

Supplementary Figure 1. General concerted evolution of eukaryotic rDNA

a. A general structure of eukaryotic rDNA clusters²⁰.
b. Deletion of homologous genes by crossover recombination^{33,58}.
c. Deletion of homologous genes by single-strand annealing³⁴.
d. A model of the rDNA number maintenance system³².
e. Copy number-controlling pathway in yeast³².



Supplementary Figure 2. Cross comparison of *R. irregularis* DAOM-18160219 orthologous genes from three genomic studies and our RIR17 assemblies.



Supplementary Figure 3. Total assembly size and predicted gene number in fungi. *Rhizophagus irregularis* genomes (RIR17 and three previously assembled genomes, JGI_v1.0, JGI_v2.0 and Lin14) and 768 genomes registered in GenBank. The fungal assembly statistics were obtained from the registered information in GenBank (ftp://ftp.ncbi.nlm.nih.gov/genomes/ASSEMBLY_REPORTS/All/).





































Supplementary Figure 4. Mapped PacBio reads of rDNA regions of RIR17 contigs and the sequence similarity of rDNAs with other rDNA regions in RIR17

The colors have the same meanings as in Fig. 3a-b.











Supplementary Figure 5. Positions and identities of JGI_v2.0 scaffold aligned against RIR17 contigs with rDNAs.

The colors have the same meanings as in Figure 3c.



Supplementary Figure 6. Synthesis-dependent strand annealing

| | | | | | | | | | CEGM complete | IA nss* |
|------------|-----------------------|-----------------------------|------------|--------------|---------|-----------|-----------|-----------|------------------|------------|
| | | IDs in public database | | | % total | # N's | | Longest | Detected | |
| Assmbly ID | Reference | (database) | # assembly | Total length | length | base | N50 (bp) | assembly | gene # | % |
| | | BDIQ01000000 | | | | | | | | |
| RIR17 | - | (INSDC) | 210 | 149,750,837 | 97.24 | 0 | 2,308,146 | 5,727,599 | 244 | 98.4 |
| | | Rhizophagus irregulare v2.0 | | | | | ~~~~~~ | | | |
| JGI_v2.0 | Chen et a. 2018 | (MycoCosm) | 1,123 | 136,807,476 | 88.84 | 6,925,426 | 336,373 | 1,375,864 | 245 | 98.8 |
| | | JEMT0000000 | | | | | | | | |
| Lin_14 | Lin et al. 2014 | (INSDC) | 30,233 | 140,695,967 | 91.36 | 30,115 | 16,434 | 198,953 | 229 | 92.3 |
| | | Rhizophagus irregulare v1.0 | | | | | | | | |
| JGI_v1.0 | Tisserant et al. 2013 | (MycoCosm) | 28,371 | 91,083,792 | 59.15 | 270,422 | 4,186 | 57,883 | 241 | 97.2 |
| | | | | | | | | | | |

Supplementary Table 1. Assmbly statistics of *R. irregularis* genomes

*All CEGMA restuls was obtained by our reanalysis on CEGMA

Supplementary Table 2. Summary of the sequencing data

| Sequence ID | DRA ID | Libraly construction and sequencing method | DNA/RNA | Read # | Read length | Total basepair (Mbp) |
|---------------|-----------|--|---------|-------------|----------------|-------------------------|
| Rir_DNA_Pac_1 | DRA004849 | PacBio RSII, P6-C4 sequence chemistry | DNA | 348,539 | *7559 | 2,635 |
| Rir_DNA_Pac_2 | DRA004878 | PacBio RSII, P6-C4 sequence chemistry | DNA | 394,261 | *8562 | 3,376 |
| Rir_DNA_Pac_3 | DRA004889 | PacBio RSII, P6-C4 sequence chemistry | DNA | 761,112 | *7500 | 5,708 |
| Rir_DNA_PE180 | DRA004835 | Hiseq PE126,TruSeq DNA prep kit | DNA | 423,041,682 | 126 | 53,303 |
| Rir_RNA_SS | DRA005204 | Hiseq PE101,TruSeq RNA prep kit | RNA | 32,245,928 | 101 | 3,257 |
| Rir_RNA_rRNA | DRA006039 | Miseq PE301,TruSeq RNA prep kit | RNA | 20,904,594 | 301 | 6,292 |

*Average read length

Supplementary Table 3. Blast hit data of eliminated contamination sequences

| Query; Illumii | na-polished assmb | ly, Databas | se; Refseo | g genome | , blast; de | efault blastn s | search | |
|----------------|-------------------|-------------------|---------------|------------------|------------------|-----------------|---------|---|
| Assmbly ID | Hit ID | Assmbly length | Identity % | Query cover % | Max bit score | Total score | E-value | Subject title |
| unitig_103 | NW_016089427 | 4223 | 99 | 100 | 7548 | 25444 | 0 | <i>Daucus carota</i> subsp. <i>sativus</i> cultivar DH1 unplaced genomic scaffold, ASM162521v1 DCARv2_B11, whole genome shotgun sequence |
| unitig_228 | NC_030383 | 2192 | 93 | 100 | 3208 | 87603 | 0 | <i>Daucus carota</i> subsp. <i>sativus</i> cultivar DH1 chromosome 3, ASM162521v1, whole genome shotgun sequence |
| unitig_85 | NW_016092113 | 14490 | 99 | 99 | 10975 | 106600 | 0 | <i>Daucus carota</i> subsp. <i>sativus</i> cultivar DH1 unplaced genomic scaffold, ASM162521v1 DCARv2_S316, whole genome shotgun sequence |
| unitig_109 | NW_016089417 | 1602 | 99 | 100 | 2826 | 52864 | 0 | <i>Daucus carota</i> subsp. <i>sativus</i> cultivar DH1 chromosome 2 genomic scaffold, ASM162521v1 2, whole genome shotgun sequence |
| unitig_88 | NW_016092113 | 14867 | 98 | 100 | 11607 | 90386 | 0 | <i>Daucus carota</i> subsp. <i>sativus</i> cultivar DH1 unplaced genomic scaffold, ASM162521v1 DCARv2_S316, whole genome shotgun sequence |
| unitig_100 | NW_016089417 | 8259 | 94 | 100 | 12676 | 539700 | 0 | <i>Daucus carota</i> subsp. <i>sativus</i> cultivar DH1 chromosome 2 genomic scaffold, ASM162521v1 2, whole genome shotgun sequence |
| unitig_83 | NW_016092113 | 46670 | 98 | 100 | 11686 | 328700 | 0 | <i>Daucus carota</i> subsp. <i>sativus</i> cultivar DH1 unplaced genomic scaffold, ASM162521v1 DCARv2_S316, whole genome shotgun sequence |
| unitig_84 | NW_016092113 | 14454 | 98 | 100 | 11673 | 103600 | 0 | <i>Daucus carota</i> subsp. <i>sativus</i> cultivar DH1 unplaced genomic scaffold, ASM162521v1 DCARv2_S316, whole genome shotgun sequence |
| unitig_101 | NC_030388 | 3879 | 95 | 100 | 6058 | 146300 | 0 | <i>Daucus carota</i> subsp. <i>sativus</i> cultivar DH1 chromosome 8, ASM162521v1, whole genome shotgun sequence |

| | | RIR17 | vs JGI2 | RIR17 v | vs Lin14 | RIR17 vs | JGI_v1.0 | JGI_v2.0 | vs Lin14 | JGI_v2.0 vs | JGI_v1.0 |
|------------|-----------------|-------------|-------------|-------------|-------------|-------------|------------|-------------|-------------|-------------|------------|
| | | RIR17 | JGI_v2.0 | RIR17 | Lin14 | RIR17 | JGI_v1.0 | JGI_v2.0 | Lin14 | JGI_v2.0 | JGI_v1.0 |
| | Total query | 210 | 1,123 | 210 | 30233 | 210 | 28,371 | 1,123 | 30,233 | 1,123 | 28,371 |
| Assmbly # | Aligned | 181 | 1,118 | 184 | 29,346 | 171 | 27,941 | 1,097 | 29,222 | 1,039 | 27,911 |
| | Unaligned | 29 | 5 | 26 | 887 | 39 | 430 | 26 | 1,011 | 84 | 460 |
| | Total bases | 149,750,837 | 136,807,476 | 149,750,837 | 140,695,967 | 149,750,837 | 91,083,792 | 136,807,476 | 140,695,967 | 136,807,476 | 91,083,792 |
| | bp | 141,467,912 | 129,748,697 | 135,095,707 | 139,499,930 | 102,512,435 | 89,901,367 | 123,572,251 | 137,934,367 | 100,065,708 | 89,599,127 |
| | % for total | | | | | | | | | | |
| Bases | Aligned bases | 94.47% | 94.84% | 90.21% | 99.15% | 68.46% | 98.70% | 90.33% | 98.04% | 73.14% | 98.37% |
| | bp | 8,282,925 | 7,058,779 | 14,655,130 | 1,196,037 | 47,238,402 | 1,182,425 | 13,235,225 | 2,761,600 | 36,741,768 | 1,484,665 |
| | % for total | | | | | | | | | | |
| | Unaligned bases | 5.53% | 5.16% | 9.79% | 0.85% | 31.54% | 1.30% | 9.67% | 1.96% | 26.86% | 1.63% |
| | Breakpoints | 57,007 | 53,835 | 109,568 | 45,089 | 116,992 | 45979 | 138,441 | 85,178 | 118,725 | 51,951 |
| Syntenic | Relocations | 982 | 622 | 219 | 82 | 4 | 48 | 668 | 238 | 68 | 79 |
| difference | Translocations | 1,980 | 1,101 | 19,502 | 766 | 28,807 | 1129 | 17,489 | 1,908 | 28,316 | 1,629 |
| | Translocations | 34 | 64 | 445 | 551 | 2 | 39 | 395 | 487 | 4 | 17 |
| | SNP* | 29, | 960 | 9,6 | 655 | 30,3 | 847 | 24, | 699 | 32,7 | 729 |
| | INDEL | 14, | 608 | 17, | 081 | 21,2 | 267 | 2,6 | 622 | 14,1 | 112 |

Supplementary Table 4. Statistics of Mummer alignment

*Supported by 20 bp over sequence identifer around the detected position

| | Gene (Longe | e mode st isoform) | Isofor | m model | Com BUS (S- | plete cOs ⊦D) | Complet single- BUSCC | te and copy Os (S) | Comple duplic BUSCO | te and ated Ds (D) | Fragme BUS(| ented COs | Miss BUSC | ng COs |
|--------------------------|----------------|-----------------------|--------|------------------------|-------------------|---------------------|-----------------------------|--------------------------|---------------------------|--------------------------|----------------|--------------|--------------|-----------|
| - | # | Average | # | Average length (AA) | # | % | # | % | # | % | # | % | # | % |
| RIR17 | 41,572 | 377 | 43,675 | 378 | 273 | 94.1 | 243 | 83.8 | 30 | 10.3 | 11 | 3.8 | 6 | 2.1 |
| RIR17 Non-provisional | 27,859 | 448 | 29,849 | 446 | 273 | 94.1 | 243 | 83.8 | 30 | 10.3 | 11 | 3.8 | 6 | 2.1 |
| JGI_v2.0 | 26,183 | 326 | - | - | 282 | 97.2 | 274 | 94.5 | 8 | 2.8 | 2 | 0.7 | 6 | 2.1 |
| Lin14 | 27,301 | 347 | 29,911 | 351 | 256 | 88.3 | 186 | 64.1 | 70 | 24.1 | 7 | 2.4 | 27 | 9.3 |
| JGI_v1.0 | 30,282 | 270 | - | - | 257 | 88.6 | 251 | 86.6 | 6 | 2.1 | 29 | 10.0 | 4 | 1.4 |

Supplementary Table 5. Predicted gene number and BUSCO completeness of *R. irregularis* DAOM-181602 genomes

Supplementary Table 6. Number of genes on repetitive regions

| | Total gana | Genes on Genes on transposon | | | | | | Other | Genes on non- |
|----------------------------|----------------------|------------------------------|-------|-------|-------|---------|---------------------|----------------------|----------------------|
| | lotal gene number | repetitive reagion | LINE | LTR | DNA | unknown | Total transposon | repetitive region | repetitive region |
| RIR17 | 41,572 | 32,665 | 3,185 | 1,735 | 8,065 | 15,287 | 24,185 | 8,480 | 8,907 |
| RIR17 Non-provisional gene | 27,859 | 22,455 | 1,462 | 1,290 | 4,354 | 10,361 | 15,099 | 7,356 | 5,404 |
| JGI_v2.0 | 26,183 | 20,106 | 252 | 334 | 1,878 | 10,043 | 11,386 | 8,720 | 6,077 |

Supplementary Table 7. Analyzed protein sequences by Orthofinder analaysis

| IDs in Figure 1 | Species name | Source |
|------------------|--|-------------------------------|
| Drosophila | Drosophila melanogaster | OrthoDB9:Metazoa* |
| Mus | Mus musculus 10090 | OrthoDB9:Metazoa* |
| Mortierella | Mortierella elongata AG-77 | INSDC:PRJNA196039 |
| R. irregularis | Rhizophagus irregularis | RIR17 |
| Phycomyces | Phycomyces blakesleeanus | INSDC: PRJNA61391 |
| Rhizopus | Rhizopus delemar RA 99-880 | OrthoDB9:Fungi* |
| Aspergillus | Aspergillus nidulans FGSC_A4 | ASpGD (s10-m04-r06) |
| Saccharomyces | Saccharomyces cerevisiae S288C | Saccharomyces Genome Database |
| Agaricus | <i>Agaricus bisporus</i> var. bisporus | OrthoDB9:Fungi* |
| Batrachochytrium | Batrachochytrium dendrobatidis JAM81 | OrthoDB9:Fungi* |
| Encephalitozoon | Encephalitozoon cuniculi | OrthoDB9:Fungi* |

*http://www.orthodb.org/?page=filelist

Supplementary Table 8. Blast search of genes involved in the degradation of plant cell wall

| | | Corresponding | | |
|---------------------------------|---------|----------------------------|------------------|------------------|
| | | gene in <i>Aspergillus</i> | | |
| | | <i>niger</i> CBS 513.88 | Blastp | tBlastn |
| Activity category in CAzy | CAZy ID | (Genbank ID) | (e-value < 1E-4) | (e-value < 1E-4) |
| | | CAK48272 | No-hit | No-hit |
| | PL4 | CAK46452 | No-hit | No-hit |
| | | CAK48551 | No-hit | No-hit |
| | | CAK48529 | No-hit | No-hit |
| | | CAK48388 | No-hit | No-hit |
| | | CAK47350 | No-hit | No-hit |
| | | CAK40523 | No-hit | No-hit |
| Polysaccharide lyases | PL1 | CAK37997 | No-hit | No-hit |
| | | AAF04492 | No-hit | No-hit |
| | GH7 | AAF04491 | No-hit | No-hit |
| | | CAK41068 | No-hit | No-hit |
| Cellobiohytdorlases | GH6 | CAK39856 | No-hit | No-hit |
| | | CAK97324 | No-hit | No-hit |
| | | CAK97151 | No-hit | No-hit |
| | | CAK46515 | No-hit | No-hit |
| | | CAK45495 | No-hit | No-hit |
| | | CAK42466 | No-hit | No-hit |
| Lytic polysaccharide mono- | | CAK41095 | No-hit | No-hit |
| oxygenases | AA9 | CAK38942 | No-hit | No-hit |
| | | CAK97324 | No-hit | No-hit |
| | | CAK46515 | No-hit | No-hit |
| | | CAK44069 | No-hit | No-hit |
| | | CAK43558 | No-hit | No-hit |
| | | CAK42930 | No-hit | No-hit |
| | | CAK42466 | No-hit | No-hit |
| Proteins with cellulose-binding | | CAK41068 | No-hit | No-hit |
| motif 1 | CBM1 | AAF04492 | No-hit | No-hit |

Query; Corresponding gene in *Aspergillus niger*, Database; all of the constructed gene models from RIR17 (for blastp search), or RIR17 genomic sequences (for tblastn search)

| | isoform ID | Mean read depth of covrage* | (rDNA) / (single-copy BUSCOs) | |
|--------|-----------------------|--------------------------------|----------------------------------|-----|
| BUSCOs | g15690.t1 | | 3 | - |
| | g16450.t1 | 160.3 | } | - |
| | g24696.t1 | 157.4 | ŀ | - |
| | g13527.t1 | 149.0 |) | - |
| | g1455.t1 | 162.1 | | - |
| | g11464.t1 | 153.4 | ļ | - |
| | g13372.t1 | 152.5 | 5 | - |
| | g31539.t1 | 155.3 | 5 | - |
| | g6363.t1 | 146.1 | | - |
| | g11459.t1 | 144.2 |) - | - |
| | Average of ten BUSCOs | 154.0 |) | - |
| rDNAs | rDNA_Consensus_IUPAC | 1733.3 | 1 1 ⁻ | 1.3 |

Supplementary Table 9. Mapped read depth of coverage to RIR17 genes.

*Calculated by the mapping of DNA short reads (Rir_DNA_PE180) to selected ten BUSCO genes and consensus rDNA sequences from ten rDNA paralogs.

| Supplemen | tary Table 10. rDNA | s in the prev | vious gen | ome asse | mblies b | ased on RN | Ammer analysis |
|-----------|---------------------|-----------------------|-----------|----------|----------|------------|-----------------|
| | | | | | | | Coresponding |
| Genome ID | Assmbly ID | rDNA type | Start | End | Strand | Score | gene on RIR17* |
| JGI_v2.0 | scaffold_373 | 28s_rRNA | 3428 | 8116 | - | 3249.9 | |
| JGI_v2.0 | scaffold_373 | 18s_rRNA | 8228 | 10038 | - | 1495.1 | C311-1 |
| JGI_v2.0 | scaffold_200 | 18s_rRNA | 86443 | 88253 | + | 1494.6 | |
| JGI_v2.0 | scaffold_200 | 28s_rRNA | 88365 | 93099 | + | 3280.4 | C312-1 |
| JGI_v2.0 | scaffold_17 | 28s_rRNA | 187376 | 191935 | - | 3283.5 | |
| JGI_v2.0 | scaffold_17 | 18s_rRNA | 192047 | 193857 | - | 1494.6 | C4-1 |
| JGI_v2.0 | scaffold_658 | 18s_rRNA | 20153 | 21963 | + | 1494.8 | C39-2(Partial) |
| JGI_v2.0 | scaffold_349 | 18s_rRNA | 122313 | 123445 | + | 164.4 | C39-1(Partial) |
| JGI_v2.0 | scaffold_159 | 28s_rRNA | 45455 | 46835 | + | 33.9 | C356-1(Partial) |
| JGI v1.0 | scaffold 3668 | 28s rRNA | 9 | 1305 | _ | 252.7 | Not analysed |
| JGI_v1.0 | scaffold_3199 | 18s_rRNA | 6 | 1498 | + | 956.7 | Not analysed |
| Lin 14 | JEMT01001954.1 | 28s rRNA | 4257 | 7785 | - | 3214.9 | Not analysed |
| Lin 14 | JEMT01015073.1 | 18s [_] rRNA | 10 | 1068 | - | 134.7 | Not analysed |
| Lin14 | JEMT01012775.1 | | 1537 | 2892 | + | 845.2 | Not analysed |

*from mummer alignment

| Supplementary | / Table 11 | . Mean intra | genomic d | lifferences | among | ten rDNA | paralogs |
|---------------|------------|--------------|-----------|-------------|-------|----------|----------|
|---------------|------------|--------------|-----------|-------------|-------|----------|----------|

| | Aligned | # differ | ence | # ind | lel | Percent identity (%) | | |
|-----------------------|-----------|----------|-------|---------|-------|----------------------|------|--|
| Region | positions | Average | SD | Average | SD | Average | SD | |
| 18SrDNA | 1811 | 1.58 | 1.09 | 0.47 | 0.50 | 99.91 | 0.06 | |
| ITS1 | 110 | 7.13 | 7.85 | 2.76 | 2.54 | 93.45 | 7.20 | |
| 5.8SrDNA | 158 | 5.29 | 5.14 | 3.00 | 3.14 | 96.65 | 3.25 | |
| ITS2 | 220 | 20.51 | 14.76 | 8.80 | 7.72 | 90.28 | 6.84 | |
| 28SrDNA | 3548 | 73.47 | 48.49 | 12.29 | 12.68 | 97.93 | 1.37 | |
| Whole 48SrDNA cluster | 5847 | 106.13 | 69.60 | 27.31 | 22.84 | 98.18 | 1.19 | |

SD = standard deviation

| | tRNAs decoding Standard 20 AA | Selenocysteine tRNAs (TCA) | Possible suppressor tRNAs (CTA, TTA) | tRNAs with undetermined/ unknown isotypes | Predicted pseudogenes | tRNAs with introns |
|-----------------------------|--|-------------------------------|---|--|--------------------------|--------------------------|
| RIR17 | 110 | 0 | 1 | 17 | 12 | 37 |
| Rhizopus oryzae | 237 | 0 | 0 | 0 | 2 | 12 |
| Phycomyces blakesleeanus | 308 | 0 | 1 | 0 | 0 | 12 |
| Mortierella elongata | 253 | 0 | 0 | 2 | 3 | 12 |
| Abisporus varbisporus | 157 | 0 | 0 | 0 | 3 | 91 |
| Saccharomyces cerevisiae | 286 | 1 | 0 | 2 | 6 | 59 |

Supplementary Table 12. Detected numbers of tRNA by tRNAscan-SE