

Complete Mitochondrial Genome Sequence of the Pezizomycete *Pyronema confluens*

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The complete mitochondrial genome of the ascomycete *Pyronema confluens* has been sequenced. The circular genome has a size of 191 kb and contains 48 protein-coding genes, 26 tRNA genes, and two rRNA genes. Of the protein-coding genes, 14 encode conserved mitochondrial proteins, and 31 encode predicted homing endonuclease genes.

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Fungal mitochondrial genomes vary greatly in size, ranging from 19 to >200 kb (1–3). They usually contain 14 genes encoding conserved proteins for oxidative phosphorylation (4); however, the number of additional genes varies greatly between species. In ascomycetes, sequenced mitochondrial genomes from the *Taphrinomycotina* and *Saccharomycotina* are <100 kb in size (5–8), whereas among the *Pezizomycotina* (filamentous ascomycetes), mitochondrial genomes can exceed 200 kb, mostly through enlargement of intronic and intergenic regions (1, 9). However, for the *Pezizomycetes*, an early diverging group of filamentous ascomycetes, no mitochondrial genomes have been described; thus, it is not clear whether the trend of genome expansion was already present in the last common ancestor of the *Pezizomycotina*. Here, the mitochondrial genome of the pezizomycete *Pyronema confluens* was sequenced to learn more about mitochondrial genome size and structure in early diverging filamentous ascomycetes.

The genomic DNA of *P. confluens* was sequenced with PacBio RS technology (BioProject no. PRJNA309361). To obtain additional mitochondrial sequences, the Illumina and 454 reads from a previous *P. confluens* genome project (10) that led to the assembly of the nuclear, but not the mitochondrial genome, were mapped to the nuclear genome with Bowtie2 (11), and nonmapping reads were extracted and the reads from bacterial contamination removed. The PacBio, Illumina, and 454 reads were assembled with SPAdes 3.6.2 (12). Among the resulting contigs, a single gapless contig of 191,189 bp contained homologs to all 14 expected conserved mitochondrial proteins. The circularity of the mitochondrial DNA was verified by PCR and Sanger sequencing of a fragment spanning the ends of the contig. Annotation was done using Prokka and MITOS (13, 14). All annotations were verified manually by BLAST analysis against GenBank (15, 16).

The genome contains genes for the conserved mitochondrial proteins for oxidative phosphorylation (*atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*), nine of which are interrupted by introns, as well as 26 tRNA genes and genes for the large and small ribosomal RNAs (*rrnL* and *rrnS*, respectively). In addition, there are 31 genes encoding putative homing endonucleases of the LAGLIDADG or GIY-YIG type, 19

of which are encoded within introns. Similar to findings in *Rhizoctonia solani* (3), truncated copies of *atp6*, *cox1*, *cox2*, *cox3*, and *nad4* reside downstream of the respective genes and are preceded by putative endonuclease genes, suggesting partial duplications of genomic regions associated with homing events. Genes for a predicted family B DNA polymerase and a reverse transcriptase often found in fungal mitochondrial plasmids (17) are also present. A gene for ribosomal protein S3 (*rps3*) was found. Similar to *Saccharomycetes*, but in contrast to many filamentous ascomycetes (18), it is not localized within an intron. In summary, the mitochondrial genome of *P. confluens* is among the largest within the fungi, indicating that a trend toward mitochondrial genome expansion was already present in the ancestor of filamentous ascomycetes.

Nucleotide sequence accession numbers. This mitochondrial genome project has been deposited in NCBI GenBank under the accession no. [KU707476](https://www.ncbi.nlm.nih.gov/nuccore/KU707476). The version described in this paper is the first version, KU707476.1.

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REFERENCES

- Mardanov AV, Beletsky AB, Kadnikov VV, Ignatov AN, Ravin NV. 2014. The 203 kbp mitochondrial genome of the phytopathogenic fungus *Sclerotinia borealis* reveals multiple invasions of introns and genomic duplications. *PLoS One* 9:e107536. <http://dx.doi.org/10.1371/journal.pone.0107536>.
- Torriani SFF, Penselin D, Knogge W, Felder M, Taudien S, Platzer M, McDonald BA, Brunner PC. 2014. Comparative analysis of mitochondrial genomes from closely related *Rhynchosporium* species reveals extensive intron invasion. *Fungal Genet Biol* 62:34–42. <http://dx.doi.org/10.1016/j.fgb.2013.11.001>.
- Losada L, Pakala SB, Fedorova ND, Joardar V, Shabalina SA, Hostetler J, Pakala SM, Zafar N, Thams E, Rodriguez-Carres M, Dean R, Vilgalys R, Nierman WC, Cubeta MA. 2014. Mobile elements and mitochondrial

- genome expansion in the soil fungus and potato pathogen *Rhizoctonia solani* AG-3. *FEMS Microbiol Lett* 352:165–173. <http://dx.doi.org/10.1111/1574-6968.12387>.
4. Lavin JL, Oguiza JA, Ramírez L, Pisabarro AG. 2008. Comparative genomics of the oxidative phosphorylation system in fungi. *Fungal Genet Biol* 45:1248–1256. <http://dx.doi.org/10.1016/j.fgb.2008.06.005>.
 5. Bullerwell CE, Leigh J, Forget L, Lang BF. 2003. A comparison of three fission yeast mitochondrial genomes. *Nucleic Acids Res* 31:759–768.
 6. Ma L, Huang DW, Cuomo CA, Sykes S, Fantoni G, Das B, Sherman BT, Yang J, Huber C, Xia Y, Davey E, Kutty G, Bishop L, Sassi M, Lempicki RA, Kovacs JA. 2013. Sequencing and characterization of the complete mitochondrial genomes of three pneumocystis species provide new insights into divergence between human and rodent pneumocystis. *FASEB J* 27:1962–1972. <http://dx.doi.org/10.1096/fj.12-224444>.
 7. Wu B, Buljic A, Hao W. 2015. Extensive horizontal transfer and homologous recombination generate highly chimeric mitochondrial genomes in yeast. *Mol Biol Evol* 32:2559–2570. <http://dx.doi.org/10.1093/molbev/msv127>.
 8. Valach M, Farkas Z, Fricova D, Kovac J, Brejova B, Vinar T, Pfeiffer I, Kucsera J, Tomaska L, Lang BF, Nosek J. 2011. Evolution of linear chromosomes and multipartite genomes in yeast mitochondria. *Nucleic Acids Res* 39:4202–4219. <http://dx.doi.org/10.1093/nar/gkq1345>.
 9. Li Y, Hu XD, Yang RH, Hsiang T, Wang K, Liang DQ, Liang F, Cao DM, Zhou F, Wen G, Yao YJ. 2015. Complete mitochondrial genome of the medicinal fungus *Ophiocordyceps sinensis*. *Sci Rep* 5:13892.
 10. Traeger S, Altegoer F, Freitag M, Gabaldon T, Kempken F, Kumar A, Marcet-Houben M, Pöggeler S, Stajich JE, Nowrousian M. 2013. The genome and development-dependent transcriptomes of *Pyronema confluens*: a window into fungal evolution. *PLoS Genet* 9:e1003820.
 11. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with bowtie 2. *Nat Methods* 9:357–359. <http://dx.doi.org/10.1038/nmeth.1923>.
 12. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <http://dx.doi.org/10.1089/cmb.2012.0021>.
 13. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <http://dx.doi.org/10.1093/bioinformatics/btu153>.
 14. Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved *de novo* metazoan mitochondrial genome annotation. *Mol Phylogenet Evol* 69:313–319. <http://dx.doi.org/10.1016/j.ympev.2012.08.023>.
 15. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2013. GenBank. *Nucleic Acids Res* 41:D36–D42.
 16. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402.
 17. Griffiths AJ. 1995. Natural plasmids of filamentous fungi. *Microbiol Res* 59:673–685.
 18. Sethuraman J, Majer A, Iranpour M, Hausner G. 2009. Molecular evolution of the mtDNA encoded rps3 gene among filamentous ascomycetes fungi with an emphasis on the ophiostomatoid fungi. *J Mol Evol* 69:372–385. <http://dx.doi.org/10.1007/s00239-009-9291-9>.