Supplementary Information for

Diploid-dominant life cycles characterize the early evolution of Fungi

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Supplemental Materials and Methods

I. Strains and nucleic acid preparations

We generated 21 high coverage genome sequences, 45 low coverage sequences, and 3 sequences with single cell/low input approaches. See Table S1 for full list of taxa and sequencing methods. Cultured strains are deposited in a cryopreserved state in the CZEUM collection (1). Details on growth and extraction of DNA from cultured strains for sequencing are in Simmons et al. (1). We extracted RNA by adding 1 mL of TRIzol reagent (Invitrogen) to ~0.5 mg finely ground frozen tissue, incubating for 5 min on ice, adding 200 μL of chloroform, inverting, incubating for 3 min on ice, and centrifuging at 12,000 x g for 15 min at 4 °C. The supernatant was removed and added to 500 μL cold isopropanol, inverted, incubated for 10 min on ice, and centrifuged at 12,000 x g for 10 min at 4 °C. The RNA pellet was washed with 1 mL 75% ethanol in DEPC-treated water, vortexed, and centrifuged at 7,500 X g for 5 min at 4 °C and dried for 5–10 min before being resuspended in 20-80 μL RNAse-free water. Completed extracts were visually confirmed by applying 2 μL to a 1% TAE electrophoresis gel run at 70 V for ~1 hr. before being stored at -80 °C.

II. Genome and Transcriptome sequencing methods

For high coverage genome sequencing we used PacBio SMRT, Illumina, or Sanger sequencing, and Illumina sequencing alone was used for sequencing genomes to low coverage. We sequenced cDNA for the genomes sequenced to high coverage to aid in annotation.

Illumina Genome Sequencing

For genomes sequenced to high coverage using the Illumina platform (Geranomyces variabilis JEL559, Chytriomyces sp. nov. MP71, Globomyces pollinis-pini Arg68, Blastocladiella britannica JEL711, Conidiobolus thromboides FSU 785, Martensiomyces pterosporus CBS 209.56, Ramicandelaber brevisporus CBS 109374, Umbelopsis ramanniana AG #, Zoophthora radicans ATCC 208865/ARSEF 4784), 100 ng of DNA was sheared to 300 bp using the Covaris LE220 and size selected using SPRI beads (Beckman Coulter). Fragments were treated with end-repair, A-tailing, and ligation of Illumina compatible adapters (IDT, Inc) using the KAPA-Illumina library creation kit (KAPA biosystems). Additionally, for Backusella circina FSU 941, R. brevisporus, and Z. radicans, 5–10 μg of DNA was sheared using Covaris g-TUBE(TM) and gel size selected for 4 kb. Sheared DNA was treated with end repair and ligated with biotinylated adapters containing loxP. Adapter ligated DNA fragments were circularized via recombination by a Cre excision reaction (NEB). Circularized DNA templates were randomly sheared using Covaris LE220 (Covaris). Sheared fragments were treated with end repair and A-tailing using 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 mate pair fragments and 9–10 cycles of PCR was used to enrich the final library (KAPA Biosystems), which was quantified by qPCR for an accurate concentration and sequenced on Illumina Hiseq. For Z. radicans, an additional 8 kb mate pair library was generated using 20 μg of genomic DNA following the same protocol. 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. Quantified libraries were prepared for sequencing on Illumina HiSeq sequencing platform using TruSeq paired-end cluster kits, v3 or v4. Flow cell sequencing was performed on Illumina HiSeq2000 or HiSeq2500 sequencer using HiSeq TruSeq SBS sequencing kits, v3 or v4, following a 2x150 (2x100 for B. circina, Z. radicans, and R. brevisporus mate pair libraries) indexed run recipe.

Low Coverage Illumina Genome Sequencing
For genomes sequenced at the U. Michigan Advanced Genomics Core, library prep was done using Nextera XT kit (Illumina), and sequencing was performed on HiSeq-4000 using paired end 150 bp mode. For genomes sequenced at the JGI, 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. 200 ng of sample DNA was sheared to 600 bp using a Covaris LE220 focused-ultrasonicator. The sheared DNA fragments were size selected by double-SPRI and then 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 libraries were then multiplexed with other libraries and sequencing was performed on the Illumina NovaSeq sequencer using NovaSeq XP V1 reagent kits, S4 flow cell, following a 2X151 indexed run recipe.

PacBio Genome Sequencing

The genomes of *Polychrytrium aggregatum* JEL109, *Chytriomyces hyalinus* JEL632, *Hyaloraphidium curvatum* SAG235-1, *Gaertneriomyces semiglobifer* Barr 43, *Entophlyctis helioformis* JEL805, *Cladochytrium polystomum* WB228, *Chytridium lagenaria* Arg66, *Triparticalcar arcticum* BR59, *Syncephalis fuscata* S228, *Obelidium mucronatum* JEL802, *Powellomyces hirtus* BR81, *Cladochytrium replicatum* JEL 714, *Fimicolychtrium jonesii* JEL569, and *Gorgonomycites haynaldii* MP57 were sequenced using Pacific Biosciences platform. For *O. mucronatum* JEL802, *P. hirtus* BR81, *C. replicatum* JEL 714, and *F. jonesii* JEL569, DNA was not sheared. For the rest, 1–5 μg of genomic DNA was sheared to 10 kb or >10 kb using Covaris g-Tubes (MegaRupter for *T arcticum* BR59). 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). Libraries were purified with AMPure PB beads. PacBio Sequencing primer was annealed to the SMRTbell template library. Version P6 sequencing polymerase was used for *G. semiglobifer* Barr 43, *E. helioformis* JEL805, *C. polystomum* WB228, *S. fuscata* S228, and *C. lagenaria* Arg66. For *B. circina*, Version XL sequencing polymerase was used. The prepared SMRTbell template libraries were sequenced on Pacific Biosciences RSII sequencer using Version C4 chemistry and 1x240 sequencing movie run times (C2 chemistry and 2 hour run times for *B. circina*). For *P. aggregatum* JEL109, *C. hyalinus* JEL632, *C. polystomum*, *T arcticum* BR5, *O. mucronatum* JEL802, *P. hirtus* BR81, *C. replicatum* JEL 714, *F. jonesii* JEL569, and *G. haynaldii* MP57, and *H. curvatum* SAG235-1, Sequel Binding kit 2.0 was used. The prepared SMRTbell template libraries were then sequenced on a Pacific Biosystems' Sequel sequencer using v3 sequencing primer, 1M v2 SMRT cells, and Version 2.0 or 2.1 sequencing chemistry with 1x360 & 1x600 sequencing movie run times.

Sanger sequencing

For genome sequencing of *A. macrogynus* isolate ATCC 38327, the Broad Institute Genomics Platform constructed three libraries, 4 and 10 kb plasmids and 40 kb Fosmids, and generated paired-end reads using Sanger chemistry. The reads were assembled using Arachne (2) Assemblez-Build 20080826 from roughly 12.9-fold total sequence coverage.

Transcriptome sequencing and assembly

For *G. haynaldii* MP57, *P. aggregatum* JEL109, and *C. replicatum* JEL 714, plate-based RNA sample prep was performed on PerkinElmer Sciclone NGS robotic liquid handling system using Illumina's TruSeq Stranded mRNA HT sample prep kit with poly-A selection of mRNA, following the protocol outlined by Illumina user guide
as follows: total RNA starting material was 1 μg per sample and 8 cycles of PCR were used for library amplification. Prepared libraries were quantified using KAPA Biosystems’ next-generation sequencing library qPCR kit and run on Roche LightCycler 480 real-time PCR instrument. Flow cell sequencing was performed on Illumina NovaSeq sequencer with NovaSeq XP V1 reagent kits, a S4 flow cell, and following a 2x150 indexed run recipe.

For all other transcriptomes sequenced (Obelidium mucronatum JEL802, Gaertneriomycetes semiglobifer Barr 43, Chytriomyces sp. nov. MP71, Globomyces pollinis-pini Arg68, Chytridi um lagenaria Arg66, Entophysctis helioformis JEL805, Blastocladiella britannica JEL711, Paraphysoderma sedebokerense JEL821, Cladochytrium polystomum WB228, Hyaloraphidium curvatum SAG235-1, Powellomyces hirtus BR81, Geranomyces variabilis JEL 559, Triparticalcar arcticum BR59, Chytridium hyalinus JEL632, Backusella circina FSU 941), stranded cDNA libraries were generated using Illumina Truseq Stranded RNA LT kit. mRNA was purified from 1 μg 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. Fragmented cDNA was treated with end-pair, A-tailing, adapter ligation, and 8-10 cycles of PCR. qPCR was used to determine concentration of libraries, which were sequenced on Illumina HiSeq. Except for U. ramanniana and B. circina, prepared libraries were quantified with KAPA Biosystems’ next-generation sequencing library qPCR kit and run on Roche LightCycler 480 real-time PCR instrument. Quantified libraries were prepared for sequencing on Illumina HiSeq sequencing platform with a TruSeq paired-end cluster kit, v4. Flow cell sequencing was performed on the Illumina HiSeq2500 sequencer using HiSeq TruSeq SBS sequencing kits, v4, following a 2x150 indexed run recipe. For U. ramanniana and B. circina, the quantified library was 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.

For all Illumina RNA-seq samples, raw reads were filtered and trimmed. Using BBduk (https://sourceforge.net/projects/bbmap/), raw reads were evaluated for artifact sequence by kmer matching (kmer=25), allowing 1 mismatch, and detected artifacts were trimmed from 3’ ends of reads. RNA spike-in reads, PhiX reads and reads containing any Ns were removed. Quality trimming was performed using a phred trimming method set at Q6. Following trimming, reads under the length threshold, being the longer of either minimum length 25 bases or 1/3 of original read length, were removed. For all samples, except O. mucronatum JEL802, filtered reads were assembled into consensus sequences using Trinity ver. 2.1.1 (v2.8.5 for C. replicatum JEL714) (3) run with the --normalize_reads (in-silico normalization routine) and --jaccard_clip (minimizing fusion transcripts derived from gene dense genomes) options. For O. mucronatum, reads were assembled into consensus sequences using Rnnotator (v. 3.4.0) (4).

Single/few cell sequencing approaches

We sequenced two samples with low DNA input methods. For Rozella multimorpha, an endoparasite of the water mold Pythium, we extracted DNA from a small number of parasite zoospores aspirated from a depression slide of infected material (5). Library prep was done using a ThruPLEX DNA-Seq Kit. We used a single cell of the alga Micrasterias cf. truncata (PSC023) infected with an endoparasitic chytrid (Fig. 1D) for single cell sequencing. The sample was prepared for sequencing according to (6) using whole genome amplification with a Qiagen REPLI-g Single Cell kit in half-volume reactions, quality control by PCR using fungal
specific rDNA primers (7), followed by library prep using NEBNext Ultra reagents, and sequencing on a NovaSeq (S4) 300 cycle.

III. Assembly and Annotation

High coverage genomes

For genomes sequenced using the Illumina platform, we filtered all raw sequence data for artifact/process contamination using the JGI QC pipeline. For all lineages except *P. sedebokerense* JEL821, *Z. radicans*, *R. brevisporus* and *B. circina*, an initial assembly of the target genome was generated using VelvetOptimiser version 2.1.7 (https://github.com/Victorian-Bioinformatics-Consortium/VelvetOptimiser.git ) with Velvet version 1.2.07 (8) as follows: "--s 61 --e 97 --i 4 --t 4, --o "-ins_length 250 -min_contig_lgth 500". Resulting assemblies were used to simulate a 2x100 bp 3000 +/- 300bp insert long mate-pair library with wgsim version 0.3.1-r13 (https://github.com/lh3/wgsim) using "-e 0 -1 100 -2 100 -r 0 -R 0 -X 0 -d 3000 -s 30". Simulated long mate-pairs were co-assembled together with original Illumina filtered fastq with AllPathsLG release version R49403 (9) to produce final nuclear assemblies. For *P. sedebokerense* JEL821 assembly was conducted using dipSPAdes version 3.10.1 (10), with parameters (-t 32 -m 120 -o spades --12) to produce a final nuclear assembly. For *R. brevisporus*, a generated 3 kb mate pair library was used instead of simulated data; similarly for *Z. radicans*, except that reads were assembled together with AllPathsLG version R49403 using HAPLODIFY=True and PLOIDY=2. For *B. circina*, AllPathsLG was run as described above using a generated 5 kb mate pair library, then improved with PacBio read data using PBjelly v12.9.14 (11).

For genomes sequenced using the PacBio platform, all filtered subread data was processed using the standard JGI QC pipeline. Subreads were assembled using Falcon (12), improved using FinisherSC (13) and polished using Arrow (https://github.com/PacificBiosciences/GenomicConsensus). See Table S2 for details on assembler versions and polishing steps taken for each lineage. Contigs less than 1000 bp were excluded from final assembly. When detected, both primary and secondary scaffolds were retained in assembly.

All assemblies were annotated using the JGI annotation pipeline (14) with the exception of the *A. macrogynus* assembly which was annotated using the Broad Institute annotation pipeline for fungal genomes (15).

Low coverage genomes

Because ploidy was unknown for most species, we generated haploidized draft assemblies using ploidy-aware assembly methods so that haploid genomes could be used for downstream heterozygosity estimation by read mapping. To take into account uncertainty of ploidy in the assembly phase, we assembled reads for each taxon with both a haploid assembly algorithm (SPAdes (16)) and a diploid assembly algorithm (dipSPAdes (10)). Based on cumulative lengths and N50 values of initial assemblies, we sorted genomes into "likely diploid" and "likely haploid" bins. Specifically, we determined a genome to be "likely diploid" if dipSPAdes length was less than or equal to 90% of SPAdes length and the dipSPAdes N50 was at least 10% higher than SPAdes N50. Otherwise, we considered assemblies to be "likely haploid". Following assignment to ploidy as above, we restarted the assembly pipeline using either SPAdes or dipSPAdes accordingly.

To assemble low coverage genomes the pipeline software Automatic Assembly For the Fungi (AAFTF) v0.2.3 (17) which runs BBduk (sourceforge.net/projects/bbmap/) to trim adaptors by quality and removal of adaptors (ref=adapters ktrim=r k=23 mink=11) and against a
contamination database (phix,artifacts,lambda and NCBI Eukaryotic contamination db), followed
by assembly with SPAdes v3.11.0 (16) using parameters --careful for the paired-end libraries
and dipSPAdes v3.11.0 using default parameters (--diploid) (10). To test whether paired-end
read merging improves the quality of assemblies and to reduce high coverage datasets we also
ran shovill (https://github.com/tseemann/shovill). Assemblies from AAFTF and were processed
to further remove vector contamination with AAFTF vecscreen using the NCBI UniVec database
(NCBI), followed by screening for bacteria contamination with sourmash v3.5.0 (18) removing
contigs that matched anything other than Blastocladiomycota or Chytridiomycota (contigs with
no matches were kept). Duplicate contigs were removed using AAFTF rmdup which were
shorter than 500 bp or aligned with 95% identity over 95% of its length to another contig in the
assembly. Assemblies were further improved with 5 rounds of polishing with Pilon v1.23 (19) by
running AAFTF polish with the original sequencing library, which is aligned back to
the assembly with bwa v0.7.17 (20). The assembly was sorted largest to smallest contig and
renumbered in this order with AAFTF sort.

Genome annotation was performed with funannotate v1.7.4 (21) which used UniProt proteins as
the informant and trained gene predictors with gene models recovered by BUSCO v2.0 using
the fungi_odb9 marker set (22–24). Funannotate uses augustus, SNAP, GeneMark.HMM+ES
and pipelines for the analyses are archived
[https://github.com/stajichlab/Chytrid_Phylogenomics].

Single cell/metagenomes

Assembly and annotation of *R. multimorpha* and PSC023 were treated using special procedures
given that they were from uncultured material and contained contaminating DNA. Removal of
Illumina adaptors and quality trimming were performed using Trimmomatic v.0.36 (25). We
assembled trimmed reads with SPAdes 3.11.1, using the “single cell” option. Contigs smaller
than 500 bp were removed. Using a recently developed single cell binning procedure, SCGid
(26), we recovered two distinct genomes, the putative chyrid parasite (*Olpidium*) of the alga and
a presumed hyperparasite of the chyrid (Rozellomycota). Gene predictions were performed
using Funannotate v1.7.4 (21). Soft masking was performed by “funannotate mask” with default
options. Gene models were predicted by “funannotate predict” in which “rhizopus_oryzae” was
specified as “--augustus_species” option.

IV. Phylogenomic methods

Gene set

To conduct genome-scale phylogenomic analyses we relied on the set of 758 conserved
markers comprising the BUSCO *fungi_odb10* database (22). Although this marker set focuses
on single copy genes, we applied a careful filtering approach to exclude detected paralogs that
were particularly common within EDF.

Gene set pruning and sequence selection

We searched our predicted proteomes against the *fungi_odb10* database using *hmmer* function included in *hmmer* (27). Instead of retaining only the sequence with the strongest hit to
each model that could introduce paralogous sequences into concatenated phylogenetic
analyses, we retained all hits to each protein model detected in each genome when the strength
of the hit was above the minimum threshold accepted by BUSCO software pipeline (i.e.,
*score_cutoffs*, included with BUSCO). This resulted in zero to many sequences per marker from
each genome and subsequently individual locus alignments, the size of which far exceeded the
137 taxa in our phylogenetic data set (e.g., 1,349 tip gene tree for *fungi_odb10* marker
6377at4751).
To filter these alignments to homologs, we employed an iterative approach that involved both automated and manual gene tree-curation steps, yielding individual locus alignments that included, at most, one sequence per taxon. Initially we removed 176 low-occupancy markers (<75% occupied), leaving 582 markers. Generation of trees before and after subsequent filtering steps involved making alignments (hmmalign), trimming alignments (trimal (28) and removal of highly gapped sequences), computing new gene trees (fasttree (29)), and evaluation of criteria as described below.

We implemented an automated gene tree filtering approach (custom scripts available at https://github.com/Michigan-Mycology/Chytrid-Phylogenomics/tree/master/scripts) that traversed trees and determined whether all tips corresponding to each genome were monophyletic. If they were all monophyletic, the best hit was taken. If tips of a genome were not monophyletic, we first compared scores of each protein (i.e., tips) to see if lack of monophyly was the result of one or more particularly low-scoring clusters of tips (<= 70% of score of highest-scoring tip for that genome). We then removed these particularly low-scoring tips and checked again for monophyly. If removal of these particularly low-scoring tips led to monophyly of the taxon, we took the highest-scoring tip among the high-scoring cluster. If monophyly did not result from removal of these particularly low-scoring tips, we still permanently removed particularly low-scoring tips, but retained all those tips with hits in the higher-scoring bin, despite their polyphyly or paraphyly. Following this first round of monophyly and taxon-specific score filtering, we regenerated gene trees.

In a second round of score-based filtering, we removed tips with scores that were lower than 1.5 standard deviations from the mean tip score calculated from all tips in each gene tree. In this way, we removed low-scoring tips from the tree that received a score higher than 70% of the taxon-specific maximum, but were low-scoring relative to the entire tree. This score filter led to the removal of another 63 markers that fell below 75% occupancy, leaving 519 markers for subsequent filtering steps. Again, we regenerated gene trees following tip removal and computed taxon monophyly across resultant trees. At this point, the only conflicting placements remaining in gene trees should have been the result of high-scoring, but non-monophyletic clusters of tips. To resolve remaining conflicts, we manually curated all 519 remaining gene trees and tagged tips and nodes for removal based on their clear paralogous position in trees, for example, a cluster of tips corresponding to fungi from multiple phyla that was positioned as an outgroup to all fungi. In 32 extreme cases, we removed entire markers from our dataset due to irreconcilable conflict in gene trees, leaving 499 markers for subsequent filtering steps.

Upon removal of these paralogous or otherwise erroneous tips from our continually-shrinking sequence set, we regenerated gene trees for one more round of manual curation that aimed to replenish data that may have been automatically removed in error. We noticed that through our automated filtering approach we had reduced representation of four taxa (Fonticula alba, Mitosporidium daphniae, Monosiga brevicollis, and Olpidium bornovanus) to very low levels (e.g., ~15% occupancy in gene trees). These taxa are understood as extreme cases both in terms of their long divergence times from the rest of the kingdom and in patterns of genome evolution (e.g., Mitosporidium daphniae). We manually selected poorly-represented taxa, for which we “spiked” tips back into our alignments from the original, unfiltered sequence set. To assure that these “spiked-in” sequences were not paralogous, we again manually looked at all gene trees to determine whether each re-inserted sequence should be kept or removed in line with the decision made by automated filtering. Through this final manual-curation step, we were able to raise all included taxa above ~40% occupancy in gene trees with confidence that paralogous sequences were not included, while poorly scoring true orthologs were retained.

Through our four-round, part-automated and part-manual iterative filtering approach, we were able to reduce the number of per-taxon sequences in each gene tree to a single sequence. For
those cases where we could not determine which sequence to use as representative sequence for a taxon, we simply removed that taxon from that gene tree. Following this final filter to remove irreconcilable taxa from our gene trees, another 12 markers fell below the 75% occupancy threshold and were removed. We generated a set of finalized gene trees for the remaining 487 markers with alignment in MAFFT 7.310 (30) and tree calculation in IQTree v2.0.5 (31) with 100 nonparametric bootstraps. Substitution models per gene tree were selected by ModelFinder (AIC/BIC highest scoring model) in IQTree.

**Concatenated phylogenetic analysis**

Our filtering approach reduced the 758 conserved marker set from fungi_odb10 to 487 markers with a mean occupancy of 82.02% and represented in up to 137 proteomes in our taxon set. We concatenated our 487 alignments into a 197,423 amino acid alignment and computed phylogenetic trees in IQTree with both unpartitioned and partitioned models. For unpartitioned analysis, we used the most frequent best (AIC/BIC) substitution model among individual alignments. For partitioned analysis, we allowed each partition to be modeled by its best-estimated model as calculated by ModelFinder. Tree topologies from the two models were identical, and therefore we used the unpartitioned analysis tree. We ran 100 nonparametric bootstraps of our unpartitioned dataset in IQTree and annotated the final tree topology with resulting support values. We also computed support measures with quartet intermode certainty using QuartetScores (32) and gene Concordance Factors using IQTree (33). All phylogenetic trees presented in this study were visualized using ggtree and ggtreeExtra in R (34, 35) (Yu et al. 2017; Yu et al. 2018; Yu 2020; Xu et al. 2021).

**Approximately unbiased test**

To assess support for alternative placements for the Blastocladiomycota, we conducted approximately unbiased (AU) tests on three topologies distinct from that of the best ML tree. The Blastocladiomycota branch was moved without rearrangement to other nodes in the tree. These alternative placements of Blastocladiomycota were: (i) diverging between Chytridiomycota and aphelids, (ii) diverging after Olpidium, and (iii) sister to the Chytridiomycota. Like the best ML tree, likelihood for these three alternative topologies were calculated under the LG+R6 substitution model. AU tests were conducted in CONSEL (36) and IQTree using default settings.

**ASTRAL**

We used our 487 gene trees selected above to generate a species tree with local posterior probabilities in ASTRAL 5.7.3 (37) using default settings.

**Time calibrated phylogeny**

We used the concatenated protein ML tree to generate a time-calibrated phylogeny with divergence times between major lineages estimated with penalized likelihood methods implemented in r8s v. 1.81 (38). Fossil-based calibration points were used to constrain minimum ages of the most recent common ancestor (MRCA) of several clades following Chang et al. (39): Blastocladiomycota=407 mya, Chytridiomycota=407 mya, Ascomycota=407 mya, Basidiomycota=330 mya, Mucorales=315 mya. We constrained the tree using a range of allowable dates for MRCA of Dikarya (500–650 mya), based on various reasonable extremes (40). Additional parameters for rate estimation were: smoothing=1000 (chosen using the cross-validation method; num_time_guesses=10; penalty=log).
Ancestral state reconstruction and gene searches

We compiled a matrix of genetic characters using searches for genes of interest, to which we added ploidy state as estimated according to section V, below. Where possible, for taxa that were included in our phylogenomic analyses but missing from AFTOL database, we determined known or observed character states. We inferred the ancestral character state of ploidy, discretized to 1.0 or >=2.0, under an ARD model via ML marginal ancestral state reconstruction with phytools in R (41). We also confirmed results of the marginal ancestral state reconstruction via stochastic ancestral state reconstruction (Bayesian MCMC) with phytools under an ER model based on 1,000 simulations.

We determined the presence of EF1-α, EFL (EF1-α-like), cell wall component (AGS, FKS1, MYSc_Myo17), sterol synthesis (ERG11, ERG5, DHCR7), and cobalamin-dependent genes (42) in genomes used to construct our phylogeny. Exemplary protein sequences were downloaded from GenBank from a search of related taxa or selection from a previously published list (42). We aligned sequences in Muscle 3.8.31 (43) and used alignments to construct hidden Markov model profiles and search genomes for homologous proteins using HMMER 3.1b2 (27). We further eliminated paralogs by aligning sequences in Muscle, creating UPGMA trees in Geneious 9.1.8, and deleting long-branch taxa from further analyses. We determined the use of amino acid selenocysteine by searching for the selenocysteine tRNA gene with Secmarker (44). Confirmation of selenocysteine use was complemented by searches for the gene phosphoseryl-tRNA kinase, a consistent marker for fungal utilization of selenocysteine (45).

We used the hidden Markov model profiles developed in Medina et al. (46) to identify the ancestral transcription factor and inhibitor (E2F and Rb) and the derived fungal transcription factor and inhibitor (SBF and Whi5) in early-diverging fungal genomes. Putative hits were validated by a Jackhmmer search (47) against the curated Swiss-Prot database to confirm homology and expected domain structure.

V. Assessing Support and Conflict for Contentious Relationships

We analyzed individual gene phylogenies for their support for alternative resolution of contentious relationships in the fungal phylogeny. We focused on the resolutions of 5 contentious relationships: the placements of Blastocladiomycota, Monoblepharidomycota, Aphelidiomycota, Neocallimastigomycota, Opидиум, and Polychytriales (48, 49). As a control, we analyzed alternative resolutions of the Ascomycota subphyla, which mostly place Taphrinomycotina as the earliest diverging lineage in mitochondrial or multilocus analyses (50–52). Our analyses focused on quartets in our ML tree distinguishing these taxa, and calculated support for alternative resolutions of these quartets in the form of local posterior probabilities (LPP), frequencies of quartets (Q), and differences in log likelihood between alternative constraint trees (deltalnL). LPP and Q values were calculated for the quartets using ASTRAL 5.7.3 (37), using modification of scripts available at https://github.com/smirarab/1kp/tree/master/scripts/hypo-test following Li et al. (49). deltalnL values were calculated for each of 487 protein alignments by searching for ML trees under topological constraints conforming to each of the three resolutions of the quartets in question using IQ-TREE with best estimated models for each protein. We compared log likelihood values among the three recovered ML trees found under constraint searches as well as an optimal, unconstrained search ML tree. We considered constraint trees recovered from each protein to be sufficient to identify a most likely quartet only when the quartet-constrained search with the highest likelihood was not more than 2 log units less likely than the unconstrained tree. In this way, a widely variable number of proteins provided support for resolution of the quartets, ranging from 20 for the Opидиум-focused quartet to 314 for the Ascomycota subphylum quartets.
VI. Ploidy Estimation

To estimate ploidy of fungal assemblies, we employed a two-pronged approach that generated: 
(i) kmer histograms by counting 23-mers in raw reads (kmer approach) and (ii) allele frequency 
histograms by counting SNPs identified by mapping reads to our draft assemblies (AF 
approach). Our AF approach required that de novo assemblies contain the single haplotype of 
haploid genomes or one haplotype of two or more haplotypes present in genomes with 2N+ 
ploidy. Since our approach was not optimized for long reads, the pipeline was only employed 
when Illumina short reads were available. Instead, for assemblies based on PacBio long reads, 
a separate approach was taken as described below. If only mRNA short reads were available 
(i.e., RNA-seq), we mapped these to our assemblies instead. We excluded from our ploidy 
analyses assemblies for which DNA or mRNA short reads were not available. Within our 137 
taxon dataset, 112 assemblies had DNA or mRNA short reads available, and we attempted to 
predict ploidy for all 112 of these. When possible for the remaining genomes, we drew 
consensus from the literature to assign ploidy or scored as ploidy uncertain.

To count 23-mers present in raw Illumina reads, we ran the *kmercountexact* algorithm included 
in bbtools on all Illumina read libraries generated in this study or in published data sets. When 
multiple Illumina read libraries were available on NCBI SRA, ENA, or JGI Genome Portal, we 
selected read libraries on a case-by-case basis (i.e., based on determined quality in past 
studies) or otherwise based on being the most voluminous raw sequence data library (Table 
S3). We used *khist* output files from individual *kmercountexact* runs to generate kmer frequency 
histograms using custom scripts and ggplot2 in R (53).

We employed a standard SNP-calling pipeline for estimating heterozygosity (SNP rate) and 
generated Variant Call Format (VCF) files that documented heterozygous positions relative to 
the reference assembly. Briefly, we mapped raw reads to their corresponding assembly using 
*bwa mem* (20), sorted and removed PCR duplicates with a variety of *samtools* utilities (54), and 
finally generated VCFs using *gatk HaplotypeCaller* specifying that DepthPerAlleleBySample be 
included in final VCFs (55). We then filtered these VCF files using custom scripts utilizing 
functions from *pyvcf* (https://github.com/jamescasbon/PyVCF) that removed homozygous 
positions and heterozygous positions with more than one alternate allele (i.e., likely artifactual). 
We filtered this SNP set further to exclude low quality SNPs by excluding SNPs that met one of 
these three criteria: i) had a measured depth (*gatk* DP parameter) outside one standard 
deviation of the genome-wide mean, ii) were on contigs outside of the genome assembly L50 
contig set (i.e., the subset of descending size-ordered contigs that account for first 50% of 
cumulative assembly size), or 3) had a measured MapQualityRankSum (MQRS) value that was 
not equal to 0; that is, we forced MappingQuality of reads bearing the reference allele to be 
identical to that of reads bearing the alternate allele. We plotted the distribution of our filtered, 
high-quality SNPs and their frequency (*gatk* AF parameter) in reference versus alternate alleles 
with *ggplot* in R.

To make our metagenomic assemblies (i.e., rozellid and *Olpidium*-like members of PSC023) 
compatible with our allele frequency mapping approach, we first mapped metagenomic reads to 
filtered draft assemblies for each member. We filtered resulting SAM files using *samtools view* 
to remove unmapped reads, pairs where one read was orphaned, and all supplementary 
alignment before extracting forward and reverse read files from filtered SAMs. We used these 
segregate read libraries as input into our standard allele frequency mapping approach as 
described above.

Histograms generated by each prong of our two-pronged approach were visualized as a pair 
and used to estimate the ploidy of 112 assembly-reads pairs in our ploidy dataset. We assessed 
validity of our method by evaluation of known diploid species *Batrachochytrium dendrobatidis* 
(56) and *Allomyces javanicus* (57), which displayed bimodal kmer frequency histograms and
unimodal allele frequency histograms centered at or around 50% allele frequency, indicative of genome-wide heterozygosity consistent with the presence of two sets of homologous chromosomes.

The variable quality of different genome assemblies affected both types of histograms (low coverage of some being a main contributor), making ploidy calls difficult in some cases. We conducted subsequent analyses to separate genomes of uncertain ploidy based on mean SNP density across the L50 contig set and the fit of the allele frequency distribution to a null binomial model. The null model is based on the expectation that a diploid assembly should have a binomial distribution centered at AF = 50% with a standard deviation relative to coverage of the underlying genome (i.e., lower coverage means higher standard deviation, and vice versa for high coverage). We measured fit of each allele frequency histogram to this expected distribution by counting the number of filtered SNPs that fell within one standard deviation of its corresponding hypothetical binomial distribution. We generated a scatter plot of genome SNP density across the L50 contig set (y-axis) and against proportion of filtered SNPs from L50 contig set falling within 1 standard deviation of the mean of each genome’s theoretical binomial distribution (x-axis). This 2D plot was visualized with ggplot2 in R. Due to low sequencing depth, four genomes were excluded from the scatter plot: Blattomyces sp. JEL0837, Fonticula alba, Lichtheimia corymbifera, and Rozella multimorpha.

To identify allelic regions in PacBio assembled genomes, we first searched the assembly against itself using blat version 34 (58) with parameters -noHead minIdentity=70. These data were then filtered to identify syntenic regions that fit the following criteria: 1) present on different scaffolds/chromosomes, 2) >95% identical to each other, 3) covered >50% of sequence space on the smaller scaffold. Genic content in these regions was then explored and likely diploid/dikaryotic genomes were determined as those where >20% of all gene models were found in allelic regions. After allelic regions were determined, we then explored other duplicated content, particularly to identify segmentally duplicated regions and other duplication events that may be too diverged to fit our criteria for identification as alleles. These were selected as duplicated regions with a minimum of three genes in each fragment and at least 50% of genes between fragments being homologs of each other (blastp e value ≤ 1e−20 and alignment coverage for both query and target > 80%). These duplicated regions could occur on the same scaffolds, or different ones. This approach revealed several lineages with relatively large proportions of their assembly in duplication, for example C. replicatum JEL 714, where 69.43% of the assembly was duplicated, but average % identity between AA sequences was only 83.29%.

Genomes were grouped into two categories, haploid mitosis and diploid or higher mitosis. The criteria for identifying genomes as diploid or higher was the following. Type 1 (Literature based): These genomes, e.g., Saccharomyces cerevisiae and Allomyces spp., have a well-defined literature showing presence of diploid mitosis. Type 2 (PacBio assembly based): These genomes showed allelism among contigs assembled from PacBio data as described above. Type 3 (High heterozygosity genomes): These genomes had detection of heterozygous SNPs from mapping of Illumina genomic sequencing reads to the haploidized reference genome assembly, with heterozygosity per base pair > 5e-4 and either kmer graphs with two peaks or allele frequency histograms with > 37% of alleles found within one standard deviation of the expected mean (50%) under a binomial model, or both. If neither kmer graphs nor allele frequencies supported diploidy, these genomes were scored as uncertain ploidy. Type 4 (Medium heterozygosity genomes): These genomes showed heterozygosity based on Illumina genomic sequencing read mapping between 5e-4 and 5e-5. If either kmer graphs showed two peaks or allele frequency histograms showed > 37% of alleles were found within one standard deviation of the expected mean (50%), the genome was considered diploid. Otherwise, the genome was scored as haploid. Type 5 (Low heterozygosity genomes): These genomes
showed heterozygosity based on Illumina genomic sequencing read mapping of < 5e-5 and were considered haploid. Type 6 (Transcriptome or single cell genomes): Because of the imbalanced nature of allele representation in these types of data, we considered genomes with < 1e-4 heterozygous positions haploid, with the remaining genomes diploid.

Supplemental Results

A number of relationships observed in our concatenated phylogeny were found to be poorly supported by both gene concordance factors and internode certainty measures, and in some cases differed between the concatenated approach and coalescence-based approach, i.e., ASTRAL tree. To gauge support from the underlying genes in our data set, we focused on querying individual genes to test whether they support one resolution relative to the alternative resolutions for each controversial relationship (59). We isolated these contentious relationships as quartets (Fig. S6) and performed constrained searches for each gene consistent with each of the three possible quartets, retaining only “decisive” genes by removal of all genes where all constraints were less likely (> 2 logL units) than unconstrained, because such genes indicate weak support for the relationships defined by the quartet. Using the quartet resolving subphyla of Ascomycota as a control for the method, we found 314 genes were decisive and 58% of the decisive genes favored the currently accepted hypothesis of Pezizomycotina and Saccharomycotina as sister clades (60), 28% support Taphrinomycotina with Saccharomycotina, and 16% support Taphrinomycotina with Pezizomycotina (Fig. S6F).

Applying this method to 5 controversial nodes of zoosporic fungi, we found that individual genes generally supported the relationship in the concatenated phylogeny, even when this conflicted with the ASTRAL tree (Fig. S6).

The Monoblepharidomycota shares a number of morphological characteristics with the Chytridiomycota, though they have divergent reproductive modes (61). In contrast the Neocallimastigomycota have a highly derived ecology and physiology, being inhabitants of ruminant guts and the only truly anaerobic lineage in fungi (62). Phylogenomic analyses have conflicted over whether Monoblepharidomycota groups with Chytridiomycota or with Neocallimastigomycota (49, 63, 64). In our concatenated tree Neocallimastigomycota grouped with Monoblepharidomycota, while in the ASTRAL tree, Neocallimastigomycota grouped with Chytridiomycota. Using our constrained searches for individual genes we found a majority (50%) of the 128 decisive genes favor Neocallimastigomycota sister to Monoblepharidomycota (Q1), with 30% of genes supporting Monoblepharidomycota sister to Chytridiomycota (Q2), and 20% of genes supporting Neocallimastigomycota with Chytridiomycota (Q3) (Fig. S6A).

Olpidium is a highly reduced endoparasite of diverse hosts, particularly plant roots, algae, and zooplankton. The discovery that Olpidium was phylogenetically distinct from other zoosporic lineages (50) was followed by a genome sequence that placed Olpidium as the most recent divergence relative to terrestrial fungi, though with considerable conflict among individual gene phylogenies (64). In both our concatenated and ASTRAL analyses Olpidium again was placed as the sister branch of terrestrial fungi. Using our constrained searches for individual genes we found that of the 206 genes in which Olpidium was present, only 20 were decisive and supported one of the three possible quartets. Of these 20, a majority (60%) supported Olpidium with the terrestrial fungi (Q1), with 25% of genes supporting Olpidium with Blastocladiomycota (Q3), and 15% of genes supporting Olpidium diverging before Blastocladiomycota (Q2) (Fig. S6B).

The placement of the Blastocladiomycota has been highly contentious (49). Most analyses place the phylum either as a branch diverging before the divergence of the Chytridiomycota or as the branch diverging after Chytridiomycota, and yet other analyses group these two
zoosporic lineages together. Morphologically, the Blastocladiomycota has traits that ally them with the terrestrial fungi: closed mitosis, the presence of a Spitzenkörper, beta 1,3 glucans in the cell wall, and a true mycelial growth in some members (65, 66). Using our data set, both concatenated and ASTRAL trees show the Blastocladiomycota branching after the Chytridiomycota. However, the internode certainty for this relationship was 0.0 and the gCF was 0.1. Following our constrained searches, we recovered 79 decisive genes, of which 44% supported Blastocladiomycota as sister to terrestrial fungi and Olpidium (Q1), 32% of genes favored Blastocladiomycota as branching before Chytridiomycota (Q2), and 24% of genes grouped Blastocladiomycota with Chytridiomycota (Q3) (Fig. S6C). To assess support for alternative Blastocladiomycota placements, we conducted approximately unbiased (AU) tests for three alternative topologies (Fig. S7) that placed Blastocladiomycota as: (i) branching between Chytridiomycota and Aphelidiomycota, (ii) branching between Olpidium and terrestrial fungi, and (iii) sister to the Chytridiomycota. We were able to reject placement of Blastocladiomycota as sister to Chytridiomycota or as branching after Olpidium, but a divergence of Blastocladiomycota between the Chytridiomycota and Aphelidiomycota was not significantly less supported than the position in the concatenated ML tree (pAU = 0.413 and pAU = 0.425 for CONSEL and IQTree, respectively).

Aphelidiomycota were only recently recognized as a fungal phylum or a fungal relative (67, 68). This lineage of endoparasites is exclusive to algae, and transcriptomics have suggested a placement as diverging as the second branch from the fungal stem after divergence of Rozellomycota+Microsporidia (69). We also recovered this relationship with both concatenated and ASTRAL analyses, but with weak support from IC (0.01) and gCF (0.15). Following constrained searches of quartets resolving the placements of aphelids, we found 95 of the 377 genes in which Paraphelidium was present as decisive. Of these, 38% supported Q1 with aphelids branching immediately before Chytridiomycota (ML result), while 35% supported aphelids with Chytridiomycota, and 27% supported aphelids as the first branch to diverge from the fungal stem.

The relationships of many of the orders of the Chytridiomycota have been difficult to resolve. Polychytriales has no clear affinities with other orders of Chytridiomycota based on zoospore ultrastructure and phylogeny (70). In our concatenated analysis, Polychytrium is sister to Chytridiales, while in the ASTRAL analysis Polychytrium is sister to Chytridiales plus Rhizophydiomycotina (the lineage of Blyttiomyces helicus, Rhizophlyctidiales, Rhizophydiiales, and Spizellomycetales). Examination of the placement of Polychytrium using constrained quartet searches found only 32 decisive trees of the 485 genes including Polychytrium. Of these, 47% supported Polychytrium as sister to Chytridiales (Q1), while 31% supported the ASTRAL relationship of Polychytrium sister to Chytridiales+Rhizophydiomycotina (Q3), and 22% supported Polychytrium as sister to Rhizophydiomycotina.
**Fig. S1.** Diversity of zoosporic eufungi. A. *Obelidium mucronatum* JEL0802 on nutrient agar. B. *Chytriomyces hyalinus* ARG121 on nutrient agar releasing zoospores in vesicular membrane. C. *Homolaphlyctis polyrhiza* JEL0142 zoospores after release. D. PSC023 in host *Micrasterias* cf. *truncata*. E. *Quaeritorhiza haematococci* JEL0916 in dual culture with *Haematococcus pluvialis*. F. *Polyrhizophydium stewartii* JEL0888 in wheat coleoptile bait. G. *Hyaloraphidium curvatum* JEL0383 in liquid medium. H. *Nowakowskiiella* sp. JEL0078 on nutrient agar. I. *Cladochytrium tenue* CCIBt4013 in onion skin. J. *Blyttiomyces helicus* on spruce pollen bait. K. *Polychytrium aggregatum* JEL0109 on nutrient agar. L. *Chytriomyces hyalinus* JEL0176 resting spore produced from sexual conjugation of two encysted zoospores on nutrient agar. M. *Allomyces macrogynus* gametophyte on sesame seed. Scale bar = 10 μm.
Fig. S2. Results of gene tree-based marker filtering before and after multiple rounds of automatic and manual filtering. Best concatenated ML tree (left), heatmap showing lack of monophyly of sequences from genome in representative marker sequences prior to filtering (middle), heatmap showing resolution of conflicts post-filtering (right). See Supplementary Methods for specifics of gene tree filtering implementation.
Fig. S3. Concatenated ML tree identical to that shown in Figure 2 with original branch lengths. For support values see Fig. S5.
Fig. S4. ASTRAL coalescent tree based on gene trees of each of 487 markers used to compute the concatenated ML tree in Figure 2. ASTRAL local posterior probabilities shown on nodes.
Fig. S5. Concatenated ML tree identical to that shown in Figure 2, except with all nodes expanded, and all support values listed on nodes. Support values are as follows: bootstrap=100 pseudoreplicates of the concatenated data set analyzed by ML, qpic=quadrpartition internode certainty calculated using QuartetScores, gCF=gene concordance factors calculated using IQTree, and LPP=local posterior probabilities as calculated in ASTRAL. The support values are listed in the following format. Above node: <bootstrap> / <qpic> Below node: <gCF> / <LPP (when applicable)>.
Fig. S6. Assessment of support for controversial nodes via quartet analyses reveals conflict among genes but also instances where the majority of decisive genes support one quartet arrangement. A) Resolution of placement of Chytridiomycota phyla. B) Placement of Olpidium relative to Blastocladiomycota. C) Primary alternative placements of Blastocladiomycota. D) Primary alternative placements of Aphelidiomycota. E) Alternative placements of Polychytrium, F) Non-contentious resolution of Ascomycota phyla. For each controversial relationship, the three quartet resolutions are shown, with Q1 (red) the relationship in the ML tree, and Q2 (blue) and Q3 (green) the alternative relationships. The bar graph indicates support for each resolution. Solid bars represent local posterior probability values from ASTRAL. Open bars indicate the proportion of gene trees in which the likelihood of the indicated quartet was the highest after ML searches imposing constraints for each quartet. Only decisive genes in which the best scoring quartet under constrained searches was less than 2 logL units different from an unconstrained search. Striped bars indicate quartet frequencies across all gene trees. In the bottom half of each panel is plotted the InL difference between the best scoring quartet and the second-best scoring quartet for decisive genes following constrained searches. Genes are ordered by average bootstrap support for each node. Best scoring Q1 likelihoods are shown as positive values, and Q2 and Q3 likelihood differences are plotted as negative values. Terrestrial Fungi = (Dikarya+Mucoromycota+Zoopagomycota). Dashed lines in upper graphs indicate the level at which support is equivalent, i.e., 33.3%.
Fig. S7. Alternative topological hypotheses on the branching position of the Blastocladiomycota (i-iii). The ML phylogeny is shown in iv. Using an approximately unbiased test of topologies, the AU p-value for each of the trees is as follows: i= 0.413, ii= 3e-36, iii= 6e-60, and iv= 0.587.
Fig. S8A. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
**Fig. S8B.** Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8C. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8D. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8E. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8F. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8G. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8H. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8J. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8K. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8L. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8M. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8N. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8O. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8P. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8Q. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8R. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8S. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8T. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
**Fig. S8U.** Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S6V. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8W. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8X. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8Y. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8Z. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8AA. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Fig. S8AB. Kmer and allele frequency histograms for 112 sets of short reads and their corresponding assembly used to estimate the ploidy of taxa included in this study.
Ancestral state reconstruction of ploidy across the fungal phylogeny. Shown at the nodes are the relative likelihoods of ancestral states using a maximum likelihood approach with the ARD model. Data are coded such that species with diploid mitosis are considered state 2, and species lacking diploid mitosis are encoded as state 1. Missing data reflect both an absence of appropriate short or long read sequencing data and lack of information from the literature.

Fig. S9. Ancestral state reconstruction of ploidy across the fungal phylogeny. Shown at the nodes are the relative likelihoods of ancestral states using a maximum likelihood approach with the ARD model. Data are coded such that species with diploid mitosis are considered state 2, and species lacking diploid mitosis are encoded as state 1. Missing data reflect both an absence of appropriate short or long read sequencing data and lack of information from the literature.
Fig. S10. Ancestral state reconstruction of ploidy across the fungal phylogeny based on maximum parsimony. The inferred state is shown at the tips while the most probable ancestral state is shown at each internal node (blue or red circles). Transitions between states are denoted with pink (haploid to diploid) or purple (diploid to haploid) edge lines. The reconstruction requires 27 past transitions between ploidy states to explain the inferred extant distribution of ploidy across the fungal phylogeny.
Table S1. List of genomes used in this study. Strains newly generated in this study are shown in Source labeled as LCG: low coverage Illumina sequencing, HCG: high coverage genome, SC: low input single cell(s) Illumina genomes. BUSCO values indicate percent completeness as estimated using the fungi_odb10 database. Ploidy and method of inference refers to whether the data support diploid mitosis (2N), haploid (1N), triploid (3N), or uncertain (?) and the source of information used. We considered primary literature as the most important source of information, and then used the heterozygosity data from genome sequencing. Dikaryotic species that lack diploid mitosis are coded as haploid, and species with alternating haploid and diploid generations are coded as diploid. Inference key: Het=genome heterozygosity estimated by short read mapping; Alleles=Allelic regions detected by PacBio assembly; Lit=literature, followed by citation.

| Species                        | Isolate_ID | Assembly Length (Mb) | GC Content | N50     | L50     | Proteins | BUSCO | NCBI Accession | Source | Ploidy and method of inference |
|--------------------------------|------------|----------------------|------------|---------|---------|----------|-------|----------------|--------|-----------------------------|
| Allomyces arbuscula            | Burma 1_F  | 26.5                 | 0.618      | 317448  | 26      | 8889     | 0.868 | PRJNA581157     | LCG    | 2N; Lit (71)              |
| Allomyces javanicus            | California 12 | 29.9             | 0.624      | 104684  | 85      | 9981     | 0.818 | PRJNA581158     | LCG    | 2N; Lit (71)              |
| Allomyces macrogyrus           | ATCC 38327 | 57.1                 | 0.568      | 1114524 | 17      | 19446    | 0.869 | PRJNA20563       | HCG    | 2N; Lit (71)              |
| Anaeromyces robustus           | S4         | 71.7                 | 0.163      | 141798  | 158     | 12832    | 0.828 | PRJNA330692      | (72)   | 1N; Het                   |
| Armillaria ostoyae             | C18_9      | 60.1                 | 0.483      | 2283935 | 9       | 22299    | 0.972 | PRJEB19205       | (73)   | 2N; Lit (74)              |
| Backusella circina             | FSU 941    | 48.6                 | 0.334      | 185938  | 78      | 17039    | 0.947 | PRJNA196083      | HCG    | 1N; Het                   |
| Basidiobolus heterosporus      | B8920      | 44.1                 | 0.397      | 2204    | 3776    | 8992     | 0.629 | PRJNA211915      | (75)   | 2N; Het                   |
| Basidiobolus meristosporus     | CBS 931.73 | 89.5                 | 0.428      | 106019  | 272     | 16110    | 0.974 | PRJNA196075      | (76)   | 1N; Het                   |
| Batrachochytrium dendrobatidis | JAM81      | 24.3                 | 0.390      | 1484462 | 6       | 8732     | 0.897 | PRJNA225502      | https://mycocosm.jgi.doe.gov/Batde5/Batde5.home.html | 2N; Lit (77) |
| Batrachochytrium dendrobatidis | JEL0423    | 23.9                 | 0.387      | 1707251 | 5       | 8819     | 0.802 | PRJNA13653       | (78)   | 2N; Lit (77)              |
| Batrachochytrium salamandrivorans | BS       | 32.6                 | 0.417      | 10956   | 814     | 10135    | 0.846 | PRJNA311566      | 61)    | 2N; Lit (78)              |
| Bifiguratus adelaidae          | AZ0501     | 19.5                 | 0.477      | 102371  | 44      | 5719     | 0.710 | PRJNA368732      | (79)   | 1N; Het                   |
| Species                                      | Accession | NCBI   | BLAST   | Genus     | HCG       | Nucleotides | PRJNA | HCG   | Remarks               |
|---------------------------------------------|-----------|--------|---------|-----------|-----------|-------------|-------|-------|-----------------------|
| Blastocladilla britannica                   | JEL711    | 19.1   | 0.586   | 23470     | 254       | 9431        | 0.723 | PRJNA331555          | HCG       | 1N; Het                |
| Blyttiomycetes helicus single-cell v1.0     | Perch Fen | 46.5   | 0.538   | 6669      | 1974      | 12167       | 0.387 | PRJNA332782          | (80)      | 2N; Lit (80)           |
| Blyttiomycetes sp. JEL0837                  | JEL0837   | 46.5   | 0.412   | 12527     | 1047      | 13891       | 0.704 | PRJNA619723          | LCG       | 1N; Het                |
| Boothiomyces macroporosum                  | PLASUS21  | 15.7   | 0.386   | 109397    | 44        | 7605        | 0.693 | PRJNA629979          | LCG       | 2N; Het                |
| Boothiomyces sp. JEL0838                   | JEL0838   | 12.8   | 0.381   | 116125    | 34        | 6264        | 0.588 | PRJNA619751          | LCG       | 2N; Het                |
| Boothiomyces sp. JEL0866                   | JEL0866   | 14.2   | 0.378   | 114768    | 36        | 7120        | 0.641 | PRJNA619753          | LCG       | 2N; Het                |
| Borealophlyctis nickersoniae               | WJD170    | 38.4   | 0.506   | 16159     | 668       | 11278       | 0.828 | PRJNA629981          | LCG       | 1N; Het                |
| Calcarisporiella thermophila               | CBS 279.70| NA     | NA      | NA        | NA        | 11703       | 0.974 | NA                | https://gb.fungalgenomics.ca/portal/ | ?          |
| Candida arabinofermentans                  | NRRL YB-2248 | 13.2   | 0.344   | 701640    | 6         | 5826        | 0.935 | PRJNA207879          | (81)      | 1N; Het                |
| Capsaspora owczarzaki                      | ATCC 30864| 28.0   | 0.528   | 1617775   | 6         | 8621        | 0.727 | PRJNA193613          | (82)      | 2N; Het                |
| Catenaria anguillulae                      | PL171     | 41.3   | 0.560   | 217825    | 41        | 12804       | 0.743 | PRJNA330705          | (76)      | 2N; Alleles            |
| Caulochytrium protostelioides              | ATCC 52028| 21.8   | 0.664   | 92490     | 68        | 6168        | 0.677 | PRJNA421608          | (80)      | 1N; Lit (80)           |
| Chytridiaceae sp. JEL0842                  | JEL0842   | 26.9   | 0.478   | 72085     | 105       | 8731        | 0.772 | PRJNA619752          | LCG       | 1N; Het                |
| Chytridiomycetes lagenaria                 | ARG066    | 42.4   | 0.453   | 216171    | 61        | 14275       | 0.666 | PRJNA331556          | HCG       | 1N; Het                |
| Chytriomyces confervae                     | CBS 675.73| 36.0   | 0.478   | 44341     | 205       | 10712       | 0.836 | PRJNA453741          | (83)      | 2N; Het                |
| Chytriomyces hyalinus ARG085               | ARG085    | 29.8   | 0.479   | 50756     | 175       | 11560       | 0.803 | PRJNA619724          | LCG       | 2N; Het                |
| Chytriomyces hyalinus ARG121               | ARG121    | 29.5   | 0.479   | 52956     | 159       | 11575       | 0.836 | PRJNA619725          | LCG       | 2N; Het                |
| Species                        | Accession | Date   | Morphology 1 | Morphology 2 | Morpheology 3 | Accession    | RefSeq | Identity | Genbank Accession | Status  |
|-------------------------------|-----------|--------|--------------|--------------|---------------|--------------|--------|----------|-------------------|---------|
| Chytriumyces hyalinus JEL0176 | JEL0176   | 0.479  | 28716        | 303          | 11511         | 0.798        | PRJNA619727 | LCG     | 2N; Het          |         |
| Chytriumyces hyalinus JEL0345 | JEL0345   | 0.477  | 41827        | 196          | 11171         | 0.786        | PRJNA619728 | LCG     | 2N; Het          |         |
| Chytriumyces hyalinus JEL0632 | JEL0632   | 0.479  | 312457       | 37           | 15516         | 0.885        | PRJNA346871 | HCG     | 1N; Alleles      |         |
| Chytriumyces sp. MP 71        | MP 71     | 0.491  | 45725        | 234          | 16054         | 0.835        | PRJNA331557 | HCG     | 2N; Het          |         |
| Cladophytrium replicatum JEL0714 | JEL0714 | 50.7   | 394289       | 42           | 16307         | 0.921        | PRJNA346872 | HCG     | ?                |         |
| Cladophytrium tenue           | GHJ CCiBt 4013-1 | 49.0 | 5135         | 2739         | 15890         | 0.439        | PRJNA630055 | LCG     | 2N; Het          |         |
| Clydaea vesicula JEL0476      | JEL0476   | 0.280  | 24845        | 277          | 8647          | 0.734        | PRJNA629973 | LCG     | 1N; Het          |         |
| Clydaea vesicula JEL0522      | JEL0522   | 0.279  | 46169        | 165          | 9112          | 0.851        | PRJNA619739 | LCG     | 1N; Het          |         |
| Coelomomyces lativittatus CIRM-AVA-1 | 21.9 | 0.323 | 6695     | 990          | 7631          | 0.487        | PRJNA631430 | JGI/STajich | 2N; Lit (84) |         |
| Coemansia reversa NRRL 1564   | 21.8      | 0.427  | 347177       | 21           | 7338          | 0.872        | PRJNA68631  | (63)    | 2N; Het          |         |
| Conidiobolus coronatus NRRL 28638 | 39.9 | 0.220 | 102411       | 113          | 10568         | 0.798        | PRJNA67455  | (63)    | 2N; Het          |         |
| Conidiobolus thromboides FSU 785 | 24.6 | 0.265 | 90842       | 82           | 8867          | 0.835        | PRJNA196084 | HCG     | 1N; Het          |         |
| Coprinopsis cinerea Okayama #7-130 | 37.5 | NA    | NA          | 13355       | 0.964          | PRJNA29797  | (85)    | 1N; Lit (86) |         |
| Dinophylyctis kinnereticum KLL_TL_06062013 | 31.5 | 0.476 | 39098      | 220          | 10332         | 0.699        | PRJNA619580 | LCG     | 2N; Het          |         |
| Drosophila melanogaster v6     | 143.7     | NA    | NA          | 13994       | 0.661          | PRJNA164    | (87)    | 2N; Lit (88) |         |
| Entophylyctis helioformis JEL0805 | 30.9 | 0.600 | 103262      | 89           | 10118         | 0.856        | PRJNA331558 | HCG     | 2N; Alleles      |         |
| Entophylyctis luteolus JEL0120 | JEL0120  | 25.9   | 16966       | 435          | 9099          | 0.662        | PRJNA619729 | LCG     | ?                |         |
| Species                                  | Accession | Length | Nucleotide | Homology | PRJ Accession | LCG | Ploidy | Alleles |
|------------------------------------------|-----------|--------|------------|----------|---------------|-----|--------|---------|
| Entophlyctis luteolus                   | JEL0129   | 27.7   | 0.480      | 68043    | 122           | 0.734 | PRJNA619730 | LCG     | 1N; Het  |
| Entophlyctis sp.                         | JEL0112   | 27.0   | 0.481      | 48027    | 154           | 0.727 | PRJNA619731 | LCG     | 1N; Het  |
| Fimicocolochytrium jonesii              | JEL0569   | 30.6   | 0.538      | 799348   | 12            | 10067 | 0.941  | PRJNA519863 | HCG     | ?        |
| Fonticula alba ATCC 38817.v2            | ATCC 38817| 31.3   | 0.551      | 2529562  | 5             | 5901  | 0.433  | PRJNA262900 | (89)    | 1N; Het  |
| Gaertneriomyces semiglobifer             | Barr043   | 20.9   | 0.495      | 575746   | 9             | 8714  | 0.900  | PRJNA331559 | HCG     | 1N; Het  |
| Geranomyces michiganensis               | JEL0563   | 23.5   | 0.532      | 269724   | 29            | 8389  | 0.892  | PRJNA619733 | LCG     | 1N; Het  |
| Geranomyces variabilis                  | JEL0379   | 23.4   | 0.544      | 225234   | 31            | 8699  | 0.927  | PRJNA619734 | LCG     | 1N; Het  |
| Geranomyces variabilis                  | JEL0389   | 24.1   | 0.543      | 475954   | 17            | 8866  | 0.942  | PRJNA619735 | LCG     | 1N; Het  |
| Geranomyces variabilis                  | JEL0566   | 23.6   | 0.544      | 397283   | 20            | 8803  | 0.937  | PRJNA619736 | LCG     | 1N; Het  |
| Geranomyces variabilis                  | JEL0567   | 23.8   | 0.543      | 473289   | 16            | 9029  | 0.934  | PRJNA619737 | LCG     | 1N; Het  |
| Geranomyces variabilis                  | JEL0559   | 23.7   | 0.543      | 231969   | 30            | 9411  | 0.935  | PRJNA334359 | HCG     | 1N; Het  |
| Gigaspora rosea DAOM 194757             | DAOM 194757| 568    | 0.265      | 232087   | 734           | 31243 | 0.937  | PRJNA430513 | (90)    | ?        |
| Globomyces pollinis-pini Arg68          | ARG068    | 21.6   | 0.350      | 50517    | 125           | 11537 | 0.897  | PRJNA331560 | HCG     | 2N; Het  |
| Globomyces sp. JEL0801                  | JEL0801   | 16.1   | 0.352      | 42882    | 105           | 6698  | 0.414  | PRJNA619749 | LCG     | 2N; Het  |
| Gonapodya prolifera                     | JEL0478   | 48.8   | 0.518      | 347324   | 42            | 13902 | 0.843  | PRJNA207863 | (63)    | 2N; Alleles|
| Gonapodya sp. JEL0774                   | JEL0774   | 35.6   | 0.511      | 71677    | 113           | 10034 | 0.574  | PRJNA619740 | LCG     | 2N; Het  |
| Genus/Moniker                          | Accession | % ID  | GenBank Accession | Identity | Accession | HCG  | Notes         |
|----------------------------------------|-----------|-------|-------------------|----------|-----------|------|---------------|
| *Gorgonomyces haynaldii*               | MP57      | 14.0  | 597596            | 8        | 7898      | 0.860| PRJNA500838   | HCG  | 1N; Het       |
| *Hesseltinella vesiculosa*             | NRRL 3301 | 27.2  | 571097            | 14       | 11139     | 0.968| PRJNA243954   | (76) | 1N; Het       |
| *Homolaphlyctis polyrrhiza*            | JEL0142   | 21.3  | 10789             | 577      | 7123      | 0.640| PRJNA68115    | (91) | 2N; Het       |
| *Hortaea weneckii*                     | EXF 2000  | 49.9  | 153735            | 100      | 15620     | 0.975| PRJNA356640   | (92) | 2N; Lit (92)  |
| *Hyaloraphidium curvatum*              | SAG235-1  | 31.9  | 722379            | 18       | 15197     | 0.852| PRJNA460970   | HCG  | 1N; Het       |
| *Irineochytrium annulatum*             | JEL0729   | 36.8  | 20474             | 470      | 11905     | 0.687| PRJNA629976   | LCG  | 1N; Het       |
| *Kappamyces sp.*                       | JEL0680   | 13.2  | 7589              | 433      | 7477      | 0.410| PRJNA619738   | LCG  | 2N; Het       |
| *Kappamyces sp.*                       | JEL0829   | 11.7  | 100276            | 39       | 5512      | 0.566| PRJNA619750   | LCG  | 2N; Het       |
| *Lichtheimia corymbifera*              | FSU 9682  | 33.5  | 367562            | 25       | 12282     | 0.873| PRJEB3978     | (93) | 2N; Het       |
| *Linderina pennispora*                 | ATCC 12442| 26.2  | 908848            | 9        | 9350      | 0.805| PRJNA721997   | (76) | 2N; Het       |
| *Linnemannia elongata*                 | AG-77     | 49.9  | 517143            | 31       | 14959     | 0.985| PRJNA196039   | (94) | 1N; Lit (94)  |
| *Lobosporangium transversale*          | NRRL 3116 | 42.8  | 672590            | 22       | 11818     | 0.964| PRJNA396744   | (76) | 1N; Het       |
| *Martensiomyces pterosporus*           | CBS 209.56| 19.8  | 117925            | 51       | 8435      | 0.904| PRJNA251778   | HCG  | 2N; Het       |
| *Mitosporidium daphniae*               | UGP3      | 5.6   | 32179             | 50       | 3322      | 0.272| PRJNA292596   | (95) | 1N; Het       |
| *Mixia osmundae*                       | IAM 14324 | 13.6  | 1194905           | 5        | 6858      | 0.946| PRJDA48573    | (96) | 1N; Het       |
| *Monosiga brevicolis*                  | MX1.v1    | 41.6  | NA                | NA       | 9203      | 0.518| PRJNA19045    | (97) | ?             |
| *Mucor circinelloides f. circinelloides*| 1006PhL   | 36.3  | 140649            | 82       | 12227     | 0.987| PRJNA172437   | (98) | 1N; Het       |
| Species                          | Accession | GenBank Accession | Length (bp) | Identity (%) | PRJ Number | Notes       |
|--------------------------------|-----------|------------------|-------------|--------------|-------------|-------------|
| Neocallimastix californiae G1  | Ga        | 193              | 443414      | 0.182        | PRJNA262392 | (72)        |
| Neurospora crassa              | OR74A     | 41.0             | NA          | NA           | PRJNA132    | (99)        |
| Nowakowskiiella sp. JEL0078    | JEL0078   | 33.1             | 2655        | 0.355        | PRJNA629968 | LCG         |
| Nowakowskiiella sp. JEL0407    | JEL0407   | 22.6             | 52255       | 0.389        | PRJNA629972 | LCG         |
| Obelidium mucronatum           | JEL0802   | 49.5             | 189008      | 0.449        | PRJNA333499 | HCG         |
| Olpidium bornovanus            | UCB_F19785| 38.7             | 2083        | 0.569        | PRJNA346684 | (64)        |
| Olpidium sp. PSC023            | PSC023    | 16.6             | 18523       | 0.490        | PRJNA633867 | SC          |
| Orbilia oligospora             | ATCC 24927| 40.1             | 2037373     | 0.443        | PRJNA245119 | (101)       |
| Paramicrosporidium saccamoebae | KSL3      | 7.3              | NA          | NA           | PRJNA331101 | (103)       |
| Paraphelidium tribonemae       | X-108     | NA               | 1343        | 0.512        | PRJNA402032 | (69)        |
| Paraphysoderma sedebokerense   | JEL0821   | 27.9             | 239846      | 0.412        | PRJNA347199 | HCG         |
| Pecoramycetes ruminatum        | C1A       | 101              | 3373        | 0.170        | PRJNA200719 | (104)       |
| Phlyctochytrium bullatum       | JEL0754   | 40.2             | 33519       | 0.539        | PRJNA619743 | LCG         |
| Phlyctochytrium planicorne      | JEL0388   | 30.0             | 79884       | 0.473        | PRJNA619744 | LCG         |
| Phycomyces blakesleeanus       | NRRL_1555 | 53.9             | 1515579     | 0.354        | PRJNA342701 | (105)       |
| Physocladia obscura            | JEL0513   | 44.1             | 11689       | 0.388        | PRJNA629974 | LCG         |
| Piptocephalis cylindrospora    | RSA_2659  | 10.7             | 11086       | 0.512        | PRJNA346814 | (80)        |
| Organism                                    | Accession | Percentage | Coverage | SRR    | BioProject | Enrichment | Notes     |
|---------------------------------------------|-----------|------------|----------|--------|------------|------------|-----------|
| *Piromyces finnis* v3.0                     | finn      | 56.5       | 749539   | 25     | 10992      | 0.865      | PRJNA330696 (72) | 1N; Het       |
| *Piromyces* sp. E2                         | E2        | 71.0       | 144455   | 143    | 14648      | 0.478      | PRJNA82799 (72) | 1N; Het       |
| *Podochytrium* sp. JEL0797                  | JEL0797   | 32.1       | 20738    | 411    | 12043      | 0.766      | PRJNA619745 LCG | ?             |
| *Polychytrium aggregatum*                   | JEL0109   | 64.9       | 389128   | 41     | 10690      | 0.950      | PRJNA346301 HCG | 2N; Alleles   |
| *Polyrhizohyphidium stewartii*              | JEL0888   | 23.3       | 11652    | 575    | 8331       | 0.774      | PRJNA629982 LCG | 1N; Het       |
| *Powellomyces hirtus*                       | Barr081   | 29.4       | 1016081  | 10     | 9359       | 0.950      | PRJNA460973 HCG | 1N; Het       |
| *Powellomyces hirtus*                       | CBS_809.83| 26.2       | 157542   | 47     | 6536       | 0.937      | PRJNA453744 (83) | 1N; Het       |
| *Puccinia graminis f. sp. tritici*          | CRL_75-36-700-3 | 88.7       | 964966   | 30     | 15800      | 0.902      | PRJNA66375 (107) | 1N; Lit (108) |
| *Quaeritorhiza haematococci*                | JEL0916   | 48.2       | 11770    | 1101   | 13723      | 0.685      | PRJNA629978 LCG | 1N; Het       |
| *Ramicandelaber brevisporus*                | CBS_109374| 25.5       | 41156    | 190    | 9281       | 0.711      | PRJNA251765 HCG | 2N; Het       |
| *Rhizoclosmatium globosum*                  | JEL0800   | 57.0       | 292246   | 51     | 15991      | 0.819      | PRJNA330693 (76) | 2N; Alleles   |
| *Rhizoclosmatium hyalinum*                  | JEL0917   | 23.5       | 2546     | 2086   | 10776      | 0.343      | PRJNA619746 LCG | 2N; Het       |
| *Rhizoclosmatium sp. JEL0117*               | JEL0117   | 30.5       | 40205    | 220    | 11857      | 0.838      | PRJNA619726 LCG | 2N; Het       |
| *Rhizophagus irregularis*                   | DAOM_181602| 150        | 2308146  | 23     | 26143      | 0.954      | PRJDB4945 (109) | 1N; Lit (110) |
| *Rhizophyctis rosea* JEL0318*               | JEL0318   | 38.9       | 21549    | 498    | 11459      | 0.760      | PRJNA629969 LCG | 1N; Het       |
| *Rhizophyctis rosea* JEL0764*               | JEL0764   | 48.1       | 104982   | 135    | 12571      | 0.781      | PRJNA619747 LCG | 1N; Het       |
| *Rhizopus delemar*                          | RA_99-880 | 46.1       | 3104119  | 6      | 17459      | 0.809      | PRJNA13066 (111) | 1N; Lit (111) |
| Species                                      | Accession Code | Length | Genomic Position | p-value | PRJ File  | Number | Heterogyne Status |
|----------------------------------------------|----------------|--------|------------------|---------|-----------|--------|-------------------|
| Rhizopus microsporus var. microsporus        | ATCC_52814     | 25.0   | 105542           | 0.927   | PRJNA330886 | (112) | 1N; Het           |
| Rozella allomycis                            | CSF55          | 13.5   | 7173             | 0.625   | PRJNA81749 | (113) | 3N; Het           |
| Rozella multimorpha                          |                | 13.6   | 5938             | 0.443   | PRJNA762434 | SC    | 2N; Het           |
| Rozella sp. PSC023                           | PSC023         | 14.7   | 17387            | 0.599   | PRJNA633868 | SC    | 2N; Het           |
| Saccharomyces cerevisiae                     | S288C          | 12.1   | NA               | 0.966   | PRJNA128   | (114) | 2N; Lit (115)     |
| Saccharomycopsis carpsularis                 | NRRL_Y-17638   | 17.8   | 289111           | 0.934   | PRJNA429441 | (116) | 1N; Het           |
| Saksenaea vasisiformis                       | B4078.G233     | 42.5   | 79983            | 0.937   | PRJNA184887 | (75)  | 1N; Het           |
| Schizosaccharomyces pombe                    | 972h-          | 12.6   | 4539804          | 0.962   | PRJNA127   | (117) | 2N; Lit (118)     |
| Siphonaria sp. JEL0065                       | JEL0065        | 37.6   | 21877            | 0.703   | PRJNA619581 | LCG   | 2N; Het           |
| Spizellomyces punctatus                      | DAOM_BR117     | 24.1   | 1465700          | 0.956   | PRJNA319336 | (119) | ?                 |
| Spizellomyces sp. palustris                  | CBS_455.65     | 22.9   | 219277           | 0.946   | PRJNA453746 | (83)  | 1N; Het           |
| Syncephalastrum racemosum                   | NRRL_2496      | 30.7   | 2374188          | 0.964   | PRJNA330704 | (76)  | 1N; Het           |
| Syncephalis fuscata                          | S228           | 29.4   | 474633           | 0.888   | PRJNA332249 | HCG   | 2N; Het           |
| Synchytrium endobioticum                     | MB42           | 21.5   | 44081            | 0.842   | PRJNA453734 | (83)  | ?                 |
| Synchytrium microbalum                       | JEL0517        | 26.2   | 518601           | 0.902   | PRJNA576245 | (83)  | 1N; Het           |
| Terramyces sp. JEL0728                       | JEL0728        | 15.6   | 101760           | 0.724   | PRJNA619748 | LCG   | 2N; Het           |
| Thoreauomyces humboldii                      | JEL0095        | 26.3   | 23424            | 0.847   | PRJNA629855 | LCG   | 1N; Het           |
| Species                          | Accession  | p-value | ID     | N  | Coverage | q-value | Assembly | Species Notes |
|---------------------------------|------------|---------|--------|----|----------|----------|----------|---------------|
| *Triparticalcar arcticum*       | Barr059    | 0.485   | 855893 | 12 | 10963    | 0.925    | PRJNA463939 | 1N; Het       |
| *Umbelopsis ramanniana*         | AG_#.Umbra1.v1 | 0.431   | 294116 | 26 | 9931     | 0.949    | PRJNA196032 | 1N; Het       |
| *Ustilago maydis*               | 521        | 0.540   | 884984 | 7  | 6764     | 0.979    | PRJNA14007  | (120) 1N; Lit (121) |
| *Yarrowia lipolytica*           | CLIB122    | NA      | NA     | NA | 6471     | 0.974    | PRJNA12414  | (122) 2N; Lit (123) |
| *Zoophthora radicans*           | ATCC_208865 | 0.319   | 544305 | 307| 14479    | 0.871    | PRJNA501640 | HCG 2N; Het   |
| *Zopfochytrium polystomum*      | WB228      | 0.533   | 222082 | 105| 16599    | 0.883    | PRJNA460969 | HCG 2N; Alleles |
**Table S2.** PacBio assembly tools used for each genome and coverage. PBA: pb-assembly=0.0.2|falcon-kit=1.2.3|pypeflow=2.1.0, FSC2: finisher SC v2.0, FSC2.1: finisherSC v2.1 (Lam et al., 2014), QV2: Quiver version smrtanalysis_2.3.0.140936.p5, AV5.0.0: Arrow version SMRTLink v.5.0.0.6792, AV5.0.1: Arrow version SMRTLink v5.0.1.9578, AV5.1.0: Arrow version SMRTLink v5.1.0.26412, AV6: Arrow version SMRTLink v6.0.0.47841, AV7: Arrow version SMRTLINK v7.0.1.66975

| Organism                        | Falcon version | Finishing | Polishing | Coverage |
|---------------------------------|----------------|-----------|-----------|----------|
| Chytridium lagenaria Arg66      | 0.4.2          | FSC2      | QV2       | 65.4     |
| Chytriomyces hyalinus JEL632    | 1.8.8          | FSC2      | AV5.0.1   | 47.82    |
| Cladochytrium replicatum JEL 714| PBA            | FSC2.1    | AV7       | 230.35   |
| Entophlyctis helioformis JEL805 | 0.4.2          | FSC2      | QV2       | 53.07    |
| Fimicolochytrium jonesii JEL569 | 1.8.8          | FSC2      | AV5.1.0   | 542.15   |
| Gaertneriomyces semiglobifer Barr 43 | 0.4.2     | FSC2      | QV2       | 435.78   |
| Gorgonomyces haynaldii MP57     | 1.8.8          | FSC2      | AV5.0.1   | 542.15   |
| Hyaloraphidium curvatum SAG235-1| 1.8.8          | FSC2      | AV5.0.1   | 312.53   |
| Obelidium mucronatum JEL802     | 1.8.8          | FSC2      | AV5.0.0   | 156.26   |
| Polychytrium aggregatum JEL109  | PBA            | FSC2      | AV6       | 279.61   |
| Powellomyces hirtus BR81        | 1.8.8          | FSC2      | AV5.0.1   | 431.14   |
| Phlyctochytrium arcticum BR59   | 1.8.8          | FSC2      | AV5.0.1   | 382.09   |
| Cladochytrium polystomum WB228  | 1.8.8          | FSC2      | AV5.0.1   | 53.25    |
| Syncephalis fuscata S228        | 0.4.2          | FSC2      | QV2       | 276.18   |
Table S3. Specific short read archive libraries used for each of the genomes for which ploidy was estimated.

| Taxon                                      | SRA Run ID |
|--------------------------------------------|------------|
| *Allomyces arbuscula*                     | SRR11025179|
| *Allomyces javanicus*                     | SRR11025671|
| *Anaeromyces robustus*                    | SRR3948236 |
| *Backusella circina*                      | SRR3747393 |
| *Basidiobolus heterosporus*               | SRR1009208 |
| *Basidiobolus meristosporus*              | SRR3786034 |
| *Batrachochytrium salamandrivorans*       | SRR3160471 |
| *Bifuratus adelaidae*                     | SRR5207255 |
| *Blastocladilla britannica*               | SRR5167031 |
| *Blyttomyces helicus*                     | SRR4184772 |
| *Blyttomyces* sp. JEL0837                 | SRR12278016|
| *Boothiomyces macroporosum*               | SRR12903493|
| *Boothiomyces* sp. JEL0838                | SRR12278256|
| *Boothiomyces* sp. JEL0866                | SRR12278258|
| *Borealophlyctis nickersoniae*            | SRR12904127|
| *Candida arabinofefermentans*             | SRR3928137 |
| *Capsaspora owczarzaki*                   | DRR183067  |
| *Chytridiaceae* sp. JEL0842               | SRR12278248|
| *Chytridium* lagenaria                    | SRR6057011 |
| *Chytriomyces confervae*                  | ERR2451316 |
| *Chytriomyces* hyalinus ARG085            | SRR12278257|
| *Chytriomyces* hyalinus ARG121            | SRR12278272|
| *Chytriomyces* hyalinus JEL0176           | SRR12278271|
| *Chytriomyces* hyalinus JEL0345           | SRR12278275|
| *Chytriomyces* hyalinus JEL632            | SRR8189667 |
| *Chytriomyces* sp. MP 71                  | SRR5167010 |
| *Cladochrytium* replicatum                | SRR11555543|
| *Cladochrytium* tenue                     | SRR12903494|
| *Clydaea vesicula* JEL0476                | SRR12903413|
| *Clydaea vesicula* JEL0522                | SRR12278222|
| *Coelomomyces* lativittatus               | SRR12904458|
| *Coemansia* reversa                       | SRR3927211 |
| *Conidiobolus* coronatus                  | SRR3927213 |
| *Conidiobolus* thromboides                | SRR1801159 |
| *Dinochytrium* kinnerteticum              | SRR12278017|
| *Entophlyctis* helioformis                | SRR6057018-SRR6057019|
| *Entophlyctis luteolus* JEL0120           | SRR12278140|
| *Entophlyctis luteolus* JEL0129           | SRR12278139|
| *Entophlyctis* sp. JEL0112                | SRR12278277|
| *Fonticula* alba                          | SRR554351  |
| *Gaertneriomyces* semiglobifer            | SRR6056997 |
| *Gaertneriomyces* sp. JEL0708             | SRR12278641|
| *Geranomyces* michiganensis               | SRR12278402|
| *Geranomyces* variabilis JEL0379          | SRR12278642|
| *Geranomyces* variabilis JEL0389          | SRR12278644|
| *Geranomyces* variabilis JEL0566          | SRR12278643|
| *Geranomyces* variabilis JEL0567          | SRR12278674|
| *Geranomyces* variabilis JEL559           | SRR8534491 |
| *Globomyces* pollinis-pini                | SRR5167032 |
| *Globomyces* sp. JEL0801                  | SRR12278247|
| Species                          | Accession     |
|---------------------------------|---------------|
| Gonapodya prolifera             | SRR427138     |
| Gonapodya sp. JEL0774           | SRR12278221   |
| Gorgonomyces haynaldii          | SRR8267450    |
| Hesseltinella vesiculosa        | SRR3503832    |
| Homolaphlyctis polyrhiza        | SRR13013247   |
| Hortaea werneckii               | SRR866616     |
| Hyaloraphidium curvatum         | SRR7517569    |
| Irineochytrium annulatum        | SRR12903421   |
| Kappamyces sp. JEL0680          | SRR12278220   |
| Kappamyces sp. JEL0829          | SRR12278250   |
| Lichtheimia corymbifera         | ERR299304     |
| Linderina pennispora            | SRR1587673    |
| Lobosporangium transversale     | SRR3990394    |
| Martensiomyces pterosporus      | SRR4125794    |
| Mitosporidium daphniæ           | SRR13260914   |
| Mixia osmundae                  | SRR427132     |
| Mucor circinelloides            | SRR770023     |
| Neocallimastix californiae      | SRR7140690    |
| Nowakowskiiela sp. JEL0078      | SRR12887672   |
| Nowakowskiiela sp. JEL0407      | SRR12903399   |
| Obellidium mucronatum           | SRR5506997    |
| Olpidium bornovanus             | SRR420852     |
| Olpidium sp. PSC023             | Pending       |
| Paraphelidium tribonemae        | SRR6014119 SRR6014120 |
| Paraphysoderma sedebokerense    | SRR6447920    |
| Pecoramyces ruminatium          | SRR5790670 SRR5790671 |
| Phlyctochytrium bullatum        | SRR12278150   |
| Phlyctochytrium planicorne      | SRR12278151   |
| Phycomyces blakesleeanus        | SRR3922754    |
| Physocladia obscura             | SRR12903417   |
| Piptocephalis cylindrospora     | SRR5621790    |
| Piromyces finnis                | SRR3948251    |
| Piromyces sp. E2                | SRR400506     |
| Podochytrum sp. JEL0797         | SRR12278144   |
| Polyrhizophydidium stewartii    | SRR12904126   |
| Powellomyces hirtus BR81        | SRR7517594    |
| Powellomyces hirtus CBS 809.83  | ERR2451317    |
| Quaeitorhiza haematococci       | SRR12903423   |
| Ramicandelaber brevisporus      | SRR4125816    |
| Rhizoclosmatium hyalinum        | SRR12278145   |
| Rhizoclosmatium sp. JEL0117     | SRR12278015   |
| Rhizophagus irregularis         | DRR063510     |
| Rhizophlyctis rosea JEL0318     | SRR12903377   |
| Rhizophlyctis rosea JEL0764     | SRR12278215   |
| Rhizopus microsporus            | SRR4063850    |
| Rozella allomycis               | Pending       |
| Rozella multimorpha             | Pending       |
| Rozella sp. PSC023              | Pending       |
| Saccharomycopsis capsularis     | SRR5559346    |
| Saksonaea vasiformis            | SRR975063     |
| Siphonoria sp. JEL0065          | SRR12278210   |
| Spizellomyces sp. palustris CBS 455.65 | ERR2451319   |
| Syncphalastrum racemosum        | SRR8843016    |
| Species                              | SRA Accession |
|-------------------------------------|---------------|
| Syncephalis fuscata                 | SRR6053271    |
| Synchytrium endobioticum            | ERR2286953    |
| Synchytrium microbalum              | ERR2451318    |
| Terramyces sp. JEL0728              | SRR12278251   |
| Thoreuomycetes humboldtii           | SRR12903376   |
| Triparticalcar arcticum             | SRR7517624    |
| Umbelopsis ramanniana               | SRR1800506    |
| Zoophthora radicans                 | SRR1587633    |
| Zopfochytrium polystomum            | SRR7141106    |
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