

The first complete mitochondrial genome of *Trichaptum biforme* (Fr.) Ryvar den 1972 within the family Trichaptaceae, Hymenochaetales

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ABSTRACT

Trichaptum biforme, a globally recognized white-rot Basidiomycete, is a member of the Hymenochaetales order. This research focused on a specimen of *T. biforme* MES02, which was obtained from the Qinling Mountain range in China, specifically from Zhashui County, Shangluo City, Shaanxi Province. Employing next-generation sequencing (NGS) technology, the whole mitochondrial genome of *T. biforme* MES02 was elucidated. This genome encompasses 15 protein-coding genes (PCGs), 24 transfer RNAs (tRNAs), and two ribosomal RNAs, spanning a total of 55,598 base pairs (bp). The nucleotide composition was determined to be A (31.80%), C (16.94%), G (18.30%), and T (32.96%), with a GC content of 35.24%. Furthermore, a phylogenetic analysis was conducted, constructing a tree from 27 mitochondrial genomes. This study presents the first insight into the phylogenetic interrelations of *T. biforme* with its alld Basidiomycetes, providing a foundational framework for further evolutionary and taxonomic inquiries within this fungal group.

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Introduction

The species of *Trichaptum* constitute a cosmopolitan assemblage of basidiomycetes, distinguished by their distinctive morphological attributes. These fungi produce fruiting bodies that display a chromatic array from purplish red to violet hues, with cylindrical spores contained within their hyphae (Thiago Kossmann et al. 2021). Initially, *Trichaptum* was categorized within the family Polyporaceae. However, subsequent taxonomic revisions have reassigned this genus to the order Hymenochaetales (Hibbett and Donoghue 1995; Larsson et al. 2006). As a prevalent genus of wood-decay fungi, *Trichaptum* is instrumental in inducing white rot across a diverse spectrum of arboreal species. This genus serves a dual ecological role, both as a catalyst in the natural wood decay process within forest ecosystems and as a mediator of ecological equilibrium through the decomposition of lignin and selective breakdown of cellulose (Blanchette 1991). *T. biforme* (Fr.) Ryvar den 1972 (Ryvar den 1972) stands out as a significant constituent of this genus, with a pivotal role in the degradation of lignin (Blanchette 1991) and specific chemical substances (Hosseini Eshghi 2011), and its lignocellulolytic enzyme system has been recently linked to efficient bioremediation of organic pollutants (Chen et al. 2021;

Xie et al. 2025). *Pallidohirschioporus biformis* is its synonymous homologue, and the name was established in 2023 (Zhou et al. 2023). Despite its ecological significance, the genetic underpinnings of *T. biforme* remain largely unexplored, underscoring the need for further research to elucidate its genetic composition and its broader implications for forest ecology and fungal taxonomy. Despite its ecological significance, the genetic underpinnings of *T. biforme* remain largely unexplored, underscoring the need for further research to elucidate its genetic composition and its broader implications for forest ecology and fungal taxonomy. Recently, large-scale mitogenomic studies have significantly expanded our understanding of fungal mitochondrial diversity. A 2025 study assembled complete mitochondrial genomes from 2,695 fungal species, nearly tripling the previously available records. Within Hymenochaetales, the mitochondrial genome of *Phellinus pomaceus* (122,850 bp) was recently characterized, revealing unique features including 24 intronic ORFs (Gao et al. 2024). Comparative mitogenomic analyses have also distinguished *Sanguangporus* species from related fungi based on mitochondrial phylogenomics (Feng et al. 2024). However, despite these advances, the family Trichaptaceae remains underrepresented in mitochondrial

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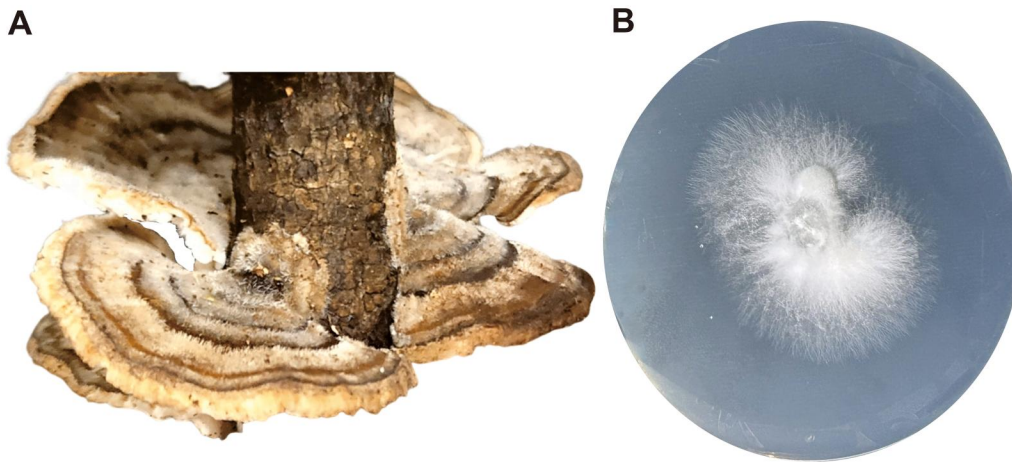


Figure 1. The fruit body of *T. biforme* collected from Shaanxi Province, China. Fruiting bodies of *T. biforme* on dead angiosperm wood, showing imbricate, effused-reflexed pilei with zonate, tomentose upper surfaces and a poroid-irpiciform hymenophore. Photograph by Jing Wei (A). Mycelial culture of *T. biforme* on agar medium, showing white, radiating aerial hyphae with a cottony texture. (B).

genomic databases (Sarhan et al. 2025), highlighting the necessity of the present study. In the present study, the complete mitochondrial genome of the *T. biforme* was sequenced and annotated, and the phylogenetic relationships with related species were analyzed by two evolutionary tree construction methods.

Materials

The specimen of *T. biforme* (Figure 1A) was collected from Niubeiliang National Forest Park, located in Qinling Mountain, Shaanxi Province, China (108°59'34.89"E, 33°52'15.30"N) and its mycelium (Figure 1B) was deposited in the Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Chemistry & Pharmacy, Northwest A&F University (<https://npcb.nwafu.edu.cn>) under the voucher number MES02 (contact Jianzhao Qi, qjz@nwafu.edu.cn). This specimen was identified by morphological characters (Figure 1A) and ITS sequence (GenBank accession no. PP872148, Figure S1). Ethical approval was not required for this study.

Methods

Total genomic DNA was extracted from the fresh mycelial tissue using the CTAB protocol. Subsequently, the sequencing was performed on an Illumina NovaSeq 6000 platform with a 2 × 150 bp paired-end read length by Biozeron Co., Ltd (Shanghai, China). For the assembly process, we employed GetOrganelle v1.7.7.1 (Jin et al. 2020), utilizing the fungal mitochondrial database (-F fungus_mt) to identify, filter, and assemble the reads associated with the target organelle. The resulting sequences were annotated using the MITOS Web Server (Donath et al. 2019), applying the genetic code 4. The predicted PCGs were further refined with the aid of the open reading frame (ORF) finder tool provided by the National Center for Biotechnology Information (NCBI, <https://www.ncbi.nlm.nih.gov/orffinder/>). Transfer RNA (tRNA) genes were identified using tRNAscan-SE v1.3.1 (Chan and Lowe 2019). Intron types were verified using the RNAweasel v5.2.1

(Lang et al. 2007) software. Lastly, the gene map was visualized using the PMGmap tool (<http://www.1kmpg.cn/pmgmap>).

A comprehensive dataset of 27 mitochondrial genomes was obtained from the NCBI database, covering a wide range of fungal species. The sample set specifically included fifteen representatives of the order Hymenochaetales, along with additional species from several other orders: three from Russulales, three from Polyporales, three from Phallales, one from Cantharellales, and two from Auriculariaceae, which were designated as outgroups. From these genomes, a total of 15 core PCGs were meticulously extracted and aligned using the MAFFT v7.526 software (Rozewicki et al. 2019), using the default parameters to ensure consistency and reliability in the alignment process. These individual protein sequences were then concatenated into a single, comprehensive dataset using the PhyloSuite v1.2.2 platform (Zhang et al. 2020). The concatenated alignment was subjected to phylogenetic analysis using two different methods: Bayesian inference (BI) and maximum likelihood (ML). The BI analysis was performed using MrBayes v3.2.7 (Ronquist et al. 2012), with the analysis running for one million generations and automatically stopping when the average standard deviation of the split frequencies fell below the 0.01 threshold. IQ-Tree v 2.0.3 (Minh et al. 2020) was used for ML analysis, and bootstrap support (BS) was determined using an ultra-fast bootstrap approximation with 1,000 replicates to assess the robustness of phylogenetic nodes. The specific methods and parameter settings used to construct the evolutionary trees, as well as the rationale for the analytical approaches chosen, are detailed in the relevant literature (Li et al. 2023).

Results

The complete mitochondrial genome sequence of *T. biforme* (GenBank accession no. OR857860) was determined to span 55,598 base pairs, with a mean coverage of 2156.84-fold, as illustrated in Figure S2, and detailed in Figure 2. This genome encodes 15 core PCGs, namely *atp6*, *atp8*, *atp9*, *cob*, *cox1*,

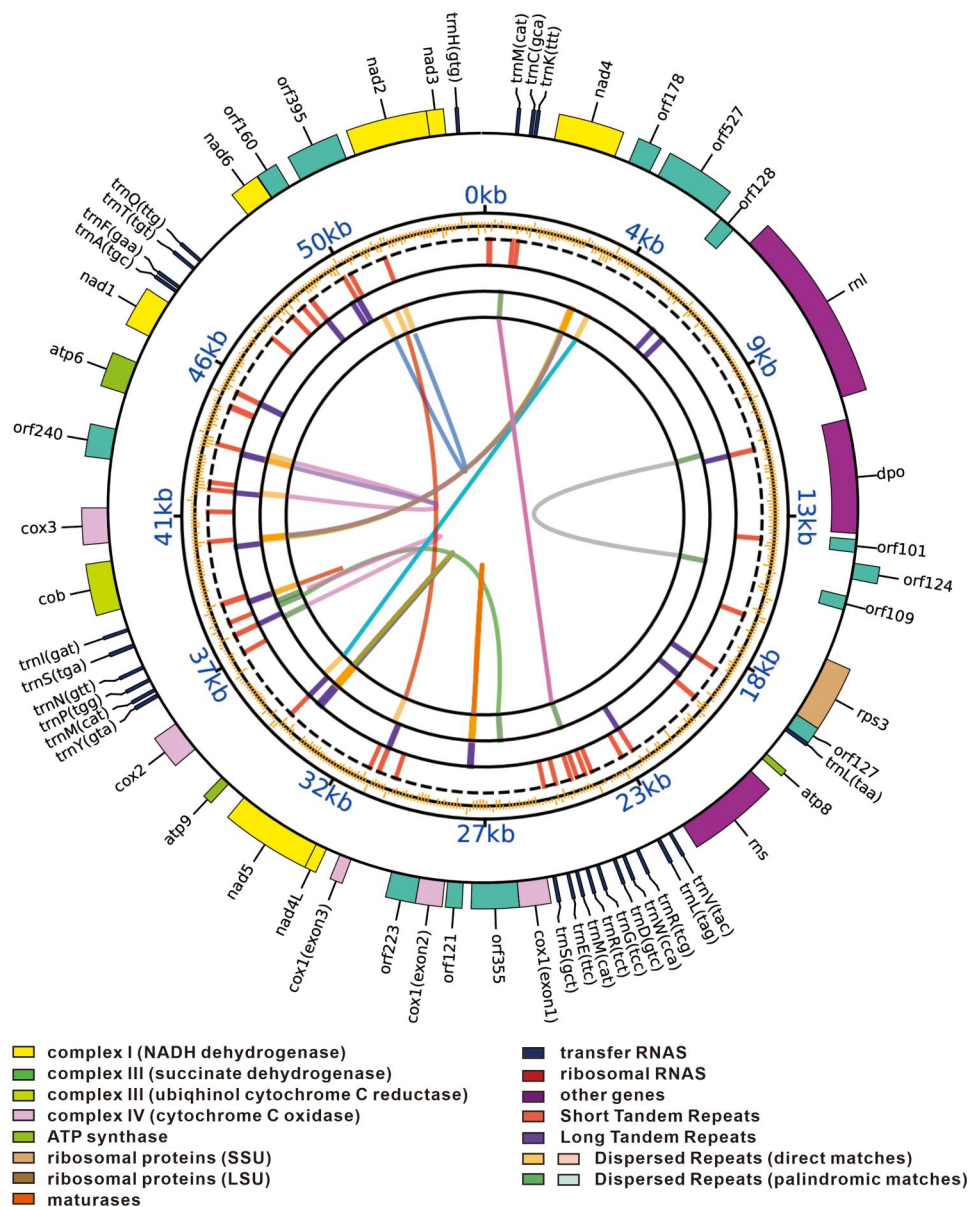


Figure 2. The diagram depicts the mitochondrial genome of *T. biforme*. Genes located on the periphery and within the outer ring are transcribed in an anticlockwise and clockwise orientation, respectively. The concentric circles illustrate the genomic scale, GC content, and the distribution of short tandem repeats, long tandem repeats, and dispersed repeats. The central parabola, highlighted in color, denotes the dispersed repeats. ORFs represent putative protein-coding regions with no assigned function.

cox2, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, and *rps3*, along with 24 tRNA genes and two rRNA genes. Except for the *cox1* gene, which contains two introns, no introns were identified in any of the other annotated protein-coding genes. Neither cis-splicing nor trans-splicing genes were found in the entire mitochondrial genome except for *cox1* (Figure S3). The base composition of the mitochondrial genome is characterized by A at 31.80%, C at 16.94%, G at 18.30%, and T at 32.96%, yielding a GC content of 35.24%. All 15 conserved PCGs initiate with the start codon ATG and terminate with the stop codon TAA.

Phylogenetic analyses, conducted using both BI and ML methods, yielded well-supported evolutionary trees. These results place *T. biforme* as a basal taxonomic unit within the family Trichaptaceae, as depicted in Figure 3. The Trichaptaceae and Hymenochaetaceae families are identified

as sister taxa within the order Hymenochaetales. This discovery offers significant insights for ongoing research into the phylogeny and evolutionary history of the Trichaptaceae family and its related taxa.

Discussion and conclusion

This study presents the first complete mitochondrial genome sequence of the wood-decay fungus *T. biforme*. To the best of our knowledge, this represents the inaugural report of a mitochondrial genome within the family Trichaptaceae. The mitochondrial genes of fungi are recognized as valuable resources for the investigation of evolutionary narratives and phylogenetic affinities, attributed to their distinctive properties (Curole and Kocher 1999; Santamaria et al. 2009; Basse 2010; Li et al. 2023). According to the MycoBank database

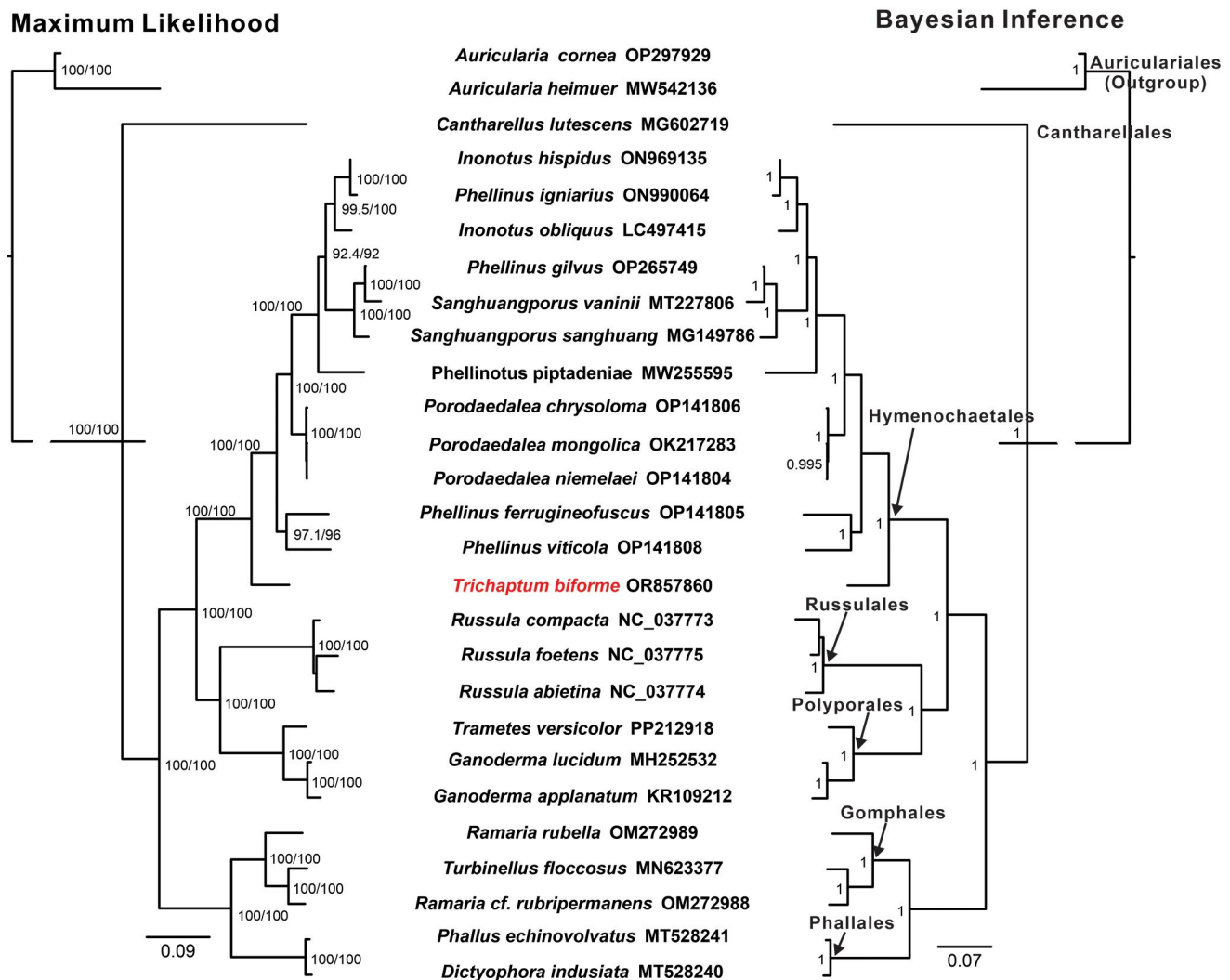


Figure 3. The phylogenetic tree based on ML (left) and BI (right) analyses of 15 mitochondrial-encoded core proteins. The 27 fungal mitogenomes were used in this phylogenetic analysis: *Auricularia cornea* (OP297929), *Auricularia heimuer* (MW542136, Wang et al. 2023), *Cantharellus lutescens* (MG602719, Sun et al. 2019), *Inonotus hispidus* (ON969135), *Phellinus igniarius* (ON990064), *Inonotus obliquus* (LC497415, Agnestisia et al. 2019), *Phellinus gilvus* (OP265749), *Sanghuangporus vaninii* (MT227806, Song et al. 2021), *Sanghuangporus sanghuang* (MG149786, Han et al. 2018), *Phellinotus piptadeniae* (MW255595, Araújo et al. 2021), *Porodaedalea chrysoloma* (OP141806, Lee et al. 2019), *Porodaedalea mongolica* (OK217283, Zhao et al. 2022), *Porodaedalea niemelaei* (OP141804, Araújo et al. 2021), *Phellinus ferrugineofuscus* (OP141805, Araújo et al. 2021), *Phellinus viticola* (OP141808), *Trichaptum bifforme* (OR857860, this article), *Russula compacta* (NC_037773, Li et al. 2018), *Russula foetens* (NC_037775, Li et al. 2018), *Russula abietina* (NC_037774, Li et al. 2018), *Trametes versicolor* (PP212918, Chen et al. 2021), *Ganoderma lucidum* (MH252532, Li et al. 2013), *Ganoderma applanatum* (KR109212, Wang et al. 2016), *Ramaria rubella* (OM272989, Li et al. 2022), *Turbinellus floccosus* (MN623377, Li et al. 2018), *Ramaria cf. rubripermanens* (OM272988, Li et al. 2022), *Phallus echinvolvatus* (MT528241, Chen et al. 2020), *Dictyophora indusiata* (MT528240, Chen et al. 2020). Mitochondrial genome information for the four species for which only GenBank accession numbers are available is available through the corresponding GenBank accession numbers. The GenBank accession number from NCBI are provided after the species names. The newly sequenced mitogenome is marked in red. Numbers near the nodes indicate bootstrap support values (>50%) and posterior probabilities (>0.95).

(consulted on 30 May 2024), the genus *Trichaptum* comprises over 70 species, and here we report only the first mitochondrial genome of the genus. The elucidation of the phylogenetic position of the genus *Trichaptum*, and by extension the family Trichaptaceae, is anticipated to be significantly advanced with the sequencing of additional mitochondrial genomes in the future.

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Conceptualization, JZQ, JW and JQJ; methodology, JW, XSH, and HWL; formal analysis, DT and JZQ; investigation, JW, XSH, and HWL; resources, JZQ and JW; writing—original draft preparation, DT and WJ; writing—review and editing, JW, JQJ, and JZQ; supervision, HWL; funding acquisition, DT and JW. All authors have read and agreed to the published version of the manuscript.

Author contributions

CRedit: **Jing Wei:** Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing; **Jian-qiao Jiang:** Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation; **Dan Tang:** Conceptualization, Data curation, Resources, Software; **Xuan-sheng Hu:** Validation, Visualization, Writing – original draft, Writing – review & editing; **Hui-wen Li:** Data curation, Formal analysis, Supervision, Validation, Visualization; **Jianzhao Qi:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Disclosure statement

No potential conflict of interest was reported by the authors.

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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> (<https://www.ncbi.nlm.nih.gov/>) under the accession no. OR857860. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1120629, SRS21535525, and SAMN41705344, respectively.

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