figure 1: **Phylogenetic relationships and genome statistics of zygomycete fungi.** (a) The maximum-likelihood tree was inferred from a phylogenomic dataset of 617 protein sequences identified in the included 181 genomes. Branches and tip labels of Mucoromycota and Zoopagomycota were colored in green and red separately. The bootstrap supports are indicated on each node relatively. Tracks from the inside to outside are mapped based on the BUSCO scores, protein-coding gene numbers, and genome size of included zygomycete fungi (detailed bootstrap values and branch lengths are shown in Supplementary Figure 1). (b) The density of protein-coding genes in each genome was plotted using genome sizes on the x-axis against the gene counts on the y-axis. Each dot was colored based on their phylogenetic placement shown in the legend.

Figure 2: **Phylogenetic backbone and highlighted genome content in zygomycete fungi.** Zygomycetes genomes that are well assembled (BUSCO score above 80%) and represent unique phylogenetic positions were selected to reconstruct the backbone phylogenomic tree. (a) The backbone phylogenomic tree of zygomycetes includes 80 taxa (rooted with *Drosophila melanogaster*). All bootstrap values (out of 100) were labeled on the branches. (b-e) Protein family domains found with striking patterns in zygomycete fungi are plotted with the copy numbers individually.

Figure 3: **Absence and presence of orthologous gene families across the Kingdom Fungi.** Orthologous gene families were examined in the genomes included in the backbone tree. The

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8,208 gene families were found present in at least 10 of the 80 taxa and thus included to examine the absence/presence pattern of genome content among different fungal lineages (a complete map showing the unfiltered 62,689 gene families was included in Supplementary Figure 2).

Figure 4: **Protein family (Pfam) domains with differentiated enrichment in Mucoromycota or Zoopagomycota.** Each dot represents a Pfam domain found in zygomycete fungi. The x-axis is the binary logarithm of the Pfam copy ratios between Zoopagomycota and Mucoromycota, and the y-axis is used to rank the Pfam domains in alphabetical order. The Pfam domains enriched in Mucoromycota are shown on the left side in cyan color, and the Zoopagomycota enriched ones are on the right side in red color. The bubbles (Pfam domains) with bigger sizes are shared by more zygomycetes members. The Pfam domains aligned on the left edge are domains only found in Mucoromycota and absent in Zoopagomycota. The domains discussed in the text were labeled with the Pfam name. A detailed chart including the names and ratios of all Pfam domains are also provided (Supplementary Table 3).

Figure 5: **Subphylum-level distribution of six Pfam domains that may contribute to the divergent evolution of zygomycete fungi.** The scales on each axis of the radar plots indicate the average copy number of the domain in each subphylum. (a-c) Pfam domains shared in all Mucoromycota subphyla and absent in the entire Zoopagomycota. (d-f) Distinct Pfam domains in Zoopagomycota subphyla and largely missing in Mucoromycota.

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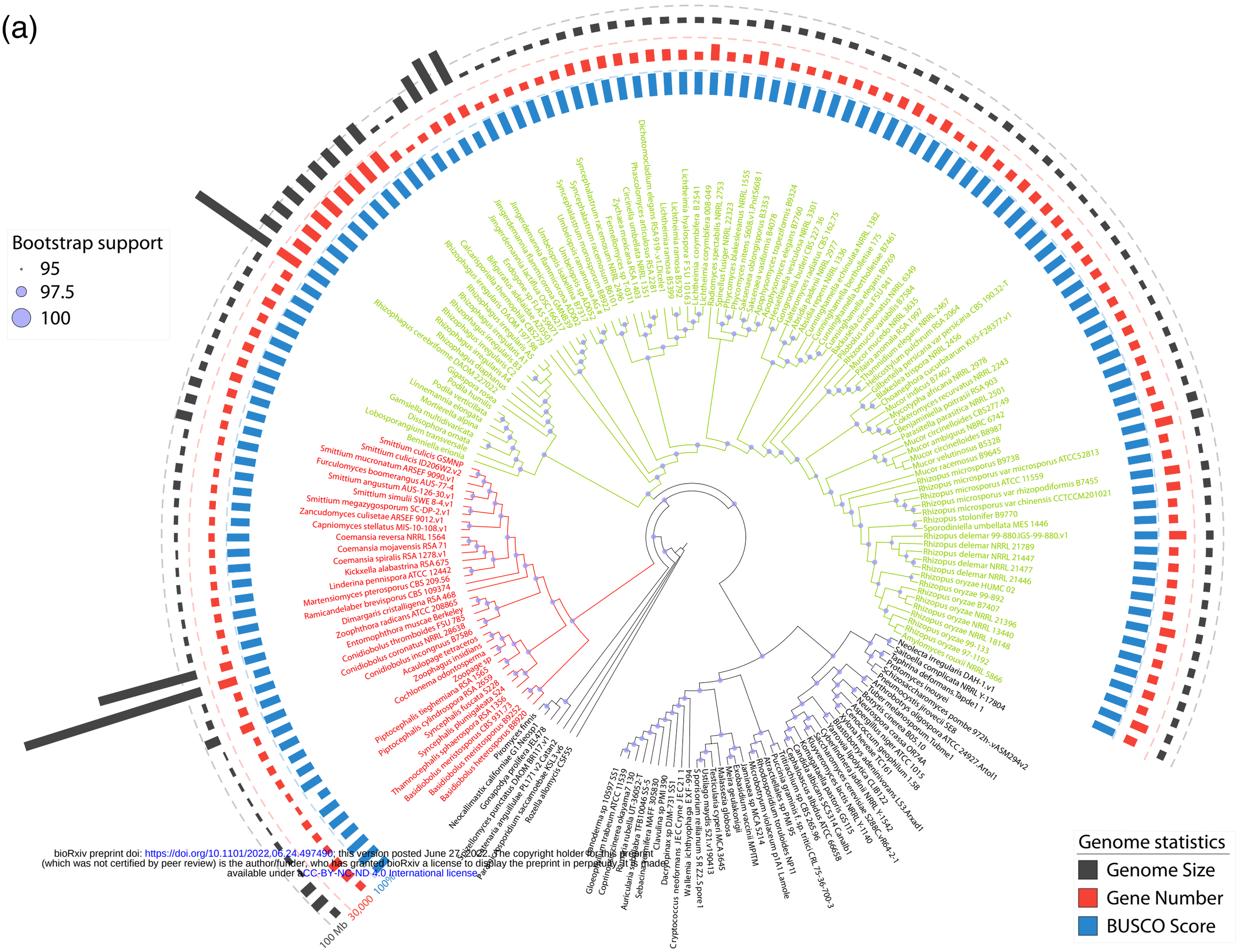
Figure 6: **Phylogenetic analysis and evolution of CotH in Kingdom Fungi.** (a) The 754 fungal CotH copies were identified from Mortierellomycotina (brown), Mucoromycotina (black), *Basidiobolus* (blue), and Neocallimastigomycota (red). The CotH phylogenetic tree was midpoint rooted and reconstructed using the maximum likelihood method with bootstrap supports (out of 100) labeled on each branch. The analysis included previously classified CotH families 1-5 (pink) to help categorize newly identified fungal CotH. (b) Reconstruction of CotH evolution in Kingdom Fungi with Notung. CotH copies identified in each genome were plotted at tree tips with proportional sizes. Nodes with more than 10 duplication events were highlighted with red bubbles and labelled with duplication (“D”) and loss (“L”) events. Node abbreviation: Muco, Mucoromycotina; Mort, Mortierellomycotina; Zoop, Zoopagomycotina.

(a)



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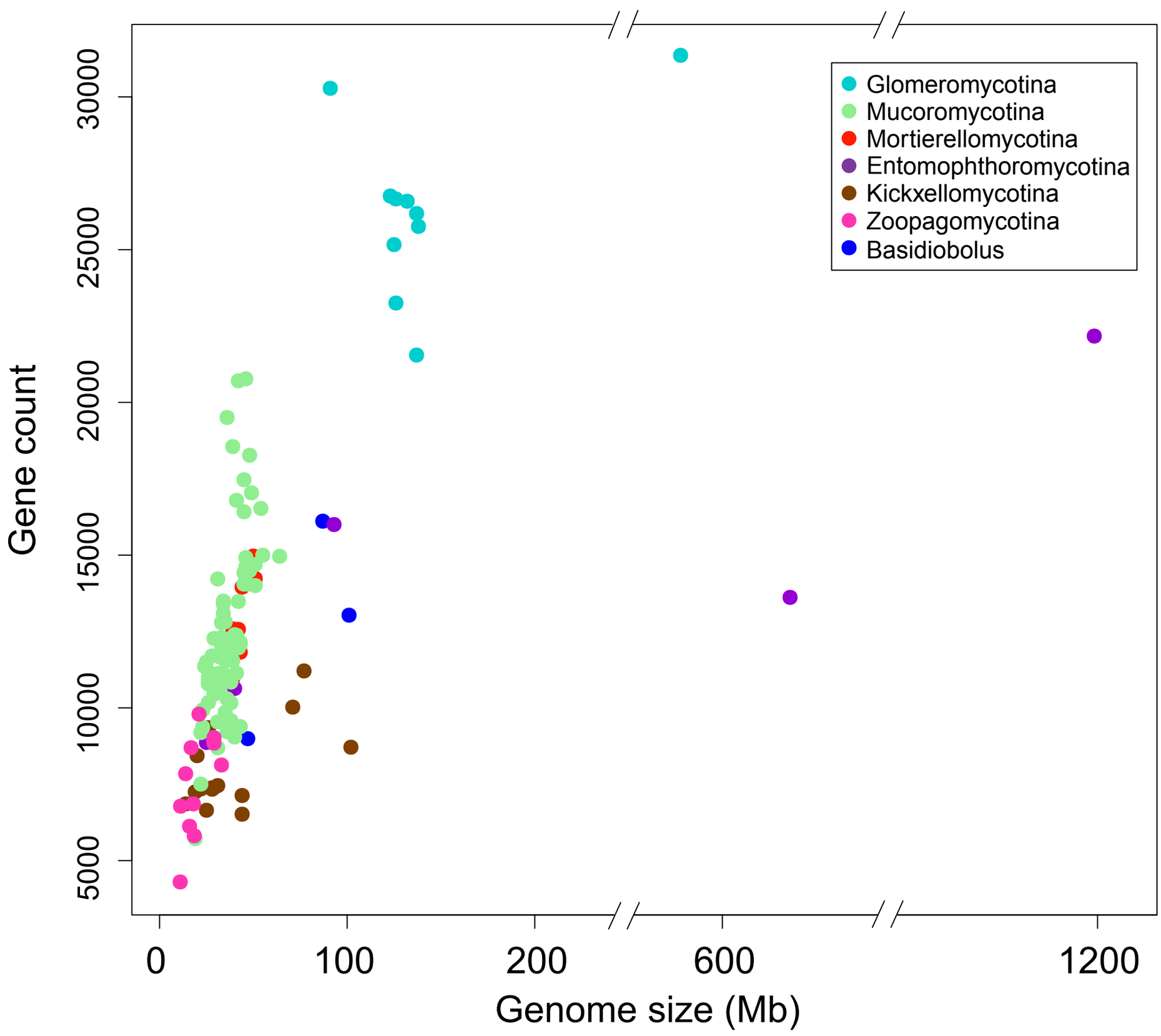
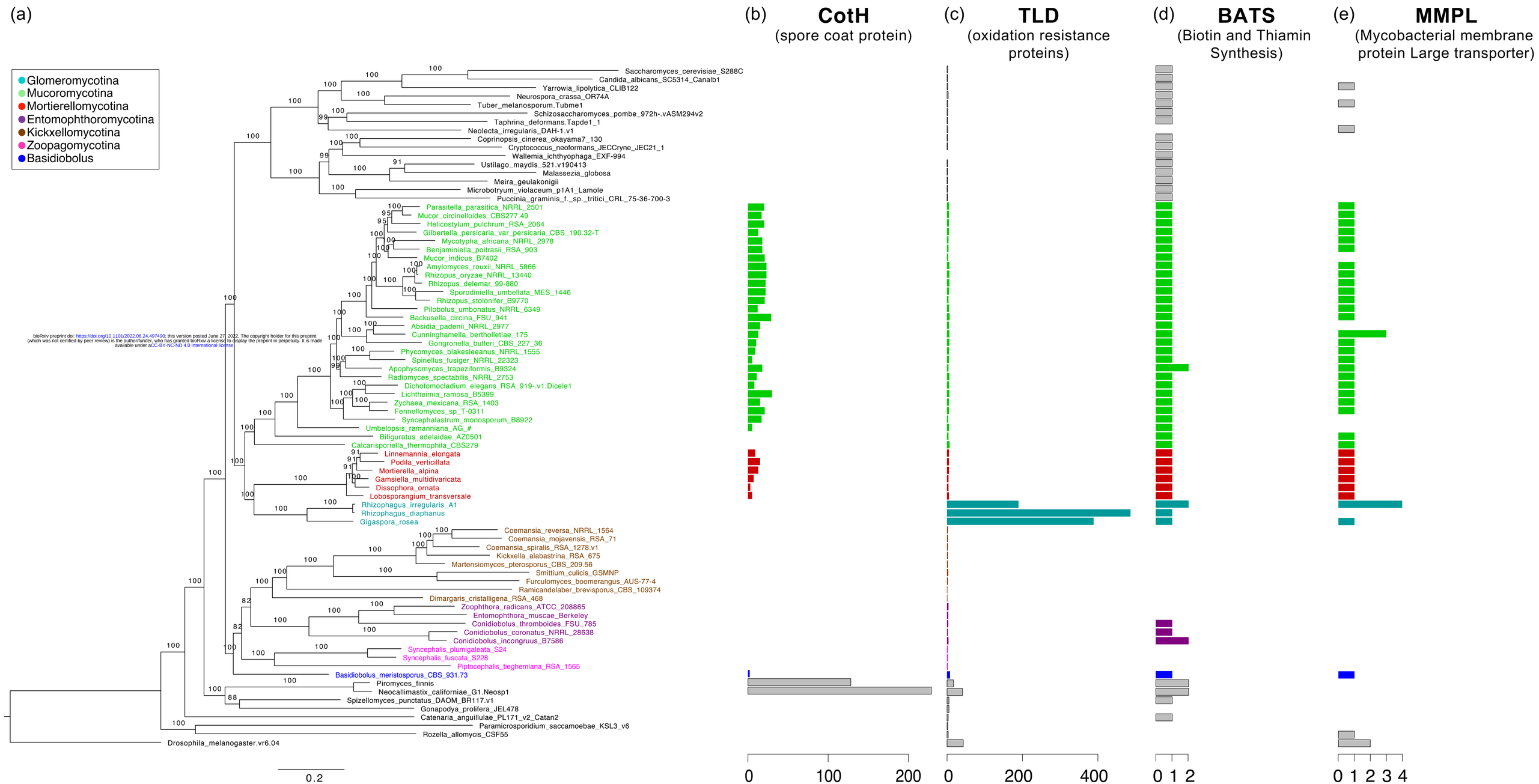
(b)





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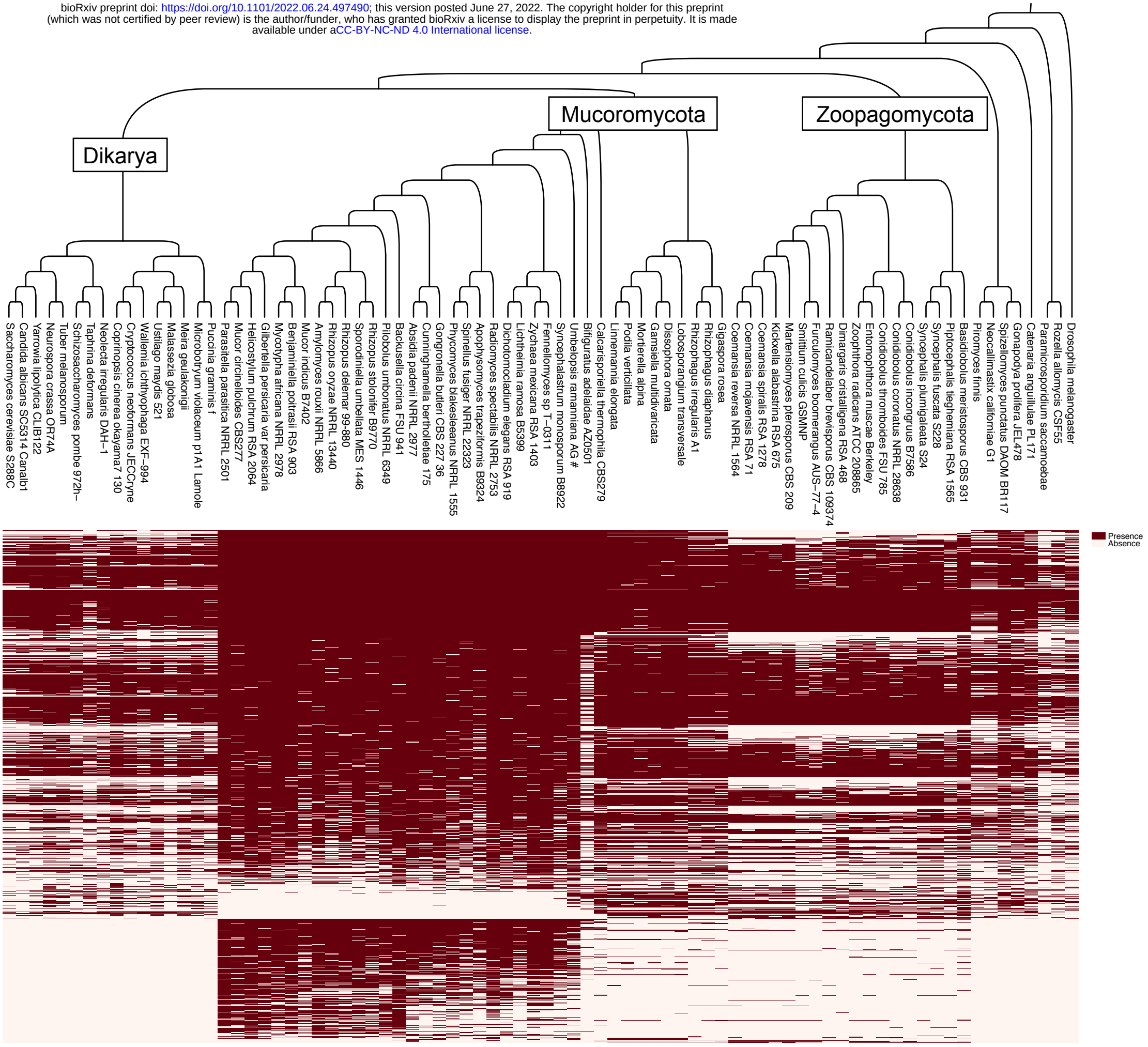
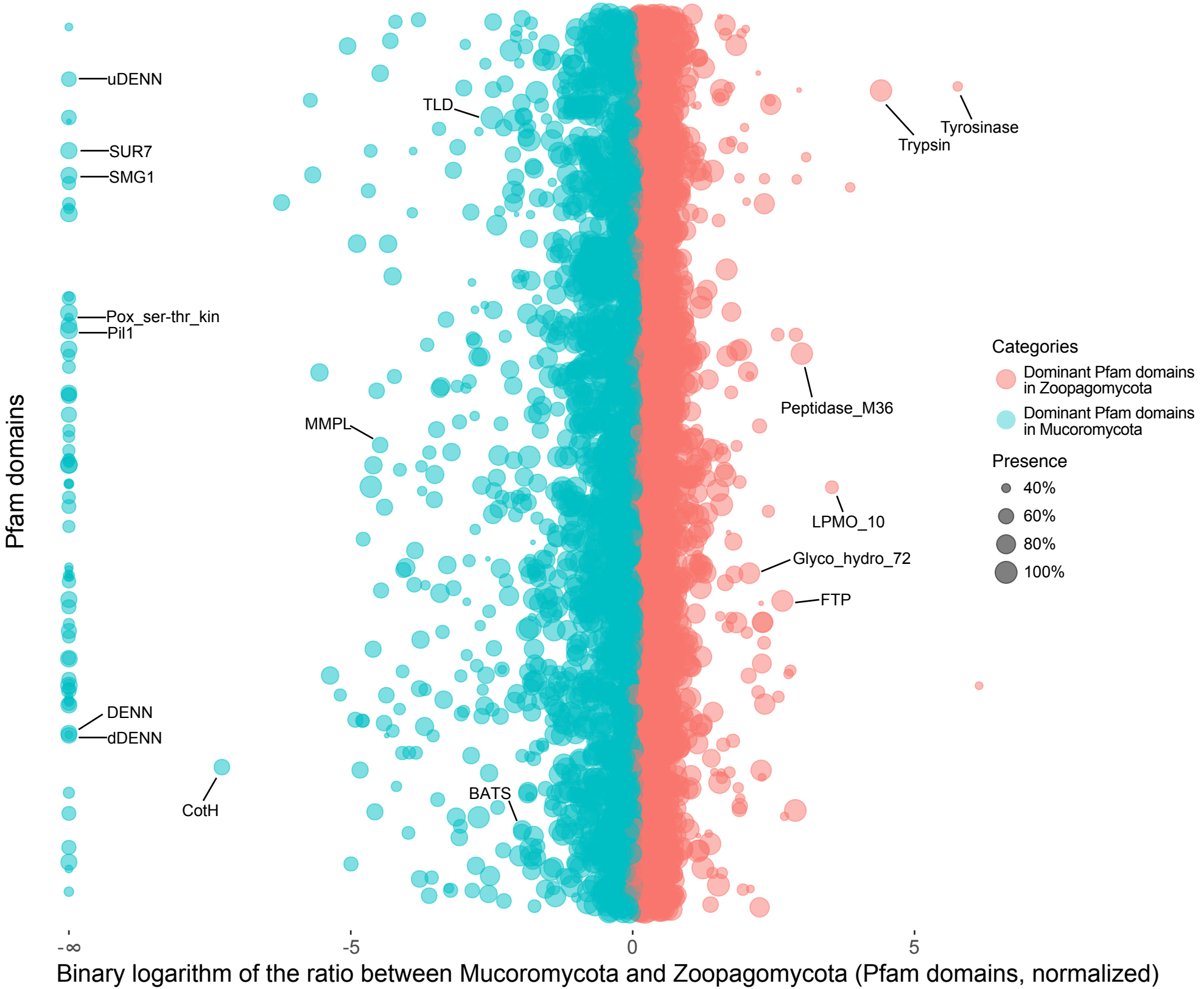
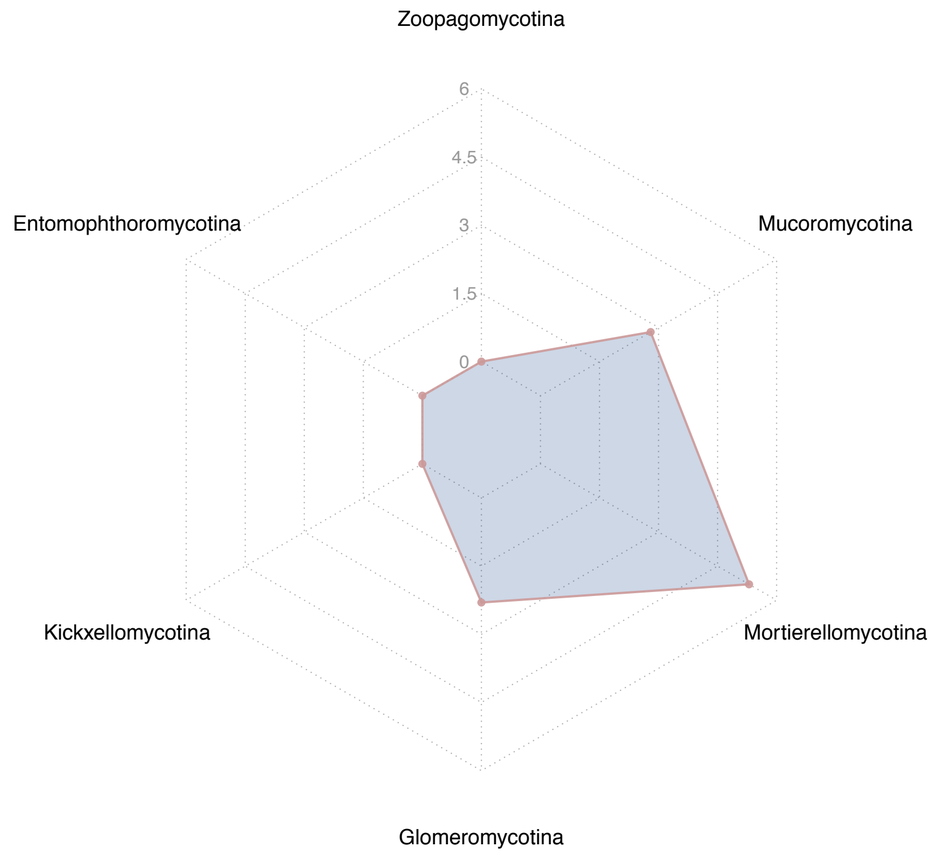
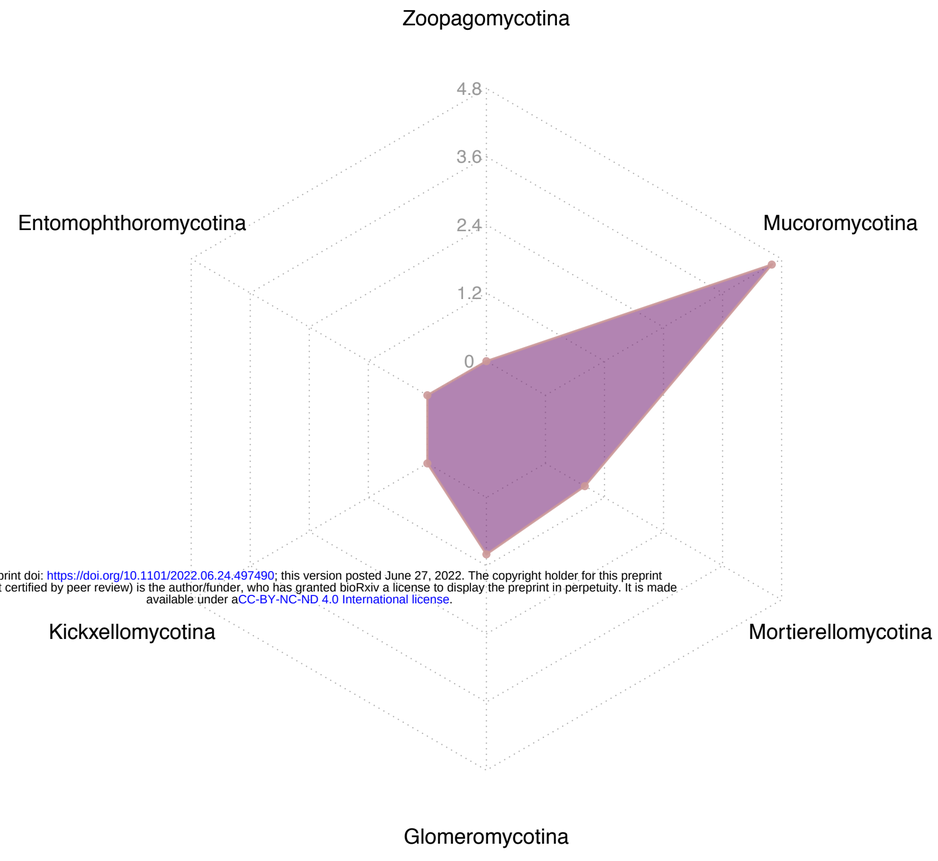


Absence and presence of orthologous gene families across the Kingdom Fungi (partial)

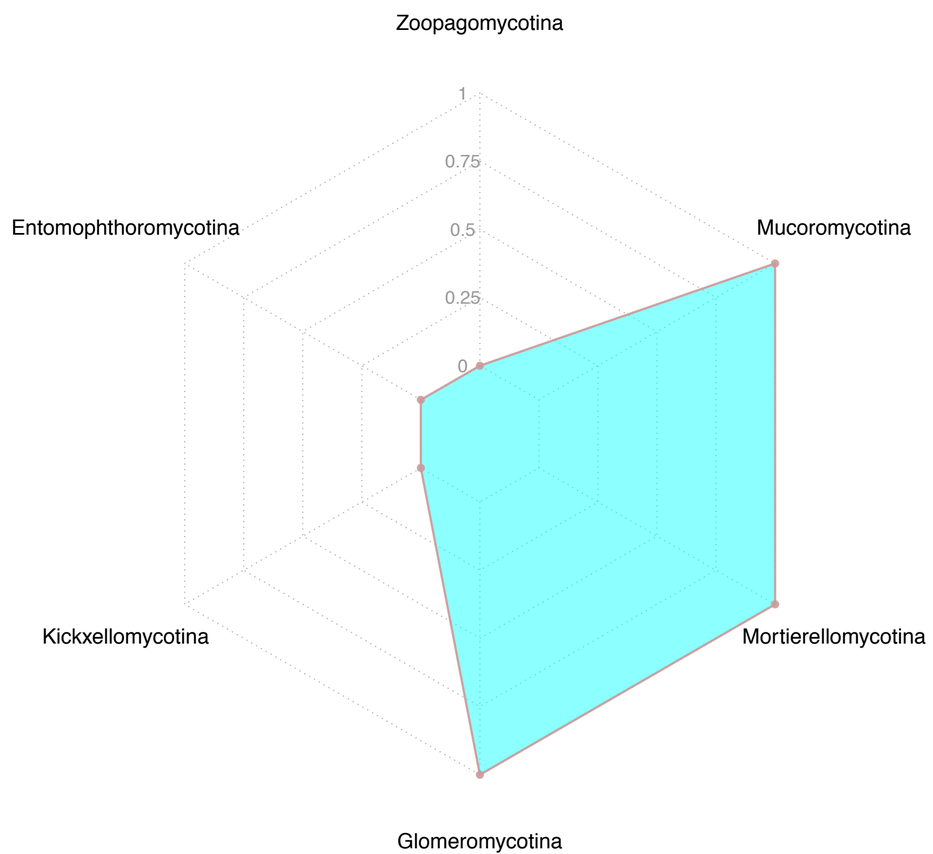

uDENN
SUR7
SMG1
Pox_ser-thr_kin
Pil1
DENN
dDENN
CotH
TLD
MMPL
BATS
Trypsin
Tyrosinase
Peptidase_M36
LPMO_10
Glyco_hydro_72
FTP
Pfam domains
-∞
-5
0
5
Binary logarithm of the ratio between Mucoromycota and Zoopagomycota (Pfam domains, normalized)
Categories
Dominant Pfam domains in Zoopagomycota
Dominant Pfam domains in Mucoromycota
Presence
40%
60%
80%
100%



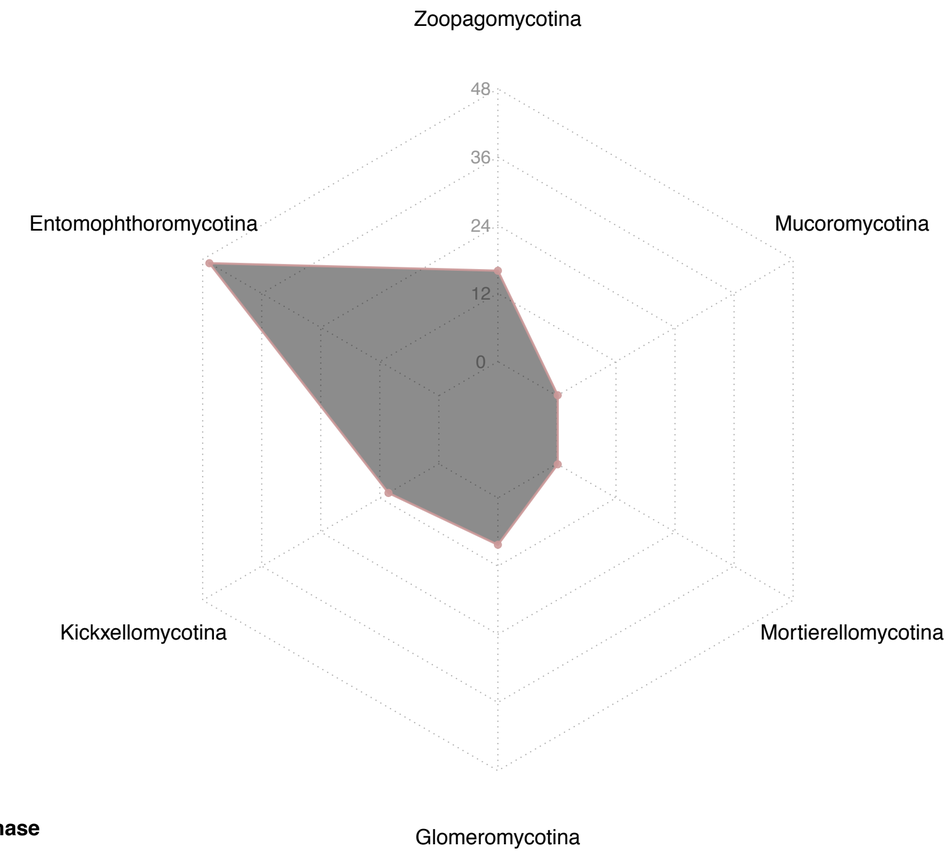
(a) Pil1



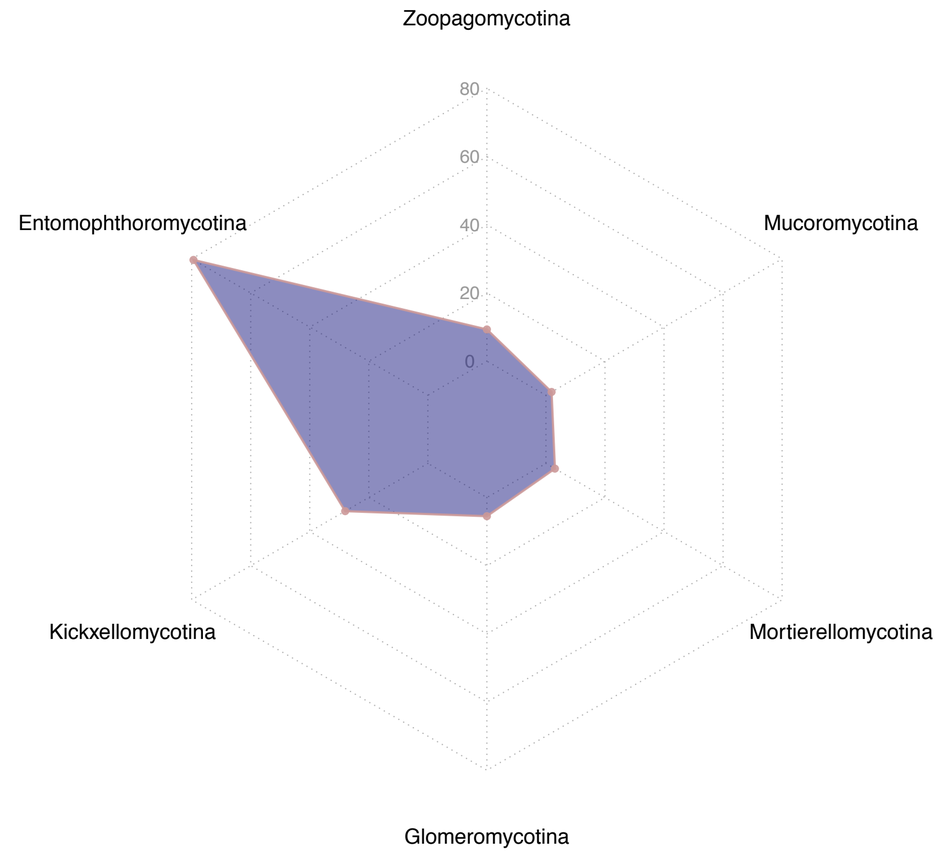
(b) SUR7



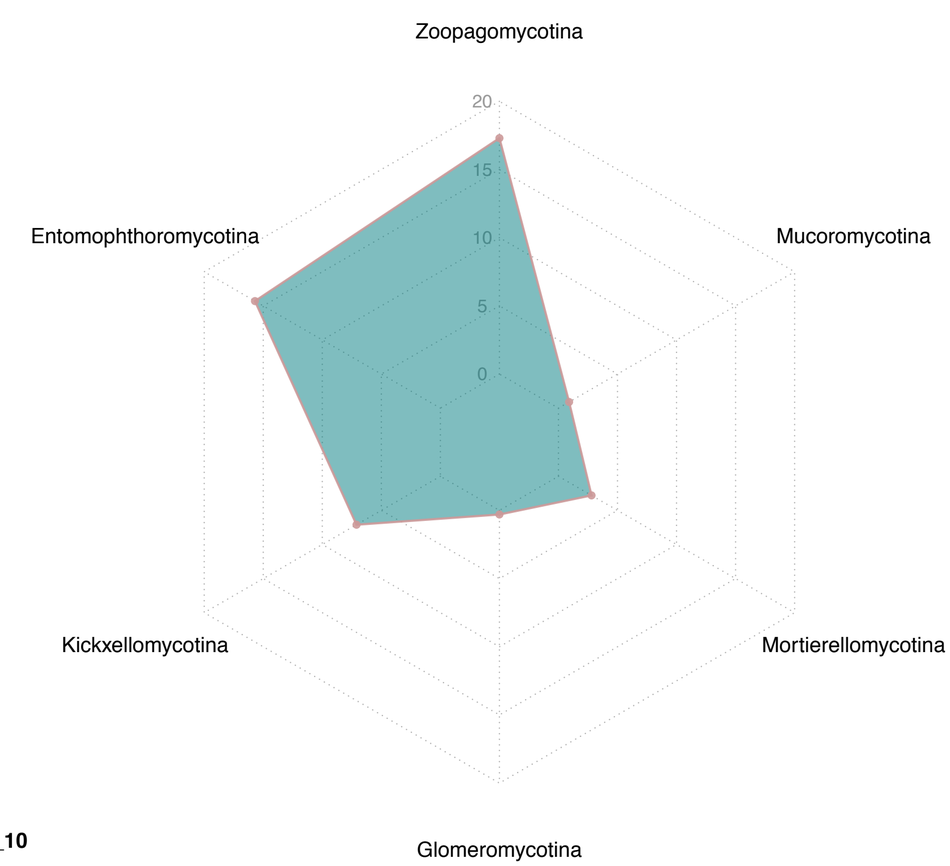
(c) SMG1



(d) Tyrosinase



(e) Trypsin



(f) LPMO_10

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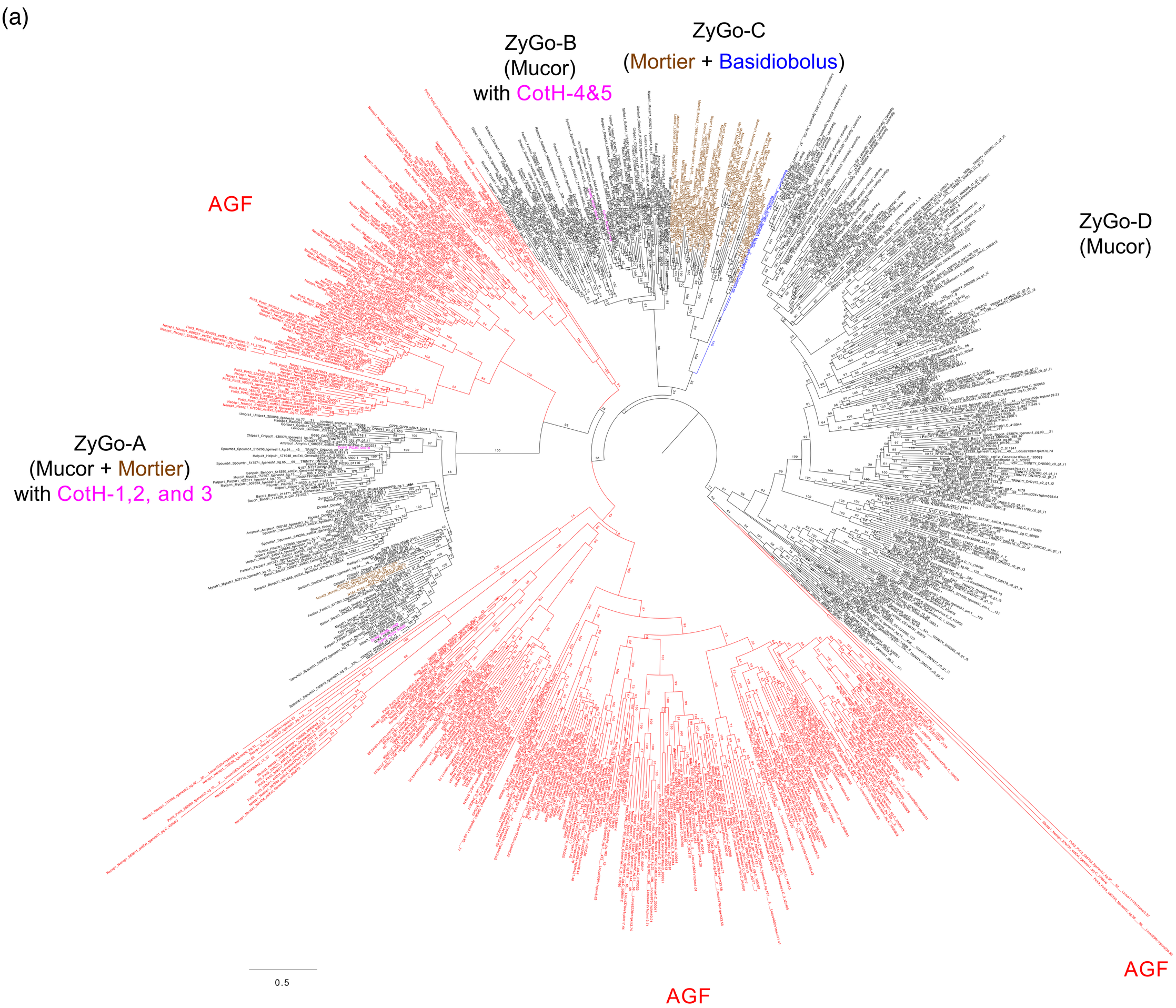
(a)


ZyGo-B
(Mucor)
with CotH-4&5
ZyGo-C
(Mortier + Basidiobolus)
AGF
ZyGo-D
(Mucor)
ZyGo-A
(Mucor + Mortier)
with CotH-1,2, and 3
0.5
AGF
AGF

(b)

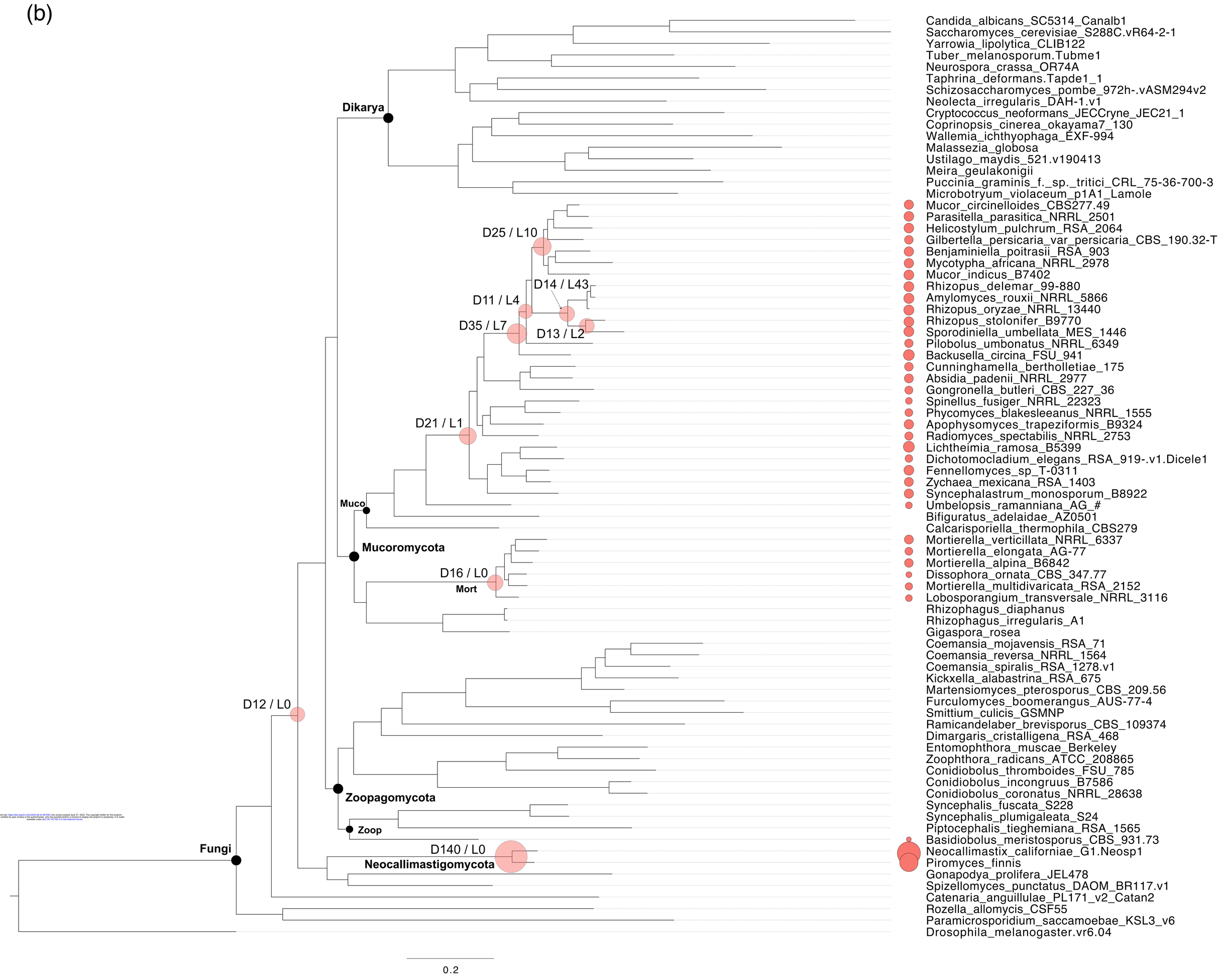

Dikarya
D25 / L10
D14 / L43
D11 / L4
D35 / L7
D13 / L2
D21 / L1
Muco
Mucoromycota
D16 / L0
Mort
D12 / L0
Zoopagomycota
Zoop
Fungi
D140 / L0
Neocallimastigomycota
0.2
Candida_albicans_SC5314_Canalb1
Saccharomyces_cerevisiae_S288C.vR64-2-1
Yarrowia_lipolytica_CLIB122
Tuber_melanosporum.Tubme1
Neurospora_crassa_OR74A
Taphrina_deformans.Tapde1_1
Schizosaccharomyces_pombe_972h-.vASM294v2
Neolecta_irregularis_DAH-1.v1
Cryptococcus_neoformans_JECCryne_JEC21_1
Coprinopsis_cinerea_okayama7_130
Wallemia_ichthyophaga_EXF-994
Malassezia_globosa
Ustilago_maydis_521.v190413
Meira_geulakonigii
Puccinia_graminis_f._sp._tritici_CRL_75-36-700-3
Microbotryum_violaceum_p1A1_Lamole
Mucor_circinelloides_CBS277.49
Parasitella_parasitica_NRRL_2501
Helicostylum_pulchrum_RSA_2064
Gilbertella_persicaria_var_persicaria_CBS_190.32-T
Benjaminiella_poitrasii_RSA_903
Mycotypha_africana_NRRL_2978
Mucor_indicus_B7402
Rhizopus_delemar_99-880
Amylomyces_rouxii_NRRL_5866
Rhizopus_oryzae_NRRL_13440
Rhizopus_stolonifer_B9770
Sporodiniella_umbellata_MES_1446
Pilobolus_umbonatus_NRRL_6349
Backusella_circina_FSU_941
Cunninghamella_bertholletiae_175
Absidia_padenii_NRRL_2977
Gongronella_butleri_CBS_227_36
Spinellus_fusiger_NRRL_22323
Phycomyces_blakesleeanus_NRRL_1555
Apophysomyces_trapeziformis_B9324
Radiomyces_spectabilis_NRRL_2753
Lichtheimia_ramosa_B5399
Dichotomocladium_elegans_RSA_919-.v1.Dicele1
Fennellomyces_sp_T-0311
Zychaea_mexicana_RSA_1403
Syncephalastrum_monosporum_B8922
Umbelopsis_ramanniana_AG_#
Bifiguratus_adelaidae_AZ0501
Calcarisporiella_thermophila_CBS279
Mortierella_verticillata_NRRL_6337
Mortierella_elongata_AG-77
Mortierella_alpina_B6842
Dissophora_ornata_CBS_347.77
Mortierella_multidivaricata_RSA_2152
Lobosporangium_transversale_NRRL_3116
Rhizophagus_diaphanus
Rhizophagus_irregularis_A1
Gigaspora_rosea
Coemansia_mojavensis_RSA_71
Coemansia_reversa_NRRL_1564
Coemansia_spiralis_RSA_1278.v1
Kickxella_alabastrina_RSA_675
Martensiomyces_pterosporus_CBS_209.56
Furculomyces_boomerangus_AUS-77-4
Smittium_culicis_GSMNP
Ramicandelaber_brevisporus_CBS_109374
Dimargaris_cristalligena_RSA_468
Entomophthora_muscae_Berkeley
Zoophthora_radicans_ATCC_208865
Conidiobolus_thromboides_FSU_785
Conidiobolus_incongruus_B7586
Conidiobolus_coronatus_NRRL_28638
Syncephalis_fuscata_S228
Syncephalis_plumigaleata_S24
Piptocephalis_tieghemiana_RSA_1565
Basidiobolus_meristosporus_CBS_931.73
Neocallimastix_californiae_G1.Neosp1
Piromyces_finnis
Gonapodya_prolifera_JEL478
Spizellomyces_punctatus_DAOM_BR117.v1
Catenaria_anguillulae_PL171_v2_Catan2
Rozella_allomycis_CSF55
Paramicrosporidium_saccamoebae_KSL3_v6
Drosophila_melanogaster.vr6.04

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Table 1. List of 131 zygomycete fungi included in the current phylogenetic and comparative genomics study

Phylum	Subphylum	Species	BUSCO Score (%)	Genome Size (Mb)	Gene Count	Source	Accession/WebPortal	Reference
Mucoromycota	Mucoromycotina	Absidia_padenii_NRRL_2977	95.5	34	13495	JGI	https://mycocosm.jgi.doe.gov/Chlpad1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Absidia_repens_NRRL_1336	95.9	46	14919	JGI	MCGE00000000.1	Mondo SJ et al., 2017
Mucoromycota	Mucoromycotina	Amylomyces_rouxii_NRRL_5866	95.5	45	14619	JGI	https://mycocosm.jgi.doe.gov/Amyrou1/	Unpublished & use with permission
Mucoromycota	Mucoromycotina	Apophysomyces_elegans_B7760	95.9	38	9584	UMD	JNDQ00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Apophysomyces_trapeziformis_B9324	96.5	35	9542	UMD	JNDP00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Backusella_circina_FSU_941	93.8	49	17039	JGI	https://mycocosm.jgi.doe.gov/Bacci1/	Amses et al. (in review)
Mucoromycota	Mucoromycotina	Benjaminiella_poitrasii_RSA_903	97.9	32.5	11659	JGI	JAMKHW000000000.1	This study
Mucoromycota	Mucoromycotina	Bifiguratus_adelaidae_AZ0501	96.2	19	5719	GenBank	MVBO00000000.1	Torres-Cruz, T. J. et al. 2017
Mucoromycota	Mucoromycotina	Blakeslea_trispora_NRRL_2456	93.1	38	11687	JGI	https://mycocosm.jgi.doe.gov/Blatri1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Calcarisporiella_thermophila_CBS279.70	94.8	28	11703	JGI	https://gb.fungalgenomics.ca/portal/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Choanephora_cucurbitarum_NRRL2744_50	93.8	35	11534	JGI	https://mycocosm.jgi.doe.gov/Chocucu1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Circinella_umbellata_NRRL_1351	95.8	51	14693	JGI	https://mycocosm.jgi.doe.gov/Cirumb1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Cokeromyces_recurvatus_NRRL_2243.0	93.8	28	10916	JGI	https://mycocosm.jgi.doe.gov/Cokrec1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Cunninghamella_bertholletiae_175	94.8	31	14220	UMD	JNEG00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Cunninghamella_bertholletiae_B7461	94.5	31	9542	UMD	JNEL00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Cunninghamella_echinulata_NRRL_1382	91.1	29	10443	JGI	https://mycocosm.jgi.doe.gov/Cunech1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Cunninghamella_elegans_B9769	94.2	31	8693	UMD	JNDR00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Dichotomocladium_elegans_RSA_919	91.4	40	12396	JGI	https://mycocosm.jgi.doe.gov/Dicele1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Endogone_sp_FLAS_59071	77.3	95.6	9366	JGI	RBNK00000000.1	Chang Y et al., 2019
Mucoromycota	Mucoromycotina	Fennellomyces_sp_T-0311	95.9	46	14128	JGI	JALLLT000000000.1	This study
Mucoromycota	Mucoromycotina	Gilbertella_persicaria_var._persicaria_CBS_190.32-T	95.2	26	10992	JGI	https://mycocosm.jgi.doe.gov/Gilper1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Gongronella_butleri	93.4	33	11004	JGI	https://mycocosm.jgi.doe.gov/Gonbut1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Halteromyces_radiatus_CBS_162.75	95.5	26	10175	JGI	https://mycocosm.jgi.doe.go	Chang et al. (in review)

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							v/Halrad1/	
Mucoromycota	Mucoromycotina	Helicostylum_pulchrum_RSA_2064	97.3	33	12794	JGI	https://mycocosm.jgi.doe.gov/Helpul1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Hesseltinella_vesiculosa_NRRL_3301	94.4	27	11141	JGI	http://genome.jgi.doe.gov/Hesve2finisherSC	Mondo SJ et al., 2017
Mucoromycota	Mucoromycotina	Jimgerdemannia_flammicorona_GMNB39	72.1	239.6	13535	JGI	RBNJ00000000.1	Chang Y et al., 2019
Mucoromycota	Mucoromycotina	Jimgerdemannia_lactiflua_OSC166217	59.7	179.7	12651	JGI	RBNI00000000.1	Chang Y et al., 2019
Mucoromycota	Mucoromycotina	Jimgerdemmania_flammicorona_AD002	66.9	231.3	13726	JGI	RQIL00000000.1	Chang Y et al., 2019
Mucoromycota	Mucoromycotina	Lichtheimia_corymbifera_008-049	94.1	36	19506	UMD	JNEE01000000.1	Chibucos MC et al., 2015
Mucoromycota	Mucoromycotina	Lichtheimia_corymbifera_B2541	94.8	36	9607	UMD	JNEU00000000.1	Chibucos MC et al., 2015
Mucoromycota	Mucoromycotina	Lichtheimia_hyalospora_FSU_10163	95.1	33	12062	JGI	PTRB00000000.1	This study
Mucoromycota	Mucoromycotina	Lichtheimia_ramosa_B5399	95.1	45	14426	UMD	JNDO00000000.1	Chibucos MC et al., 2015
Mucoromycota	Mucoromycotina	Lichtheimia_ramosa_B5792	94.9	42	13483	UMD	JNEP00000000.1	Chibucos MC et al., 2015
Mucoromycota	Mucoromycotina	Mucor_ambiguus_NBRC_6742	97.2	40.7	11343	GenBank	BBKB00000000.1	Lebreton et al., 2020
Mucoromycota	Mucoromycotina	Mucor_circinelloides_B8987	98.3	36	10295	UMD	JNDM00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Mucor_circinelloides_CBS277.49	97.3	36	11719	JGI	https://mycocosm.jgi.doe.gov/Muccir1_3/	Navarro-Mendoza et al., 2019
Mucoromycota	Mucoromycotina	Mucor_indicus_B7402	96.6	39	11544	UMD	JNEK00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Mucor_mucedo_NRRL_3635	96.9	45	14052	JGI	JALPTJ000000000.1	This study
Mucoromycota	Mucoromycotina	Mucor_racemosus_B9645	97.6	64	14961	UMD	JNEI00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Mucor_velutinosus_B5328	97.3	35	9855	UMD	JNDK00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Mycotypha_africana_NRRL_2978	94.5	29	10917	JGI	https://mycocosm.jgi.doe.gov/Mycafr1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Parasitella_parasitica	96.6	33	12306	JGI	JALLKT000000000.1	This study
Mucoromycota	Mucoromycotina	Phascolomyces_articulosus	95.2	48	14495	JGI	https://mycocosm.jgi.doe.gov/Phaart1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Phycomyces_blakesleeanus_NRRL_1555	94.5	54	16528	JGI	AMYC00000000.1	Corrochano LM et al., 2016
Mucoromycota	Mucoromycotina	Phycomyces_nitens_S608	92.7	38	11985	JGI	https://mycocosm.jgi.doe.gov/PnitS608_1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Pilaira_anomala_RSA1997	96.5	35	12817	JGI	https://mycocosm.jgi.doe.gov/Pilano1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Pilobolus_umbonatus_NRRL_6349	96.5	35	10983	JGI	https://mycocosm.jgi.doe.gov/Pilumb1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Radiomyces_spectabilis_NRRL_2753	95.2	30	10905	JGI	JALLLU000000000.1	This study
Mucoromycota	Mucoromycotina	Rhizomucor_variabilis_B7584	96.2	33	11811	UMD	JNES00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_delemar_99-880	96.2	46	20770	JGI	AACW00000000.2	Chibucos MC et al., 2016 & Ma LJ et al. 2009 (reannotated)

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Mucoromycota	Mucoromycotina	Rhizopus_delemar_NRRL_21446	93.5	38	12034	UMD	JNED00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_delemar_NRRL_21447	95.5	38	12216	UMD	JNEC00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_delemar_NRRL_21477	94.5	40	12044	UMD	JNEA00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_delemar_NRRL_21789	96.9	41	11140	UMD	JNDY00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_microsporus_ATCC_11559	96.5	24	11355	JGI	MCOJ00000000.1	Lastovetsky OA et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_microsporus_B9738.N156	98.6	75	20957	GenBank	JNEJ00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_microsporus_var_chinensis_CCTC CM201021	92.1	45	16419	JGI	ANKS00000000.1	Wang D et al., 2013
Mucoromycota	Mucoromycotina	Rhizopus_microsporus_var_rhizopodiformis _B7455	95.2	48	18269	UMD	JNER00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_microsporus_var_microsporus_A TCC52813	97.6	26	10905	JGI	MCGZ00000000.1	Mondo SJ et al., 2017
Mucoromycota	Mucoromycotina	Rhizopus_oryzae_97-1192	96.2	42	20706	UMD	JNEF00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_oryzae_99-133	96.6	41	12373	UMD	JNDX00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_oryzae_99-892	93.8	39	18552	UMD	JNEB00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_oryzae_B7407	96.2	43	12151	UMD	JNDL00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_oryzae_HUMC_02	95.1	40	12144	UMD	JNDZ00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_oryzae_NRRL_13440	96.6	43	12077	UMD	JNDU00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_oryzae_NRRL_18148	96.6	47	14051	UMD	JNDV00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_oryzae_NRRL_21396	96.3	42	11967	UMD	JNDW00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Rhizopus_stolonifer_B9770	95.2	38	10844	UMD	JNDS00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Saksenaea_oblongisporus_B3353	95.5	40	9047	UMD	JNEV00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Saksenaea_vasiformis_B4078	95.8	43	9387	UMD	JNDT00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Spinellus_fusiger_NRRL_22323	91.8	38	10164	JGI	JALLLS000000000.1	This study
Mucoromycota	Mucoromycotina	Sporodiniella_umbellata_MES_1446	92.4	26	10781	JGI	https://mycocosm.jgi.doe.go v/Spoumb1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Syncephalastrum_monosporum_B8922	96.2	29	8910	UMD	JNEN00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Syncephalastrum_racemosum_B6101	94.1	29	9019	UMD	JNDN00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Syncephalastrum_racemosum_NRRL_2496	95.2	31	11124	JGI	MCGN00000000.1	Mondo SJ et al., 2017
Mucoromycota	Mucoromycotina	Thamnidium_elegans	97.2	34	13104	JGI	https://mycocosm.jgi.doe.go v/Thaele1/	Chang et al. (in review)
Mucoromycota	Mucoromycotina	Umbelopsis_isabellina_B7317	95.2	22	7504	UMD	JNEQ00000000.1	Chibucos MC et al., 2016
Mucoromycota	Mucoromycotina	Umbelopsis_ramanniana_AG_#	97.3	23	9931	JGI	http://genome.jgi.doe.gov/ Umbra1	Amses et al. (in review)
Mucoromycota	Mucoromycotina	Umbelopsis_sp._nov._AD052	94.4	23	9369	JGI	https://mycocosm.jgi.doe.go v/Umbsp_AD052_1/	Chang et al. (in review)

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Mucoromycota	Mucoromycotina	Zychaea_mexicana_RSA_1403	94.1	51	14004	JGI	https://mycocosm.jgi.doe.gov/Zycmex1/	Chang et al. (in review)
Mucoromycota	Mortirellomycotina	Dissophora_ornata_CBS_347.77	95.2	39	12584	JGI	https://mycocosm.jgi.doe.gov/Disorn1/	Chang et al. (in review)
Mucoromycota	Mortirellomycotina	Lobosporangium_transversale_NRRL_3116	94.5	43	11822	JGI	MCFF00000000.1	Mondo SJ et al., 2017
Mucoromycota	Mortirellomycotina	Mortierella_alpina_B6842.N164	94.8	39	10977	UMD	AZCI00000000.1	Etienne KA et al., 2014
Mucoromycota	Mortirellomycotina	Linnemannia_elongata_AG-77	94.5	50	14969	JGI	LYLZ00000000.1	Uehling J et al., 2017
Mucoromycota	Mortirellomycotina	Podila_humilis_PMI_1414	86.5	36	12012	JGI	https://mycocosm.jgi.doe.gov/Morhum1/	Chang et al. (in review)
Mucoromycota	Mortirellomycotina	Gamsiella_multidivaricata_RSA_2152	94.1	38	12248	JGI	https://mycocosm.jgi.doe.gov/Mormul1/	Chang et al. (in review)
Mucoromycota	Mortirellomycotina	Benniella_erionia_GBAus27b	93.1	44	13953	JGI	https://mycocosm.jgi.doe.gov/MorGBAus27b_1/	Chang et al. (in review)
Mucoromycota	Mortirellomycotina	Podila_verticillata_NRRL_6337	95.1	42	12569	Broad Institute	AEVJ00000000.1	Vandepol et al., 2020
Mucoromycota	Glomeromycotina	Gigaspora_rosea	95.5	568	31291	JGI	QKWP00000000.1	Morin E et al., 2019
Mucoromycota	Glomeromycotina	Rhizophagus_cerebriforme_DAOM_227022	94.2	137	21549	JGI	QKYT00000000.1	Morin E et al., 2019
Mucoromycota	Glomeromycotina	Rhizophagus_diaphanus	94.1	126	23252	JGI	QZLH00000000.1	Morin E et al., 2019
Mucoromycota	Glomeromycotina	Rhizophagus_irregularis_A1	87.9	126	26659	JGI	LLXH00000000.1	Chen ECH et al., 2018
Mucoromycota	Glomeromycotina	Rhizophagus_irregularis_A4	87.2	138	25760	JGI	LLXI00000000.1	Chen ECH et al., 2018
Mucoromycota	Glomeromycotina	Rhizophagus_irregularis_A5	87.6	132	26585	JGI	LLXJ00000000.1	Chen ECH et al., 2018
Mucoromycota	Glomeromycotina	Rhizophagus_irregularis_B3	87.6	125	25164	JGI	LLXK00000000.1	Chen ECH et al., 2018
Mucoromycota	Glomeromycotina	Rhizophagus_irregularis_C2	87.9	123	26756	JGI	LLXL00000000.1	Chen ECH et al., 2018
Mucoromycota	Glomeromycotina	Rhizophagus_irregularis_DAOM_197198	87.9	137	26183	JGI	AUPC00000000.1	Chen ECH et al., 2018
Zoopagomycota	Zoopagomycotina	Acaulopage_tetraceros	86.9	11.2	6781	GenBank	QZWU00000000.1	Davis et al. 2019
Zoopagomycota	Zoopagomycotina	Cochlonema_odontosperma	72.8	16.8	8696	GenBank	QZWT00000000.1	Davis et al. 2019
Zoopagomycota	Zoopagomycotina	Piptocephalis_cylindrospora_RSA_2659_single-cell	46.9	11	4301	JGI	QPFT00000000.1	Ahrendt SR et al. 2018
Zoopagomycota	Zoopagomycotina	Piptocephalis_tieghemiana_RSA_1565_CoCulture	89.6	18.5	5811	UCR	JAMKHV000000000.1	This study
Zoopagomycota	Zoopagomycotina	Syncephalis_fuscata_S228	86.2	29	8846	JGI	https://mycocosm.jgi.doe.gov/Synfus1/	Amses et al. (in review)
Zoopagomycota	Zoopagomycotina	Syncephalis_plumigaleata_NRRL_S24	84.4	33	8130	JGI	https://mycocosm.jgi.doe.gov/Synplu1/	Chang et al. (in review)
Zoopagomycota	Zoopagomycotina	Thamnocephalis_sphaerospora_RSA_1356_single-cell	72.4	18	6857	JGI	QUVU00000000.1	Ahrendt SR et al. 2018
Zoopagomycota	Zoopagomycotina	Zoopage_sp.	71.7	13.9	7844	GenBank	QZWS00000000.1	Davis et al. 2019
Zoopagomycota	Zoopagomycotina	Zoophagus_insidians	83.4	21	9794	GenBank	QZWR00000000.1	Davis et al. 2019

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Zoopagomycota	Kickxellomycotina	Capniomyces_stellatus_MIS-10-108	78.2	25	6649	GenBank	LUVW00000000.1	Wang et al., 2016a
Zoopagomycota	Kickxellomycotina	Coemansia_mojavensis_RSA_71	93.4	14	6859	JGI	https://mycocosm.jgi.doe.gov/Coemoj1/	Chang et al. (in review)
Zoopagomycota	Kickxellomycotina	Coemansia_reversa_NRRL_1564	93.8	22	7347	JGI	JZJC00000000.1	Chang Y et al., 2015
Zoopagomycota	Kickxellomycotina	Coemansia_spiralis_RSA_1278	93.5	19	7247	JGI	https://mycocosm.jgi.doe.gov/Coespi1/	Chang et al. (in review)
Zoopagomycota	Kickxellomycotina	Dimargaris_cristalligena_RSA_468_single-cell	88.3	31	7456	JGI	QRFA00000000.1	Ahrendt SR et al., 2018
Zoopagomycota	Kickxellomycotina	Furculomyces_boomerangus_AUS-77-4	87.6	28	7338	GenBank	MBFT00000000.1	Wang et al., 2018
Zoopagomycota	Kickxellomycotina	Kickxella_alabastrina_RSA_675	82.4	22	7475	JGI	https://mycocosm.jgi.doe.gov/Kicala1/	Chang et al. (in review)
Zoopagomycota	Kickxellomycotina	Linderina_pennispora_ATCC_12442	76.6	26	9351	JGI	MCFD00000000.1	Mondo SJ et al., 2017
Zoopagomycota	Kickxellomycotina	Martensiomyces_pterosporus_CBS_209.56	95.1	20	8435	JGI	http://genome.jgi.doe.gov/Marpt1	Amses et al. (in review)
Zoopagomycota	Kickxellomycotina	Ramicandelaber_brevisporus_CBS_109374	89	26	9281	JGI	http://genome.jgi.doe.gov/Rambr1	Amses et al. (in review)
Zoopagomycota	Kickxellomycotina	Smittium_angustum_AUS-126-30	86.9	28	7385	GenBank	MBFU00000000.1	Wang et al., 2018
Zoopagomycota	Kickxellomycotina	Smittium_culicis_GSMNP	82.4	77	11209	JGI	LSSN00000000.1	Wang et al., 2016b
Zoopagomycota	Kickxellomycotina	Smittium_culicis_ID-206-W2	79.7	71	10024	JGI	LSSM00000000.1	Wang et al., 2016b
Zoopagomycota	Kickxellomycotina	Smittium_megazygosporum_SC-DP-2	78	44	7132	GenBank	MBFS00000000.1	Wang et al., 2018
Zoopagomycota	Kickxellomycotina	Smittium_mucronatum_ALG-7-W6	77.6	102	8712	JGI	LSSL00000000.1	Wang et al., 2016b
Zoopagomycota	Kickxellomycotina	Smittium_simulii_SWE-8-4	77.3	44	6519	GenBank	MBFR00000000.1	Wang et al., 2018
Zoopagomycota	Kickxellomycotina	Zancudomyces_culisetae_COL-18-3	78.6	29	7387	JGI	LSSK00000000.1	Wang et al., 2016b
Zoopagomycota	Entomopthoromycotina	Basidiobolus_heterosporus_B8920	74.1	47	8992	UMD	JNET00000000.1	Chibucos MC et al., 2016
Zoopagomycota	Entomopthoromycotina	Basidiobolus_meristosporus_B9252	91.3	101	13034	UMD	JNEO00000000.1	Chibucos MC et al., 2016
Zoopagomycota	Entomopthoromycotina	Basidiobolus_meristosporus_CBS_931.73	95.5	87	16111	JGI	MCFE00000000.1	Mondo SJ et al., 2017
Zoopagomycota	Entomopthoromycotina	Conidiobolus_coronatus_NRRL_28638	84.9	40	10635	JGI	JXYT00000000.1	Chang Y et al., 2015
Zoopagomycota	Entomopthoromycotina	Conidiobolus_incongruus_B7586	92.1	93	16001	UMD	JNEM00000000.1	Chibucos MC et al., 2016
Zoopagomycota	Entomopthoromycotina	Conidiobolus_thromboides_FSU_785	84.4	25	8867	JGI	http://genome.jgi.doe.gov/Conth1	Amses et al. (in review)
Zoopagomycota	Entomopthoromycotina	Entomophthora_muscae_Berkeley	67.6	1.2Gb	22505	GenBank	QTSX00000000	Elya et al., 2018
Zoopagomycota	Entomopthoromycotina	Zoophthora_radicans_ATCC_208865	90	655.2	14479	JGI	https://mycocosm.jgi.doe.gov/Zoorad1/	Amses et al. (in review)

Table 2. Summary of phylum-level and subphylum-level lineage-specific genes and Pfam domains in zygomycete fungi

	Phylum-level (>10 taxa)		Subphylum-level (>1 taxa)					
	Mucoromycota	Zoopagomycota	Mucoromycotina	Mortierellomycotina	Glomeromycotina	Kickxellomycotina	Entomorphthoromycotina	Zoopagomycotina
lineage-specific genes	171	9	7779	2742	5572	1706	2209	1186
Pfam domains	2	0	32	11	24	0	5	1

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