

The first complete mitochondrial genome of *Macalpinomyces bursus* (Ustilaginales: Ustilaginaceae) and insights into its phylogeny

Peng Wang^a, Tianhao Lu^a and Jingwei Huang^{a,b}

^aSchool of Preclinical Medicine, Chengdu University, Chengdu, Sichuan, P.R. China; ^bKey Laboratory of Coarse Cereal Processing, Ministry of Agriculture and Rural Affairs, Sichuan Engineering & Technology Research Center of Coarse Cereal Industrialization, School of Food and Biological Engineering, Chengdu University, Chengdu, Sichuan, P.R. China

ABSTRACT

In the present study, the complete mitochondrial genome of *Macalpinomyces bursus* (Berk.) Vanky 2002 was sequenced and assembled. The complete mitochondrial genome of *M. bursus* was 49,024 bp in length, with the GC content of 30.4%. The *M. bursus* mitochondrial genome contained 27 protein-coding genes, 2 ribosomal RNA (rRNA) genes, and 22 transfer RNA (tRNA) genes. Phylogenetic analysis based on combined mitochondrial gene dataset indicated that the *M. bursus* exhibited a close relationship with species from the genera *Ustilago*, *Sporisorium*, and *Anthracoecystis*.

ARTICLE HISTORY

Received 25 April 2021
Accepted 11 June 2021

KEYWORDS



Mitochondrial genome; phylogenetic analysis; evolution

The genus *Macalpinomyces* is a group of plant pathogenic fungi (Li et al. 2017). The three genera, including *Ustilago*, *Sporisorium*, and *Macalpinomyces*, represent an unresolved complex (McTaggart et al. 2012a, 2012b). These smut fungi from *Ustilaginomycotina* contain about 540 described species. Species from the complex often possess characters that occur in more than one genus, creating uncertainty for species placement (McTaggart et al. 2016). Previous studies have shown that it is difficult to identify genus from this complex only by morphology (McTaggart, Shivas, Geering, Callaghan, et al. 2012; Stoll et al. 2005). Mitochondrial genomes have been widely used in the phylogenetic analysis of fungal species (Li, He, et al. 2020; Wang, Song, et al. 2020; Zhang et al. 2017). However, up to now, no complete mitochondrial genome from the genus *Macalpinomyces* has been reported. The complete mitochondrial genome of *Macalpinomyces bursus* (Berk.) Vanky 2002 will promote the understanding of phylogeny, evolution, and taxonomy of this important fungal genus.

The specimen (*M. bursus*) was collected from Yunnan, China (101.24 E; 24.89 N). The specimen was then deposited at Collection Center of Chengdu University (contact information: Jingwei Huang, email: huangjingwei1003@qq.com) under the voucher number of Mbur_s99. The complete mitochondrial genome of *M. bursus* was sequenced and *de novo* assembled according to previous described methods (Li, Ren, et al. 2019; Li, Xiang, et al. 2019; Wang, Song, et al. 2020; Wang, Wang, et al. 2020). First, we extracted the total genomic DNA of *M. bursus* using a Fungal DNA Kit (D3390-00, Omega Bio-Tek, Norcross, GA, USA). Then, the extracted genomic DNA was purified using a Gel Extraction Kit (Omega

Bio-Tek, Norcross, GA, USA). The purified DNA was stored in Chengdu University (No. DNA_ Mbur_s99). We constructed sequencing libraries using a NEBNext® Ultra™ II DNA Library Prep Kit (NEB, Beijing, China). Whole genomic sequencing of *M. bursus* was conducted using the Illumina HiSeq 2500 Platform (Illumina, San Diego, CA). We *de novo* assembled the mitochondrial genome of *M. bursus* using NOVOPlasty v4.3.1 (Dierckxsens et al. 2017; Li, Ren, et al. 2020). The complete mitochondrial genome of *M. bursus* was annotated according to previous described methods (Li et al. 2018; Li, Li, et al. 2021). Briefly, the protein-coding genes, rRNA genes, tRNA genes, and introns of the *M. bursus* mitochondrial genome were annotated using MITOS (Bernt et al. 2013) and MFannot (Valach et al. 2014), both based on the genetic code 4. We also predicted protein-coding genes (PCGs) or open reading frames (ORFs) based on the NCBI ORF Finder (Coordinators 2017), and annotated by BLASTP searches against the NCBI non-redundant protein sequence database (Bleasby and Wootton 1990). The tRNA genes in the *M. bursus* mitogenome were also predicted with tRNAscan-SE v1.3.1 (Lowe and Chan 2016).

The complete mitochondrial genome of *M. bursus* is 49,024 bp in length. The base composition of the *M. bursus* mitochondrial genome is as follows: A (34.15%), T (35.45%), G (15.09%), and C (15.31%). The complete mitochondrial genome of *M. bursus* contains 27 protein-coding genes, 2 ribosomal RNA genes (*rms* and *rnl*), and 22 transfer RNA genes. The genome size and GC content of *M. bursus* were the lowest in the *Ustilago*, *Sporisorium*, and *Macalpinomyces* complex. A total of 6 introns were detected in the PCGs of *M. bursus* mitogenome, including Mbu.cox1P386, Mbu.cox1P709, Mbu.cox1P971,

CONTACT Jingwei Huang  huangjingwei1003@qq.com  School of Preclinical Medicine, Chengdu University, 2025# Chengluo Avenue Chengdu 610106, Sichuan, China

© 2021 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

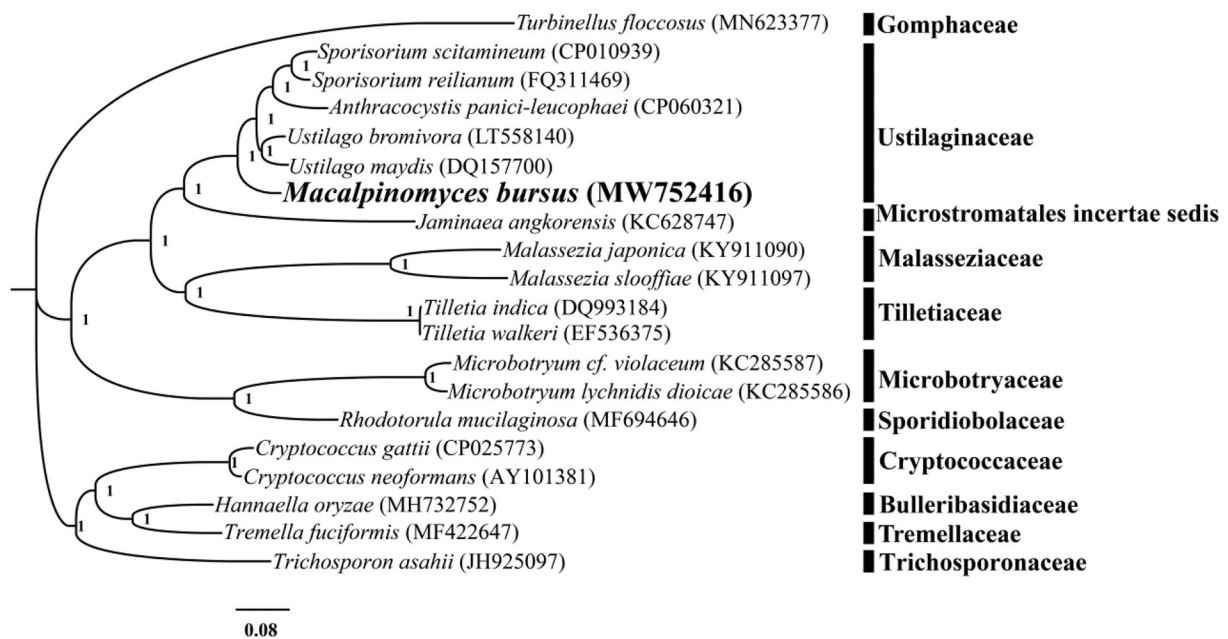


Figure 1. Bayesian phylogenetic analysis of 20 species based on the combined 14 core protein-coding genes. Accession numbers of mitochondrial sequences used in the phylogenetic analysis are listed in brackets after species.

Mbu.cobP393, Mbu.cobP429, and Mbu.cobP490, which were named according to previous studies (Zhang and Zhang 2019). We constructed a phylogenetic tree for 20 basidiomycete species to investigate the phylogenetic status of *M. bursus*. *Turbinellus floccosus* from the order Gomphales was set as outgroup (Cheng et al. 2021). The Bayesian analysis (BI) method was used to construct phylogenetic tree based on the combined 14 core protein-coding genes according to previous described methods (Li, Wu, et al. 2021; Li, Yang, et al. 2020). Briefly, single mitochondrial genes were first aligned using MAFFT v7.037 (Katoh et al. 2019), and then, we concatenated these alignments to a gene set using the SequenceMatrix v1.7.8 (Vaidya et al. 2011). Best-fit models of evolution and partitioning schemes for the gene set were determined according to PartitionFinder 2.1.1 (Lanfear et al. 2017). MrBayes v3.2.6 (Ronquist et al. 2012) was used to analyze the phylogenetic relationships of the 20 basidiomycete species based on the combined gene set. As shown in the phylogenetic tree (Figure 1), the mitochondrial genome of *M. bursus* exhibited a close relationship with species from the genera *Ustilago*, *Sporisorium*, and *Anthracocystis*.

Disclosure statement

The authors have declared that no competing interests exist.

Funding

The study was supported by Innovation and Entrepreneurship of Chengdu University Students [grant number S202011079105].

Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI at (<https://www.ncbi.nlm.nih.gov/>)

under the accession no. MW752416. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA724891, SRR14320034, and SAMN18864185, respectively.

References

- Bernt M, Donath A, Juhling F, Externbrink F, Florentz C, Fritzsche G, Putz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. *Mol Phylogenet Evol.* 69(2): 313–319.
- Bleasby AJ, Wootton JC. 1990. Construction of validated, non-redundant composite protein sequence databases. *Protein Eng.* 3(3):153–159.
- Cheng J, Luo Q, Ren YH, Luo Z, Liao WL, Wang X, Li Q. 2021. Panorama of intron dynamics and gene rearrangements in the phylum Basidiomycota as revealed by the complete mitochondrial genome of *Turbinellus floccosus*. *Appl Microbiol Biotechnol.* 105(5):2017–2032.
- Coordinators NR. 2017. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res.* 44(D1):D7–D19.
- Dierckx N, Mardulyn P, Smits G. 2017. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic Acids Res.* 45(4):e18.
- Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Brief Bioinform.* 20(4):1160–1166.
- Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Mol Biol Evol.* 34(3):772–773.
- Li Q, Chen C, Xiong C, Jin X, Chen Z, Huang W. 2018. Comparative mitogenomics reveals large-scale gene rearrangements in the mitochondrial genome of two *Pleurotus* species. *Appl Microbiol Biotechnol.* 102(14):6143–6153.
- Li Q, He X, Ren Y, Xiong C, Jin X, Peng L, Huang W. 2020. Comparative mitogenome analysis reveals mitochondrial genome differentiation in ectomycorrhizal and asymbiotic amanita species. *Front Microbiol.* 11: 1382.
- Li Q, Li L, Feng H, Tu W, Bao Z, Xiong C, Wang X, Qing Y, Huang W. 2021. Characterization of the complete mitochondrial genome of Basidiomycete yeast *Hannaella oryzae*: intron evolution, gene rearrangement and its phylogeny. *Front Microbiol.* 12:646567.
- Li Q, Ren Y, Shi X, Peng L, Zhao J, Song Y, Zhao G. 2019. Comparative mitochondrial genome analysis of two ectomycorrhizal fungi

- (Rhizopogon) reveals dynamic changes of intron and phylogenetic relationships of the subphylum Agaricomycotina. *IJMS*. 20(20):5167.
- Li Q, Ren Y, Xiang D, Shi X, Zhao J, Peng L, Zhao G. 2020. Comparative mitogenome analysis of two ectomycorrhizal fungi (*Paxillus*) reveals gene rearrangement, intron dynamics, and phylogeny of basidiomycetes. *IMA Fungus*. 11(1):12.
- Li Q, Wu P, Li L, Feng H, Tu W, Bao Z, Xiong C, Gui M, Huang W. 2021. The first eleven mitochondrial genomes from the ectomycorrhizal fungal genus (*Boletus*) reveal intron loss and gene rearrangement. *Int J Biol Macromol*. 172:560–572.
- Li Q, Xiang D, Wan Y, Wu Q, Wu X, Ma C, Song Y, Zhao G, Huang W. 2019. The complete mitochondrial genomes of five important medicinal *Ganoderma* species: features, evolution, and phylogeny. *Int J Biol Macromol*. 139:397–408.
- Li Q, Yang L, Xiang D, Wan Y, Wu Q, Huang W, Zhao G. 2020. The complete mitochondrial genomes of two model ectomycorrhizal fungi (*Laccaria*): features, intron dynamics and phylogenetic implications. *Int J Biol Macromol*. 145:974–984.
- Li YM, Shivas RG, McTaggart AR, Zhao P, Cai L. 2017. Ten new species of *Macalpinomyces* on *Eriachne* in northern Australia. *Mycologia*. 109(3): 408–421.
- Lowe TM, Chan PP. 2016. tRNAscan-SE On-line: integrating search and context for analysis of transfer RNA genes. *Nucleic Acids Res*. 44(W1): W54–W57.
- McTaggart AR, Shivas RG, Boekhout T, Oberwinkler F, Vanky K, Pennycook SR, Begerow D. 2016. *Mycosarcoma* (Ustilaginaceae), a resurrected generic name for corn smut (*Ustilago maydis*) and its close relatives with hypertrophied, tubular sori. *IMA Fungus*. 7(2):309–315.
- McTaggart AR, Shivas RG, Geering AD, Callaghan B, Vanky K, Scharaschkin T. 2012. Soral synapomorphies are significant for the systematics of the *Ustilago-Sporisorium-Macalpinomyces* complex (Ustilaginaceae). *Persoonia*. 29:63–77.
- McTaggart AR, Shivas RG, Geering AD, Vanky K, Scharaschkin T. 2012a. A review of the *Ustilago-Sporisorium-Macalpinomyces* complex. *Persoonia*. 29:55–62.
- McTaggart AR, Shivas RG, Geering AD, Vanky K, Scharaschkin T. 2012b. Taxonomic revision of *Ustilago*, *Sporisorium* and *Macalpinomyces*. *Persoonia*. 29:116–132.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol*. 61(3):539–542.
- Stoll M, Begerow D, Oberwinkler F. 2005. Molecular phylogeny of *Ustilago*, *Sporisorium*, and related taxa based on combined analyses of rDNA sequences. *Mycol Res*. 109(Pt 3):342–356.
- Vaidya G, Lohman DL, Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics*. 27(2):171–180.
- Valach M, Burger G, Gray MW, Lang BF. 2014. Widespread occurrence of organelle genome-encoded 5S rRNAs including permuted molecules. *Nucleic Acids Res*. 42(22):13764–13777.
- Wang X, Song A, Wang F, Chen M, Li X, Li Q, Liu N. 2020. The 206 kbp mitochondrial genome of *Phanerochaete carnosae* reveals dynamics of introns, accumulation of repeat sequences and plasmid-derived genes. *Int J Biol Macromol*. 162:209–219.
- Wang X, Wang YJ, Yao W, Shen JW, Chen MY, Gao M, Ren JN, Li Q, Liu N. 2020. The 256 kb mitochondrial genome of *Clavaria fumosa* is the largest among phylum Basidiomycota and is rich in introns and intronic ORFs. *IMA Fungus*. 11(1):26. 10.1186/S43008-020-00047-7 doi: Artn 26
- Zhang S, Zhang YJ. 2019. Proposal of a new nomenclature for introns in protein-coding genes in fungal mitogenomes. *IMA Fungus*. 10(15):15.
- Zhang YJ, Zhang HY, Liu XZ, Zhang S. 2017. Mitochondrial genome of the nematode endoparasitic fungus *Hirsutella vermicola* reveals a high level of synteny in the family Ophiocordycipitaceae. *Appl Microbiol Biotechnol*. 101(8):3295–3304.