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MITOGENOME ANNOUNCEMENT

The complete mitochondrial genome of a wood-decaying fungus *Vanderbylia fraxinea* (Polyporaceae, Polyporales)

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ABSTRACT

Vanderbylia fraxinea (Bull.) D.A. Reid, 1973 is an important wood-inhabiting fungus that plays a significant role in nutrient recycling in most forest ecosystems. In this study, the complete mitochondrial genome of *V. fraxinea* was characterized through *de novo* assembly using Illumina sequencing data and genome annotation. The mitochondrial genome is a circular molecule of 115,473 bp with a GC content of 28.66%. It comprises a total of 62 genes. Among these, 36 are protein-coding genes including 21 free-standing open reading frames (ORFs), 24 transfer RNA genes, and two ribosomal RNA genes. Core gene set commonly found in fungal mitochondrial genomes is also present in this genome, such as the apocytochrome b (*cob*), three subunits of the cytochrome c oxidase (*cox1*, *cox2*, and *cox3*), seven subunits of the NADH dehydrogenase (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*), and three subunits of the ATP synthase (*atp6*, *atp8*, and *atp9*), as well as ribosomal RNA subunits (*rns* and *rnl*) and a set of transfer RNA genes. Phylogenetic analysis of the protein-coding sequences from the mitochondrial genome revealed a close relationship between *V. fraxinea* and the *Ganoderma* species within the Polyporaceae family.

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Introduction

The genus *Vanderbylia* D.A. Reid belongs to the phylum Basidiomycota, the class Agaricomycetes, the order Polyporales, and the family Polyporaceae (Kirk et al. 2001; Cui et al. 2019). It is a large, cosmopolitan genus typified by *Polyporus medulla-panis* (Jacq.) Fr., which is characterized by poroid basidiomata. It is a thick-walled, ellipsoid that distinctly truncates basidiospores and displays varying dextrinoid and amyloid reactions with a cyanophilous nature. The species within this genus exhibit a diverse range of ecological characteristics, functioning as saprophytes and either parasitic or pathogenic fungi (Dai et al. 2007, 2009; Younes et al. 2007; Wang et al. 2012). In most cases, this group possesses the ability to decompose cellulose, hemicellulose, and lignin within the plant cells as wood-inhabiting fungi, playing a significant role in nutrient recycling in most forest ecosystems (Dai 2012).

Vanderbylia fraxinea is either a parasite or a saprotroph growing on a broad range of hosts including *Castanea*, *Fraxinus*, *Juglanas*, *Platanus*, *Populus*, *Prunus*, *Quercus*, *Robinia*, *Salix*, and *Ulmus* (Kotlaba 1984; Kreisel 1987; Ryvarden and Gilbertson 1994). Among those within the genus *Vanderbylia*, it is considered an important group of fungi given their potential applications in bio-engineering. In particular, *V. fraxinea* can accumulate amounts of heavy metals, displaying high tolerance to Cd, Hg, and Cu. These characteristics position this fungus as a potential tool for monitoring environmental pollution (Sturini et al. 2017). Given the importance of this taxon, we investigated the phylogenetic relationship between *V. fraxinea* and the related genera based on the complete mitochondrial genome of the fungus.

Materials and methods

Samples of *V. fraxinea* were collected from Jinju, in the Gyeongsangnam province, South Korea (35°10′51.0″N 128°05′43.0″E) (Figure 1) and deposited to the culture collection (GNUFP) of Gyeongsang National University, South Korea (accession no. GNUFP287, gnu.ac.kr, Prof. Keumchul Shin, kcshin@gnu.ac.kr). A voucher specimen was deposited to the herbarium of the Korea National Arboretum (KH; voucher no. KA23-0002C, kna.forest.go.kr, Dr. Chang Sun Kim, changsun84@korea.kr). Genomic DNA was extracted from the

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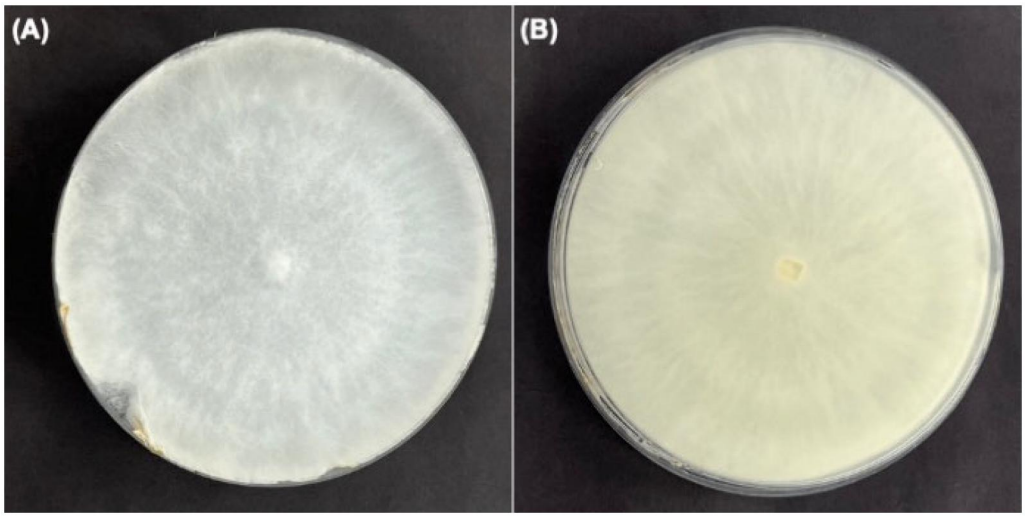

Figure 1. Colony morphology of the isolate of *Vanderbylia fraxinea*, GNUFP287, on the potato dextrose agar (PDA); (A) obverse and (B) reverse. The photo provided by Prof. Shin, Keumchul, was taken during cultivation of *V. fraxinea* after collection made from *Prunus* × *yedoensis*.

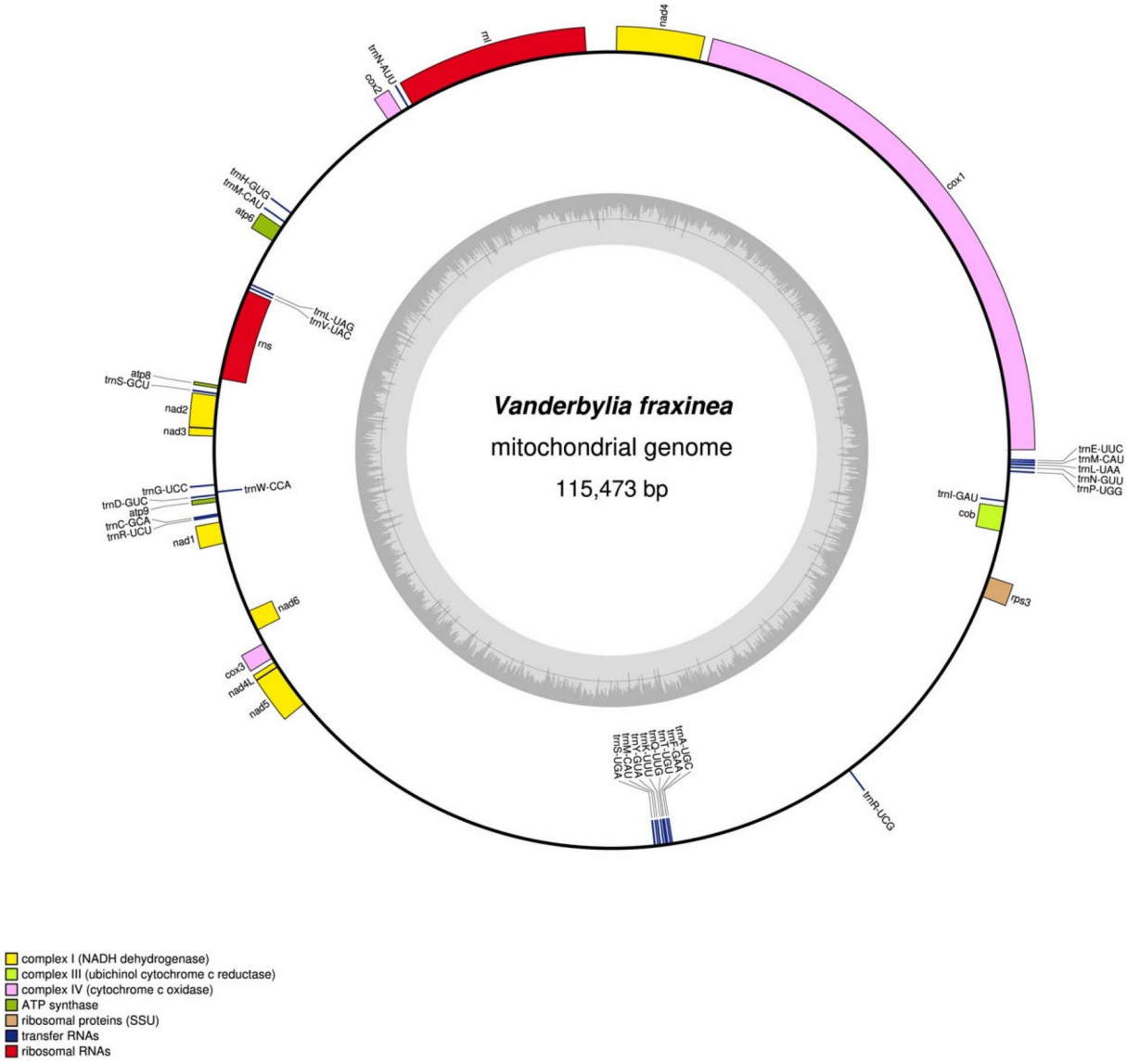

Figure 2. Mitochondrial genome map of *Vanderbylia fraxinea*. The map was prepared using OGDRAW program (https://chlorobox.mpimp-golm.mpg.de/OGDraw.html) (Lohse et al. 2007). Genes transcribed clockwise and counterclockwise are indicated on the outside and inside of the large circle, respectively. GC contents are indicated on the inner circle. This genome map does not show ORFs.

Table 1. The annotated gene list of mitochondrial genome in *Vanderbylia fraxinea*.

Gene groups	Gene names	Gene numbers
Complex I (NADH dehydrogenase)	*nad1*, *nad2*, *nad3*, *nad4*[z], *nad4L*, *nad5*, *nad6*	7
Complex II (succinate dehydrogenase)		0
Complex III (ubiquinol-cytochrome c reductase)	*cob*	1
Complex IV (cytochrome c oxidase)	*cox1*[z], *cox2*, *cox3*	3
Complex V (ATP synthase)	*atp6*, *atp8*, *atp9*	3
Cytochrome c biogenesis		0
Large subunit ribosomal proteins		0
Small subunit ribosomal proteins	*rps3*	1
Maturase		0
Transport membrane protein		0
Ribosomal RNAs	*rnl*[z], *rns*[z]	2
Transfer RNAs	*trnA-UGC*, *trnC-GCA*, *trnD-GUC*, *trnE-UUC*, *trnF-GAA*, *trnG-UCC*, *trnH-GUG*, *trnI-GAU*, *trnK-UUU*, *trnL-UAA*, *trnL-UAG*, *trnM-CAU*, *trnN-AUU*, *trnN-GUU*, *trnP-UGG*, *trnQ-UUG*, *trnR-UCG*, *trnR-UCU*, *trnS-GCU*, *trnS-UGA*, *trnT-UGU*, *trnV-UAC*, *trnW-CCA*, *trnY-GUA*	24
Other genes	*orf157*, *orf215*, *orf250*, *orf261*, *orf297*, *orf298*, *orf300*, *orf307*, *orf315*, *orf323*, *orf326*, *orf334*, *orf342*, *orf348*, *orf362*, *orf368*, *orf376*, *orf385*, *orf395*, *orf415*, *orf749*	21

[z]Genes consisting of multiple exons.

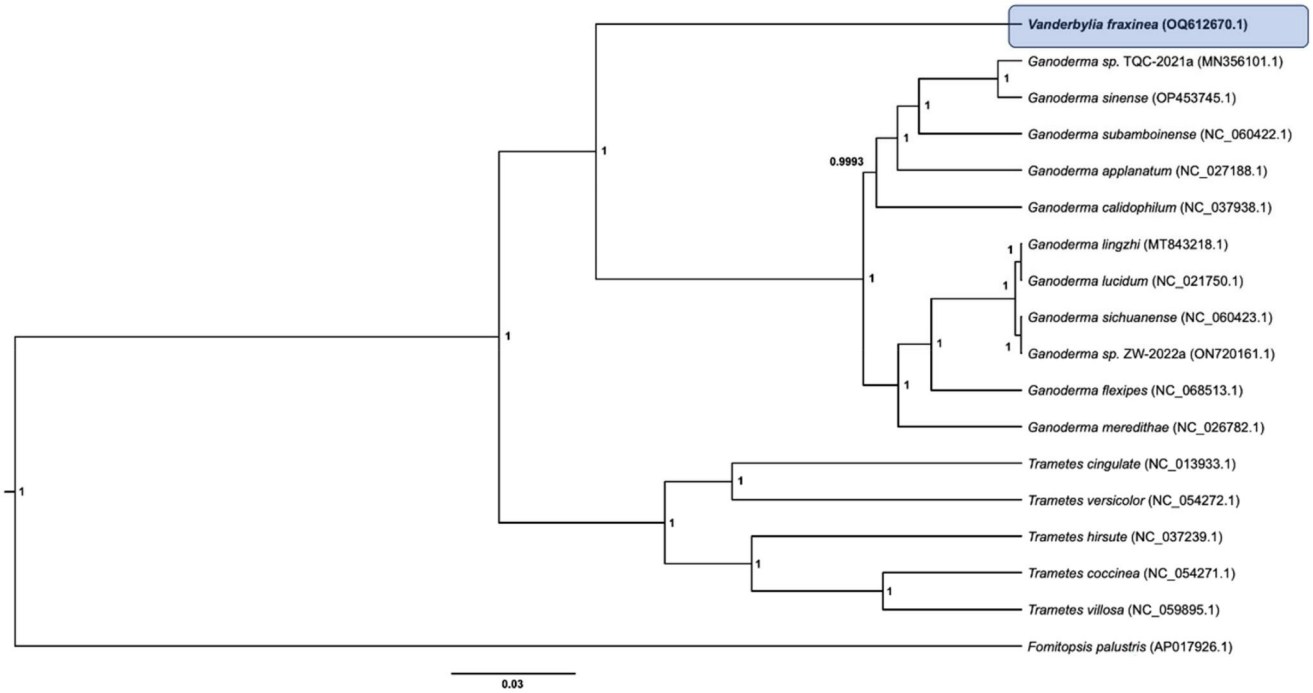

Figure 3. Phylogenetic tree of mitochondrial genomes of *Vanderbylia fraxinea* and its related species. Protein-coding sequences conserved in the mitochondrial genomes of 18 species were multiple-aligned using MAFFT (http://mafft.cbrc.jp/alignment/server/index.html) and used to generate phylogenetic tree using Bayesian inference method of BEAST2 (Bouckaert et al. 2019) with default parameters. Posterior probability values are on the branches. The isolate, *V. fraxinea* (GNUFP287), obtained in this study was represented by bold letters. GenBank accession nos. of mitochondrial genome sequences used for this tree are indicated within parentheses.

isolate, GNUFP287, obtained from fungal mycelium harvested using fungal Maxwell 16 DNA purification kits (Promega, Madison, WI). An Illumina paired-end (PE) library was constructed and subsequently sequenced using the Illumina NovaSeq platform (San Diego, CA), producing 151 bp PE reads. Raw sequencing data of 3.4 Gb were generated and trimmed using the quality_trim program in the CLC Assembly Cell package ver. 4.2.1 (QIAGEN, Hvidovre, Denmark) with Phred scores >20. The trimmed data were used for the *de novo* assembly of the mitochondrial genome, as described in the previous studies (Lee et al. 2018; Cho et al. 2022, 2023). In brief, trimmed high-quality read sequences of 3.0 Gb were *de novo* assembled using the clc_novo_assemble program with default parameters in the CLC Assembly Cell. From the assembled contigs, mitochondrial contigs were selected and ordered through BLAST-based similarity searches (*E*-value cut-off 1e-5) against the mitochondrial sequences available in the NCBI organelle genome resources (https://www.ncbi.nlm.nih.gov/genome/organelle/). The selected mitochondrial contigs were merged and gap-filled through a series of read mapping and extension steps, resulting in the generation of a complete, circularized mitochondrial genome. Sequence error

was investigated and subsequently corrected through read mapping of the trimmed sequence data and manual curation. The complete mitogenome sequence was annotated using the GeSeq (Tillich et al. 2017) and Artemis (Carver et al. 2012) programs with reference mitochondrial genomes (GenBank accession nos. KR109212.1, KP410262.1, MN356101.1, MT843218.1, and NC_021750.1). In addition, gene annotation was reconfirmed through manual curation, involving BLAST searches against the reference mitochondrial genomes.

Conserved protein-coding sequences were identified among the mitochondrial genomes of *V. fraxinea* and related 17 species and concatenated for each species. The concatenated sequences were multiple-aligned using MAFFT ver. 7 (http://mafft.cbrc.jp/alignment/server/index.html) (Katoh et al. 2019) with default parameters and used as input data for phylogenetic analysis. Phylogenetic analysis was performed using a Bayesian inference method of BEAST2 ver. 2.6.7 (Bouckaert et al. 2019), with default parameters (Site Substitution Model = Gamma Site Model (Gamma Category = 4; GTR), Chain length of MCMC = 10,000,000, Burn-in = 10%, Model = Yule model).

Results and discussion

The mitochondrial genome of *V. fraxinea* is a circular molecule of 115,473 bp consisting of 28.66% GC content (GenBank accession no. OQ612670) and a ×2948 mean coverage of trimmed sequencing data (Figure 2). In total, 62 genes were predicted in this mitochondrial genome comprising 36 protein-coding genes, which included 21 free-standing open reading frames (ORFs), 24 transfer RNA genes, and two ribosomal RNA genes (Table 1). Core genes commonly found in fungal mitochondrial genomes are also identified, which include the apocytochrome b (*cob*), three subunits of the cytochrome c oxidase (*cox1*, *cox2*, and *cox3*), seven subunits of the NADH dehydrogenase (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*), and three subunits of the ATP synthase (*atp6*, *atp8*, and *atp9*), as well as untranslated genes of the small and large ribosomal RNA subunits (*rns* and *rnl*, respectively) and 24 transfer RNA (*trnA-UGC* to *trnY-GUA*) genes. Most genes consist of a single exon, but four genes consist of multiple exons, such as *cox1* (16 exons and 15 introns), *nad4* (three exons and two introns), *rnl* (three exons and two introns), and *rns* (two exons and one intron).

A phylogenetic tree based on a Bayesian inference method with 13 conserved protein-coding sequences of *atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad5*, and *nad6* revealed that *V. fraxinea* is close to the *Ganoderma* species in the Polyporaceae family (Figure 3).

The complete mitochondrial genome sequence generated in this study will provide valuable genetic information for understanding the phylogenetic relationship of this species at the molecular level.

Acknowledgements

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Ethics statement

Ethical approval for the study was obtained from the National Institute of Forest Science, South Korea. The collection and analysis of plant material were performed in accordance with guidelines provided by the National Institute of Forest Science, South Korea.

Authors contributions

Sung-Eun Cho and Dong-Hyeon Lee conceived the research project and drafted the manuscript. Sanggon Lee, Misong Kim, and Keumchul Shin contributed to cultivation management and collection of fungal samples. Sung-Eun Cho, Dong-Hyeon Lee, Heonil Kang, Sang-Tae Seo, Namkyu Kim, and Keumchul Shin performed the data analysis and revised the manuscript critically for intellectual content. All authors contributed to the final approval of the version to be published and all agree to be accountable for all aspects of the work.

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 under the accession no. OQ612670. The associated BioProject, BioSample, and SRA numbers are PRJNA943535, SAMN33730946, and SRR23823732, respectively.

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