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MITOGENOME REPORT

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The complete mitochondrial genome of the basidiomycetous fungus, *Tinctoporellus epimiltinus* strain RS1

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ABSTRACT

Tinctoporellus epimiltinus is widely known as a wood-decaying fungus. In the present study, we identified the complete mitochondrial genome of this species using next-generation sequencing technology. Our findings revealed that the genomic structure is a circular molecule with a size of 51,878 bp. Consistent with most Basidiomycota species, it consists of 14 core protein-coding genes, one ribosomal protein gene (*rps3*), 26 transfer RNA genes, and small and large ribosomal RNA (*rns* and *rnl*) genes. Seven additional open reading frames were identified. These included two sequences similar to DNA polymerases, an endonuclease-like sequence, and four hypothetical proteins. The mitochondrial genome exhibited a nucleotide composition of A (36.24%), C (12.04%), G (13.18%), and T (38.55%), resulting in a 25.21% GC content. A phylogenetic tree constructed using the combined mitochondrial gene dataset provided insight into the phylogenetic relationships of this species within the context of Basidiomycota and its members.

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KEYWORDS
Basidiomycota; Illumina sequencing; phylogenetic analysis

Introduction

Tinctoporellus epimiltinus, a species primarily distributed across the pantropical regions (Yuan and Wan 2012), was initially identified as *Polyporus epimiltinus* by Berkeley and Broome in 1873. In 1979, Ryvarden proposed the monospecific genus *Tinctoporellus*, designating *T. epimiltinus* as the type species. Since then, this nomenclature has been widely used. *T. epimiltinus* is a well-known wood-decaying fungus characterized by white rot and a reddish zone-line appearance (Kubayashi et al. 2001). Alongside *T. epimiltinus*, the introduction of additional species has expanded the genus *Tinctoporellus*. In addition, a recent phylogenetic analysis revealed that the type species of *Tinctoporellus* is nested within the same clade as *Porogramme*, and *Tinctoporellus* is considered a synonym for *Porogramme* (Mao et al. 2023). In terms of biotechnological applications, *T. epimiltinus* has shown potential for decolorizing industrial textile effluents (Sanchez-Lopez et al. 2008). Despite these advancements, molecular research on *Tinctoporellus* remains in the early stages of development. Although the draft genome of *T. epimiltinus* has been reported (Subramaniam et al. 2019), the complete mitochondrial genome of this genus has not been sequenced. Mitochondria play a pivotal role in the growth and development of fungi (Basse 2010) and serve as valuable genetic markers (Dong et al. 2021). This study presents the first report of the mitochondrial genome of *T. epimiltinus* and contributes to a deeper understanding of the genetics of this species.

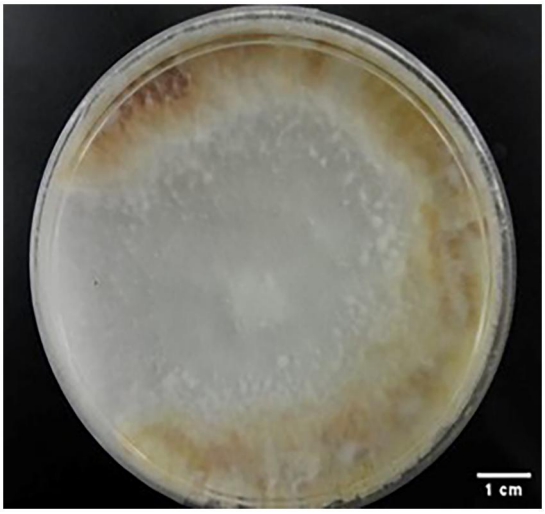

Figure 1. The sample of *T. epimiltinus* strain RS1 which was grown on potato dextrose agar (PDA) plate. The photograph, taken by Ranjita Subramaniam, depicts the stage where the mat is predominantly white, transitioning to light orange and vinaceous at the margins in an irregular pattern.

Materials

The fungal specimen designated *T. epimiltinus* strain RS1 (Figure 1) was isolated using fungal colony purification techniques. It grew alongside colonies of *Trichoderma* spp. in a mixed culture plate. Originally sourced from soil samples from an oil palm plantation in Lahad Datu, Sabah, Malaysian Borneo

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(N5.0608, E118.9198), the specimen was cultured in pure form. The isolate was deposited in the Biotechnology Research Institute collection repository, Universiti Malaysia Sabah (www.ums.edu.my/ipbv2; Vijay Kumar is the contact person: vijay@ums.edu.my) under the voucher number UMS/BRI/TE-RS1/2019.

Methods

Genomic DNA extracted using a modified cetyltrimethylammonium bromide (CTAB) method was converted into a sequencing-ready library and subsequently sequenced on an Illumina MiSeq platform with 150-bp paired-end reads (Subramaniam et al. 2019). To ensure robust mitogenome validation, two independent approaches were employed for mitochondrial DNA assembly. In the first method, raw reads were subjected to adapter removal *via* Trimmomatic-0.33 (Bolger et al. 2014). NOVOPlasty version 4.3.3 (Dierckxsens et al. 2017) was then used to assemble the mitochondrial genome of *T. epimiltinus* using *Trametes cingulata* (NCBI accession no. NC_013933.1) as the seed sequence. To assess the depth coverage, Python scripts outlined by Ni et al. (2023) were employed. In the second approach, the CLC Genomic Workbench version 6.5.1 (CLC, Inc., Aarhus, Denmark) was used for assembly, producing a nuclear genome (previously reported by Subramaniam et al. 2019) along with a single mitogenome contig. The circularity of the mitogenome was confirmed by Sanger sequencing of the region connecting both ends of the contig. The supplementary material includes a list of primers (Supplementary Table S1) used for PCR amplification and Sanger sequencing, the generated Sanger sequences (Supplementary Figure S1), and the alignment of the Sanger sequences to the mitochondrial contig (Supplementary Figure S2). Finally, contigs from both approaches with different start and end points were compared using a BLASTn pairwise alignment, which revealed

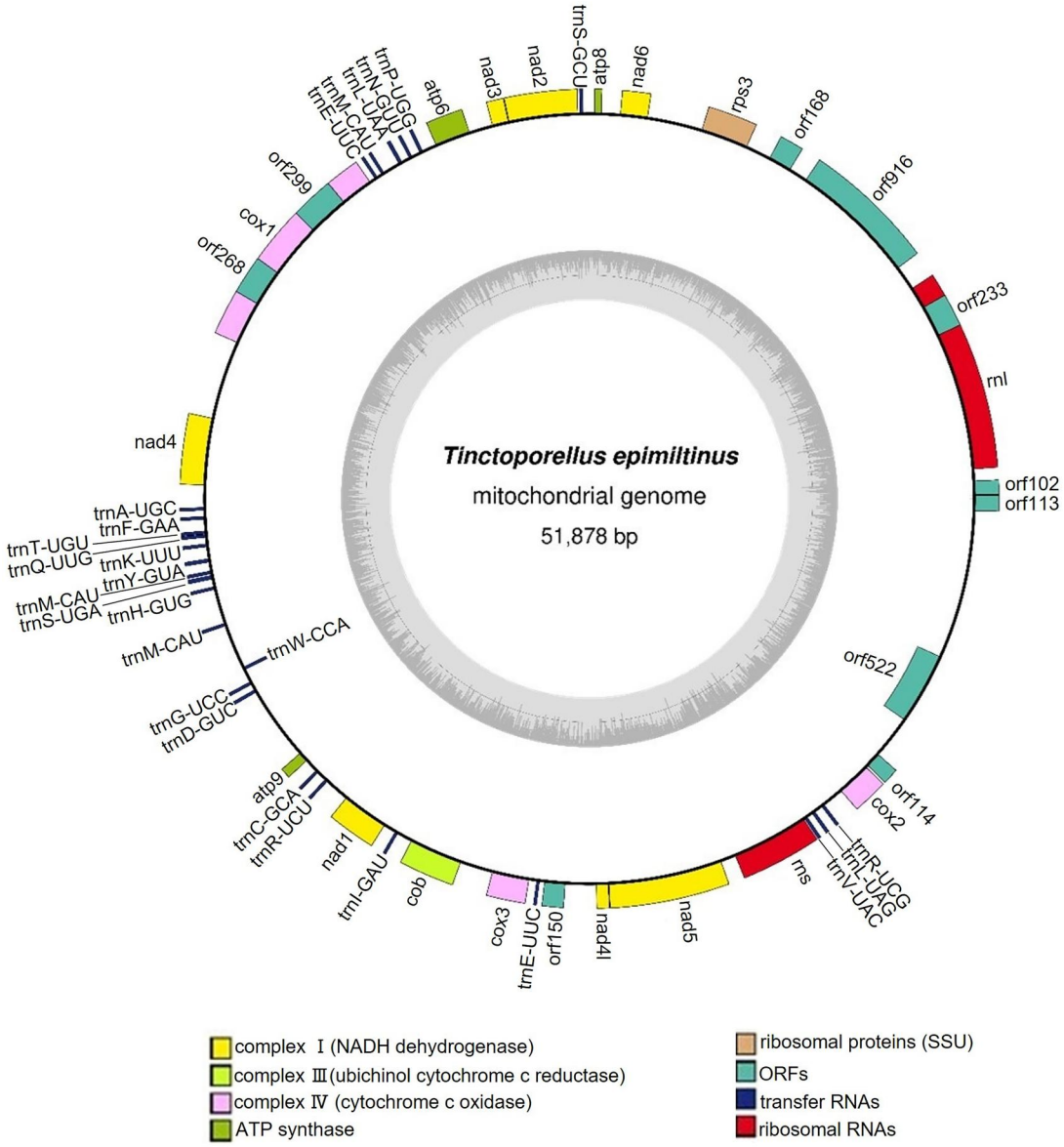

Figure 2. The circular map of the mitogenome of *T. epimiltinus* strain RS1, prepared using OGDRAW program version 1.3.1 (Greiner et al. 2019) (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html). Genes located on the clockwise strand are depicted outside the circle, while genes on the anti-clockwise strand are shown inside. GC and AT contents across the genome are shown with dark and light shading, respectively.

identical lengths and sequences. Gene annotation was performed on the NOVOPlasty-derived contig using Mfannot (https://megasun.bch.umontreal.ca/apps/mfannot/). The presence of introns has been verified by Rnaweasel (https://megasun.bch.umontreal.ca/apps/rnaweasel/). The exon–intron boundaries of protein-coding genes (PCGs) were manually refined using annotated reference sequences of *Trametes cingulata* and compared with species from NCBI BlastP searches. Transfer RNA (tRNA) genes were annotated using tRNAscan-SE 2.0 (Lowe and Chan 2016) (http://lowelab.ucsc.edu/tRNAscan-SE/). Gene structures containing introns were visualized using PMGmap (http://www.1kmpg.cn/pmgmap) (Zhang et al. 2024). Based on 14 PCGs from *T. epimiltinus* and other members of Basidiomycota, a maximum likelihood phylogeny was inferred using MegaX version 10.2.6 (Kumar

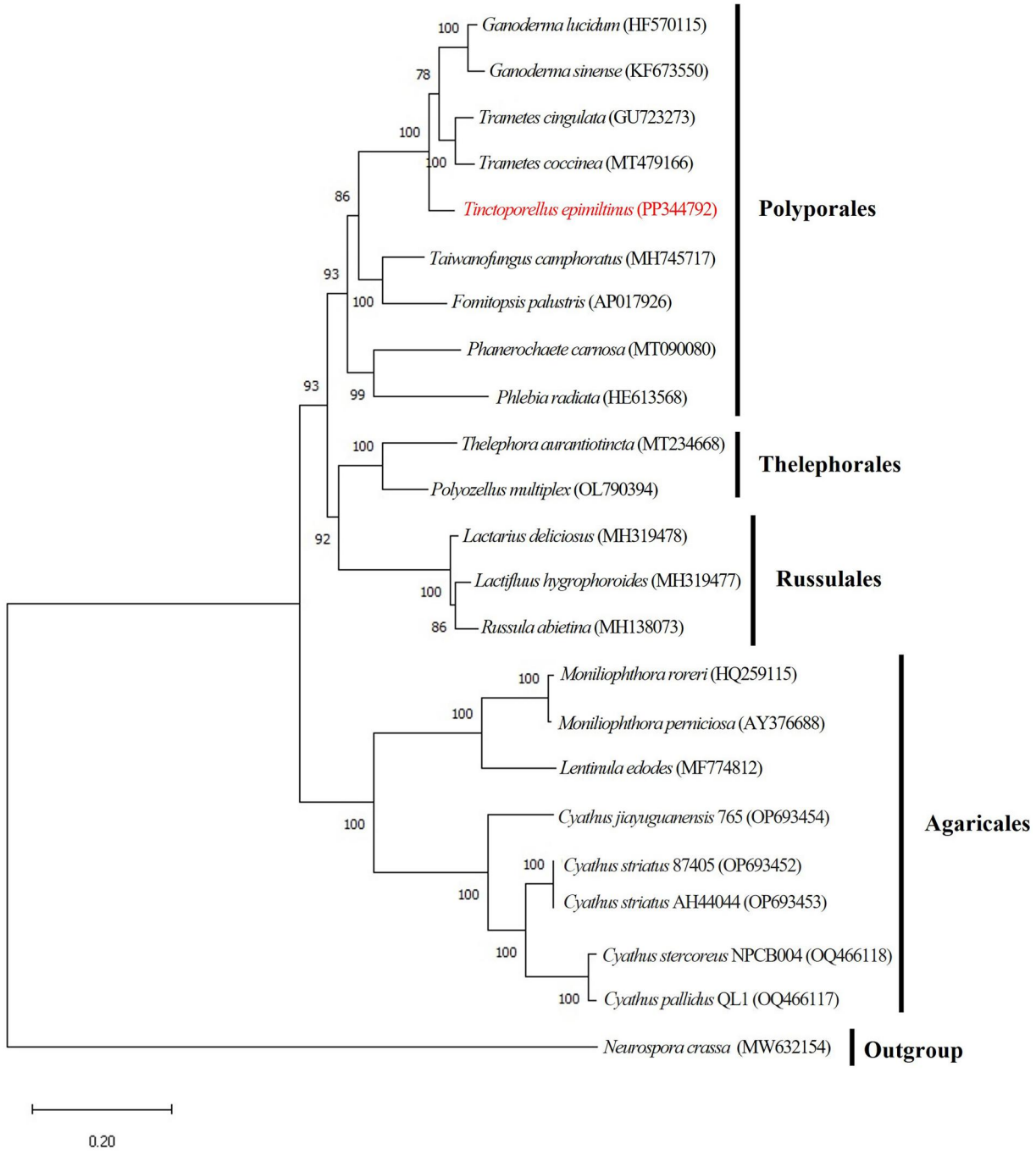

Figure 3. Phylogenetic tree of *T. epimiltinus* strain RS1 and related taxa based on maximum likelihood method using concatenated amino acid sequences of 14 core protein-coding genes, including *atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4l*, *nad5*, and *nad6*. *Neurospora crassa* was used as an outgroup. The bootstrap values are indicated at each node. GenBank accession numbers are displayed in brackets. The following species are included: *Tinctoporellus epimiltinus* (this study), *Ganoderma lucidum* (Li et al. 2013), *Ganoderma sinense* (unpublished), *Trametes cingulata* (Haridas and Gantt 2010), *Trametes coccinea* (Chen et al. 2021), *Taiwanofungus camphoratus* (Wang et al. 2020), *Fomitopsis palustris* (Tanaka et al. 2017), *Phanerochaete carnosa* (Wang et al. 2020), *Phlebia radiata* (Salavirta et al. 2014), *Thelephora aurantiotincta* (Chen et al. 2021), *Polyozellus multiplex* (Liu et al. 2022), *Lactarius deliciosus* (Li et al. 2019), *Lactifluus hygrophoroides* (Li et al. 2019), *Russula abietina* (Li et al. 2018), *Moniliophthora roreri* (Costa et al. 2012), *Moniliophthora perniciosa* (Formighieri et al. 2008), *Lentinula edodes* (Song et al. 2019), *Cyathus jiayuguanensis* 765 (Li et al. 2023), *Cyathus striatus* 87405 (Li et al. 2023), *Cyathus striatus* AH44044 (Li et al. 2023), *Cyathus stercoreus* NPCB004 (Li et al. 2023), *Cyathus pallidus* QL1 (Li et al. 2023), *Neurospora crassa* (Monteiro et al. 2021).

et al. 2018), with the substitution model LG + G + F and 1000 bootstrap replications.

Results

The complete mitochondrial genome sequence of *T. epimiltinus* strain RS1 has a total length of 51,878 bp with an average coverage of 3895.54 × (Supplementary Figure S3). The nucleotide composition was 36.24% A, 12.04% C, 13.18% G, and 38.55% T, with a GC content of 25.21%. The genome has been validated to be circular in structure and consists of 14 core PCGs associated with respiratory chain complexes (*atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4l*, *nad5*, and *nad6*), one ribosomal protein gene (*rps3*), 26 tRNA genes, and two rRNA genes (*rns* and *rnl*). The gene map is shown in Figure 2. Notably, LAGLIDADG endonuclease sequences were detected in the intronic regions of *cox1* (*orf299* and *orf268*) and *rnl* (*orf233*). The structures of the cis-spliced introns are shown in Supplementary Figure S4. An overlapping region (one base pair) was detected between *nad4l* and *nad5* (1 bp). Additionally, seven open reading frames (ORFs) were identified, including two similar to DNA polymerase genes (*orf916* and *orf522*), one free-standing LAGLIDADG endonuclease sequence (*orf168*), and four others that encode hypothetical proteins (*orf102*, *orf113*, *orf150*, and *orf114*). All mitogenomic genes were positioned on the plus strand, except for *orf522* and *tRNA-Trp*. Fourteen PCGs and *rps3* were initiated with the start codon ATG, whereas the stop codons were either TAA (8 out of 15 genes) or TAG (7 out of 15 genes). Furthermore, phylogenetic analysis placed *T. epimiltinus* within the order Polyporales, exhibiting close relationships with members such as *Ganoderma* and *Trametes* (Figure 3), thus providing valuable insights into its evolutionary context.

Discussion and conclusion

In this study, we successfully sequenced and assembled the complete mitochondrial genome of *T. epimiltinus* strain RS1. The consistent lengths and sequences of the mitogenome obtained from the two different assembly approaches validated the accuracy of the genome. The identification of 14 core PCGs, along with the *rps3* protein gene, aligned with the typical genetic composition of members of Basidiomycota. Intriguingly, we also discovered intronic regions among the genes *cox1* and *rnl* that housed endonuclease-like sequences, whereas others remained intronless. Endonuclease-like sequences within introns imply a mechanism for the variable mitochondrial genome sizes (Haridas and Gantt 2010). The mitogenome of *T. epimiltinus* also exhibits an overlapping region between two genes (*nad4l* and *nad5*); this phenomenon is not unique to *Tinctoporellus*, as it has been observed in several fungal mitochondrial genomes (Cai and Scofield 2020; Li et al. 2020). Overlap between genes has been proposed to potentially extend genetic information within a restricted genome size (Sun et al. 2020). The phylogeny also highlighted *T. epimiltinus*' relationship with other members of Basidiomycota, particularly its clustering among Polyporales. Notably, *T. epimiltinus* shares a close relationship with *Ganoderma* and *Trametes*. The insights gained from this study offer initial perspectives on the mitochondrial genetics of *Tinctoporellus*, and provide valuable information for future research on this genus.

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Author contributions

RS, VSK and SS were responsible for sample collection, data analysis, interpretation, and manuscript preparation. VSK conceptualized the project, provided critical revisions to the manuscript, and approved the final version for publication. All authors participated in discussions, critically reviewed the results, and contributed to the final version of the manuscript.

Ethical approval

This research does not involve any ethical issues. The sample collection for this study did not require any permissions, as the specimen is neither an endangered nor protected species and there are no ethical considerations involved in using them in experiments.

Disclosure statement

The authors have declared that no competing interests exist.

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Data availability statement

The genome sequence data, supporting the results of this study, can be openly accessed on the NCBI GenBank at https://www.ncbi.nlm.nih.gov/ with accession no. PP344792. The corresponding BioProject, SRA, and BioSample are PRJEB11981, ERS992373, and SAMEA3685224, respectively.

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