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MITOGENOME ANNOUNCEMENT

Complete mitochondrial genomic sequence of *Auricularia delicata* (Auriculariaceae), an edible Chinese mushroom

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ABSTRACT

Auricularia delicata (Mont.) Henn. 1893 is an edible and medicinal jelly mushroom popular in China. Here, we report the assembly and annotation of a complete *A. delicata* mitochondrial genome based on data sequenced using an Illumina NovaSeq 6000 platform. The length of the complete circular *A. delicata* mitochondrial genome is 189,696 bp, with a GC content of 34.1%. The *A. delicata* mitochondrial genome contains 60 genes, including 32 protein-coding genes, 26 tRNA genes, and two rRNA genes. Phylogenetic analysis indicated that *A. delicata* clustered with the *Auricularia* group, alongside *A. auricula-judae* and *A. heimuer*. Additionally, *A. delicata* was found to be genetically distant from other species of Polyporales, Russulales, and Agaricales. This genome will provide an invaluable reference for the continued study and utilization of *A. delicata* and other *Auricularia* species.

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KEYWORDS
Auricularia delicata; mitochondrial genome; phylogeny; edible mushroom; jelly fungus

1. Introduction

Auricularia delicata (Mont.) Henn. 1893 is a jelly mushroom utilized as a source of food and medicine across certain regions of Africa and eastern Asia, and particularly in China and India (Wangkheirakpam et al. 2018; Li et al. 2020; Muharagi et al. 2020). *A. delicata* grows on fresh-cut wood, decaying logs, and tree trunks across several temperate, tropical, and subtropical areas (Looney et al. 2013). *A. delicata* belongs to a species complex which can be roughly divided between American and Australian groups (Looney et al. 2013). Through advancements in fungal cultivation techniques, *A. delicata* is being successfully cultivated in China and other countries (Qian et al. 2020). To address gaps in our understanding of the phylogeny and evolutionary history of this economically important fungal species, here, we report the first complete *A. delicata* mitochondrial genome. This genome will provide an invaluable reference for the continued study and utilization of *A. delicata* and other *Auricularia* species.

2. Materials and methods

2.1. Materials

An *A. delicata* specimen found growing on a decaying stump was collected from Nanning, Guangxi Province, China (108°15′15.2604″N; 22°51′4.3272″E). A voucher specimen (No. 2018051504) was stored at the Guangxi Academy of Agricultural Sciences (GXAAS) (Wang Xiaoguo, wangxiaoguo2005@163.com). The species identity of the specimen was verified through morphological investigation of the fruiting body as well as internal transcribed spacer (ITS) sequencing (Figure 1).

2.2. DNA extraction and sequencing, and genome assembly and annotation

Total genomic DNA was extracted with a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). Sequencing was performed with an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA). The library was constructed with a Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA), with an average read length of 350 bp. A total of 2.63 Gb of raw sequencing reads were cleaned and edited with the NGS QC Tool Kit (Patel and Jain 2012). After quality control, a total of 2.61 Gb of clean reads, with a >100-fold coverage depth, were utilized for further analyses (Figure S1). The clean data exhibited a GC content of 58.48%, a Q20 of 97.81%, and a Q30 of 93.85%, indicating high quality sequencing and assembly. The high-quality reads were assembled into a complete *A. delicata* mitochondrial genome with the SPAdes v.3.11.0 *de novo* assembler (Bankevich et al. 2012). The complete

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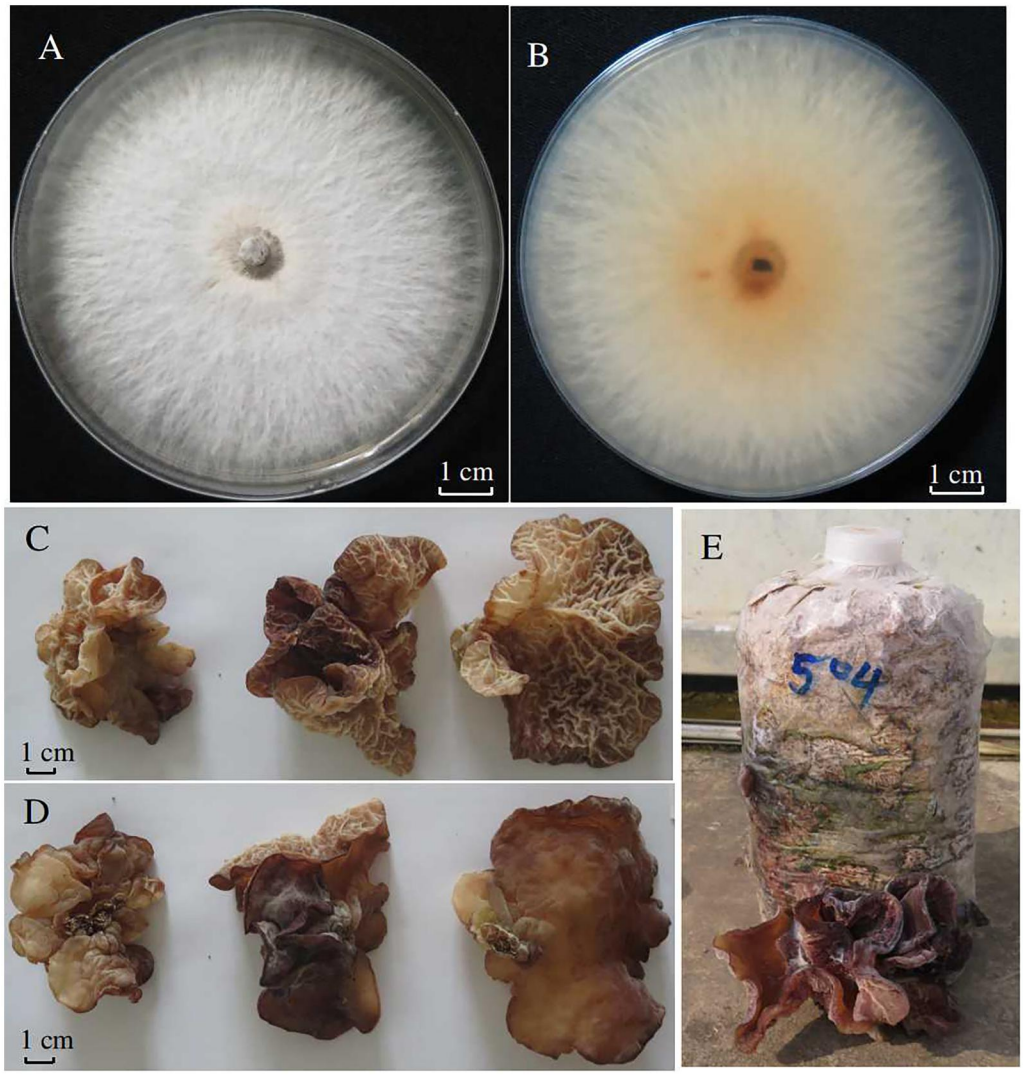

Figure 1. Photos of the *A. delicata* specimen used in this study. (A) A topside view of the *A. delicata* mycelium; (B) an underside view of the *A. delicata* mycelium; (C) an underside view of the *A. delicata* fruiting body; (D) a topside view of the *A. delicata* fruiting body; (E) cultivation of *A. delicata* in a grow bag. Although the appearance of *A. delicata* fruiting bodies can vary significantly, they are generally large, dark purple-brown, soft, floppy, and bowl-shaped. The underside of the *A. delicata* fruiting body is porous, which differentiates this species from other *Auricularia* species. All photographs were taken by Dr. Wang Xiaoguo with a Canon EOS M50 camera in a laboratory in Nanning, China (108°14′ 37″ N; 22°50′ 51″ E).

A. delicata mitochondrial genome was annotated using MITOS (Bernt et al. 2013). A circular gene map of the *A. delicata* mitochondrial genome was created with Organellar Genome DRAW v1.3.1 (Greiner et al. 2019).

2.3. Phylogenetic analysis

We obtained 24 complete Agaricomycetes mitochondrial genomes by searching the National Center for Biotechnology Information (NCBI) nonredundant (nr) database. A total of 14 homologous protein-coding genes (PCGs) from each sequence were selected for comparison. The *A. delicata* mitochondrial genome was aligned with MAFFT 7.037 (Katoh and Standley 2013). The phylogenetic estimation model (GTR + F+R2) was selected using ModelFinder. A maximum-likelihood (ML) tree was constructed with IQtree 1.6 (Trifinopoulos et al. 2016), with *Cantharellus cibarius* (KC573037) used as the outgroup. Finally, the phylogenetic tree was constructed with MrBayes (Ronquist et al. 2012).

3. Results

3.1. Characteristics of the A. delicata mitochondrial genome

The complete, circular *A. delicata* mitochondrial genome (GenBank accession number OM995805) was found to be 189,696 bp in length, with a GC content of 34.1% and a base composition of 32.0% A, 33.9% T, 17.4% G, and 16.7% C. The *A. delicata* mitochondrial genome was found to contain 60 genes, including 32 PCGs (53.3%), 26 tRNA genes (43.3%), and two rRNA genes (*rrnS* and *rrnL*) (3.3%). The 32 conserved PCGs were found to encode apocytochrome b (*cob*), three ATP synthase subunits (*atp6*, *atp8*, and *atp9*), three cytochrome oxidase subunits (*cox1*, *cox2*, and *cox3*), and seven

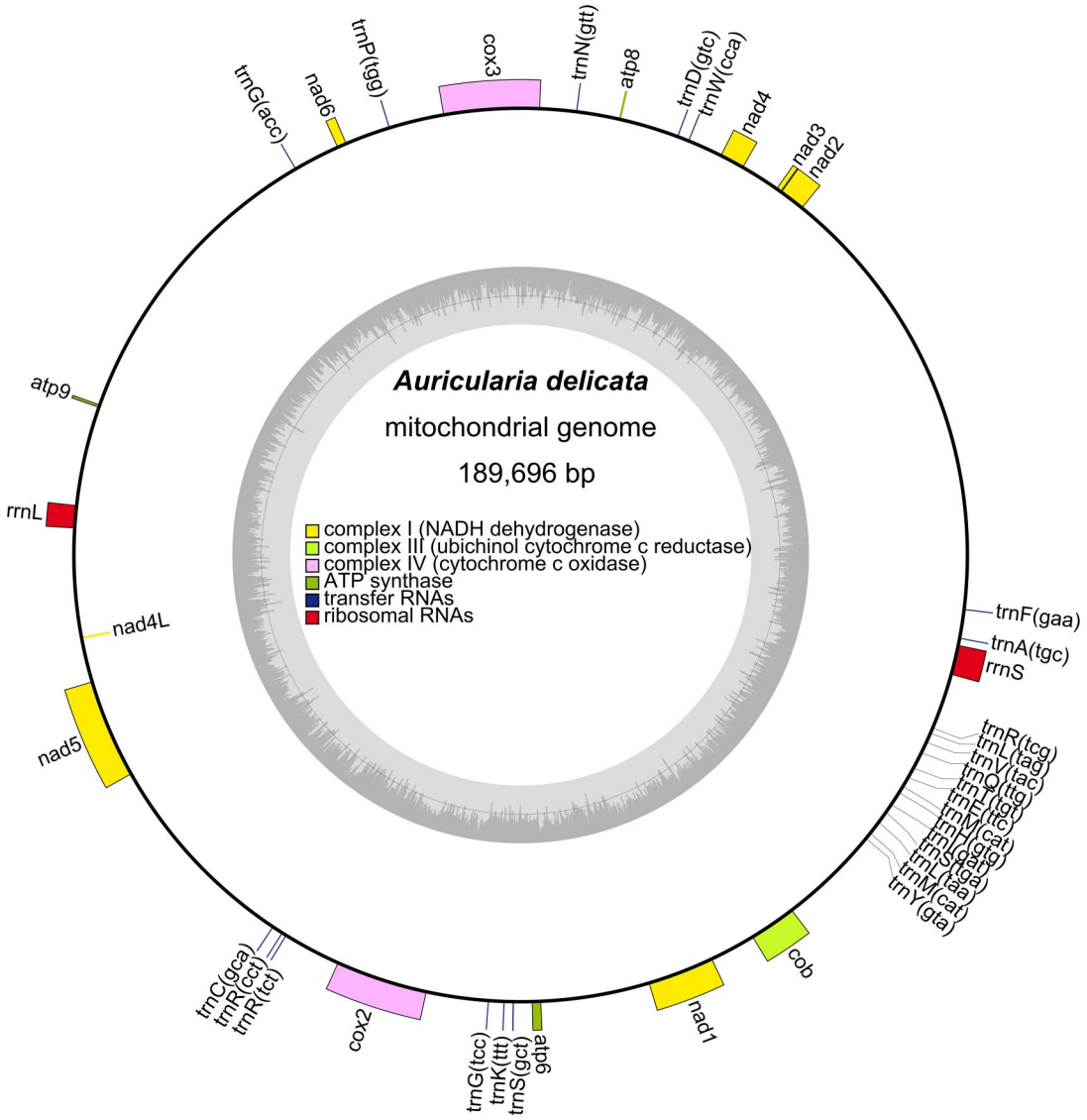

Figure 2. Circular map of the *A. delicata* mitochondrial genome. Different colored blocks represent different categories of genes (listed in center). Blocks outside the ring represent forward strand genes and blocks inside the ring represent reverse strand genes. The inner grayscale circle indicates the GC content, with the center line representing the 50% threshold.

NADH: ubiquinone reductase subunits (*nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*). The circular *A. delicata* mitochondrial genome map is shown in Figure 2, with different colored blocks representing different categories of genes. Blocks outside the ring represent forward strand genes and blocks inside the ring represent reverse strand genes. The majority of PCGs, tRNA genes, and rRNA genes are encoded on the forward strand, with the exception of *nad4L*. The inner grayscale circle indicates the GC content, with the center line representing the 50% threshold.

3.2. *Phylogeny of* A. delicata

We produced a ML phylogenetic tree based on a comparison of 14 core PCGs encoded in the mitochondrial genomic sequences of *A. delicata* and 24 other Agaricomycetes (Figure 3). As expected, *A. delicata* clustered with the *Auricularia* group with 100% bootstrap values, alongside *A. auricula-judae* (MN510416) and *A. heimuer* (MW542136.1) (Fang et al. 2019). Interestingly, the *A. delicata* mitochondrial genome was considerably larger (189,696 bp) than that of *A. auricula-judae* (40,586 bp) and smaller than that of *A. heimuer* (209,153 bp) (Fang et al. 2019). Additionally, *A. delicata* was found to be genetically distant from other species of Polyporales, Russulales, and Agaricales.

4. Discussion and conclusions

At present, there are few published *Auricularia* mitochondrial genome assemblies available for study. The complete *A. delicata* mitochondrial genome reported here adds to the number of *Auricularia* mitochondrial genomes available for future work. Additionally, our contributed genome will help resolve the phylogeny of *Auricularia* through comparison with a previously published analysis of *Auricularia* rDNA ITS barcode sequences, which included *A. delicata* and *A. auricula-judae*

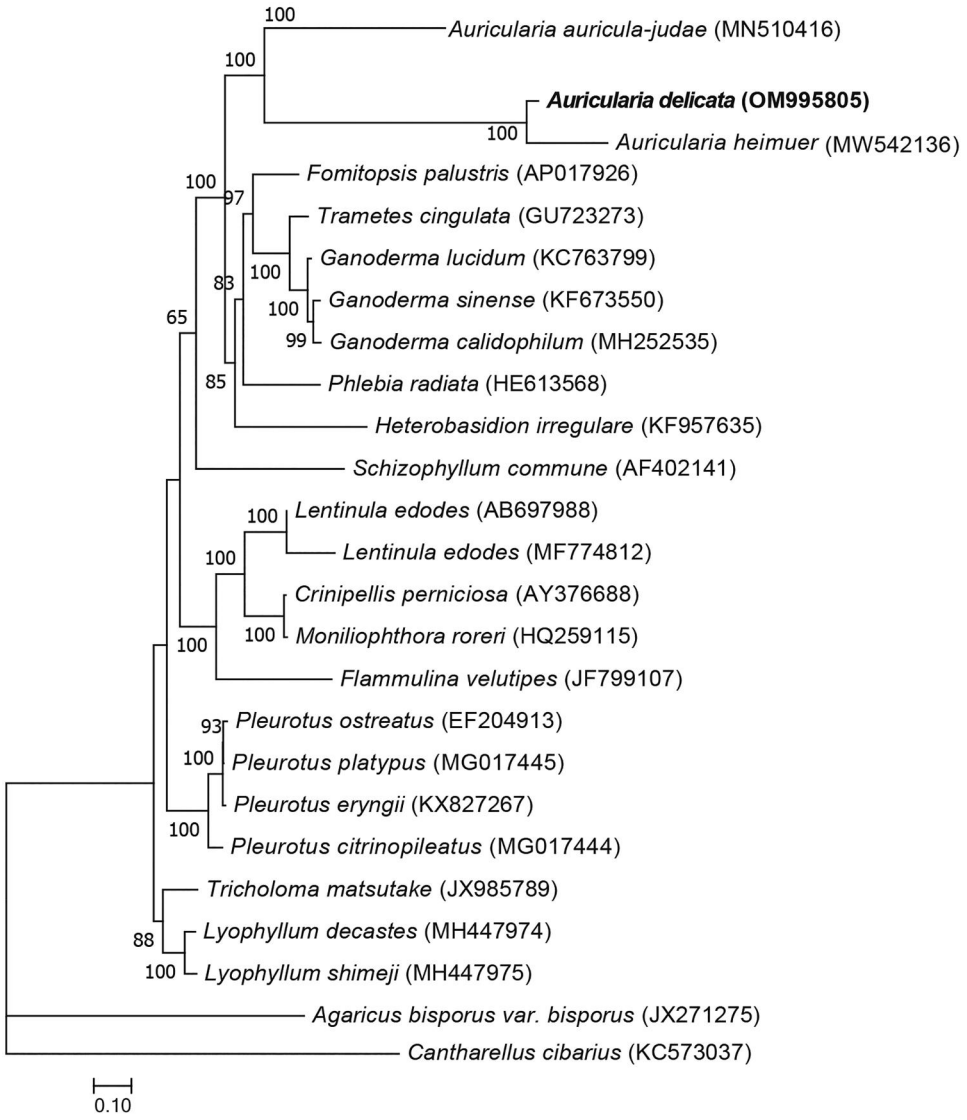

Figure 3. Phylogenetic tree showing the evolutionary relationships between *A. delicata* and 24 other Agaricomycetes species, with *Cantharellus cibarius* used as the outgroup. The tree was produced using the ML method (IQ tree 1.6) to compare 14 homologous PCGs encoded in the mitochondrial genomic sequences of each compared species. Node values indicate bootstrap support (1000 replicates).

(Looney et al. 2013). This genome will provide an invaluable reference for the continued study and utilization of *A. delicata* and other *Auricularia* species.

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Author contributions

Xiao-guo Wang participated in the design of this study and drafted the manuscript; Shi-yan Wei and Liang-liang Qi both collected and isolated *Auricularia delicata* specimen and provided assistance for data acquisition and analysis; Zai-feng Yang, Jun Tang, and Zeng-liang Liu provided assistance for data acquisition, data analysis, and statistical analysis; Sheng-jin Wu provided interpretation of data collection for the work and was responsible for ensuring that the descriptions were accurate and agreed upon by all authors. All authors have read and approved the content of this manuscript.

Ethics statement

Specimen collection conformed to international ethical requirements, and did not cause damage to the local environment. Both the process and purpose of this experimental research were in line with the rules and regulations of our institute. There were no ethical issues or other conflicts of interest related to this study.

Disclosure statement

No potential conflicts of interest were reported by any authors.

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Data availability statement

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

GenBank under the accession number OM995805. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA805016, SRR17966153, and SAMN25819720, respectively.

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