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Characterization and phylogenetic analysis of the *Talaromyces liani* (kamyschko) Yilmaz, Frisvad & Samson, 2014 (Eurotiales: trichocomaceae) mitochondrial genome

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ABSTRACT

The filamentous fungus *Talaromyces liani* (Kamyschko) Yilmaz, Frisvad & Samson, 2014, has attracted considerable interest in biotechnology due to its diverse industrial applications and physiological characteristics. However, the mitochondrial genome of *T. liani* remains uncharacterized. Here, we present the complete mitochondrial genome of *T. liani*, comprising 38,000 bp with a GC content of 24.61%. This genome includes 15 core protein-coding genes, 4 independent ORFs, 6 intronic ORFs, 26 tRNAs, and 2 rRNA genes. Phylogenetic analysis using Bayesian inference (BI) revealed the evolutionary relationships among 15 fungi from Eurotiales, strongly supporting distinct clades and indicating that *T. liani* most closely related to *T. pinophilus*.

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1. Introduction

The fungal species *Talaromyces liani*, belonging to the Eurotiomycetes class and Trichocomaceae family, has attracted considerable attention in biotechnology for its physiological characteristics and diverse industrial uses [1,2]. This fungus is known for its capacity to produce a wide array of bioactive compounds and enzymes, such as antibiotics, antioxidants, and anti-inflammatories, which display potent biological activities [3,4]. In the pharmaceutical industry, *T. liani* is being actively investigated as a potential source of new antibiotics and anticancer agents [5,6]. Furthermore, its enzymes are under scrutiny for industrial applications, including biofuel production and waste management [7,8]. Given its distinctive metabolic abilities and strong adaptability, *T. liani* is a valuable microbial resource [9]. Its potential in various sectors, such as medicine, bioenergy, and environmental remediation, suggests that further research and development on this fungal species could yield significant advancements in biotechnology [9].

Eukaryotes harbor a mitochondrial genome that is essential for governing growth and development, preserving cellular homeostasis, and facilitating responses to environmental cues [10–12]. It has been suggested that the mitochondrial genome serves as a beneficial tool for studying fungal phylogeny [13–16]. The mitochondrial genome characteristics of fungi belonging to the *Talaromyces* genus have been inadequately elucidated, with only three mitochondrial genomes reported thus far [17–19]. This study presents the first complete mitochondrial genome of *T. liani*, contributing to a better understanding of the genomic features of this important fungal group.

2. Materials and methods

2.1. Sample collection

A specimen of *T. liani* was isolated from soil in Chengdu, Sichuan, China (103.67°E, 30.60°N) in 2023. The specimens were identified through morphological analysis and nuclear genome molecular markers (including ITS, elongation factor, and beta-tubulin) according to previous studies [20–22]. The specimens were cataloged at the Culture Collection Center of Chengdu University with voucher number Rmic1. For additional information, please contact Jingwei Huang at huangjingwei@cdu.edu.cn (Figure 1).

2.2. Mitochondrial genome assembly and annotation

A fungal DNA extraction kit from Omega Bio-Tek (Norcross, GA, USA) was used for DNA extraction from *T. liani*, while the NEBNext® Ultra™ II DNA Library Prep Kit (NEB, Beijing, China)

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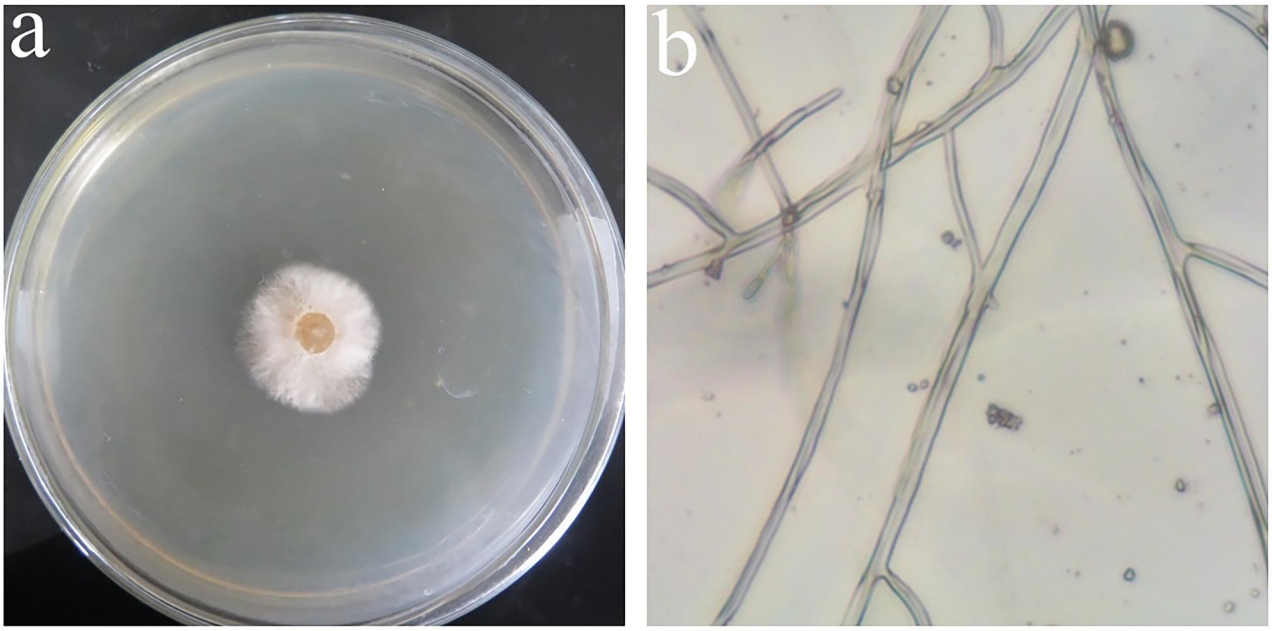

Figure 1. The colony morphology (a) and microscopic hyphal morphology (b) of *Talaromyces liani*. The photo of the species was taken by Jingwei Huang using Camera (Canon EOS 5D mark IV, Canon Inc., Japan) and stereoscope (SZX7, Olympus, Japan).

was used for sequencing library preparation in accordance with the manufacturer's instructions. Subsequent whole-genome sequencing was carried out on the Illumina HiSeq 2500 Platform (Illumina, San Diego, CA, USA). To maintain data accuracy, low-quality sequences were filtered out using ngsShoRT [23], and adapter reads were removed with AdapterRemoval v2 [24]. The mitochondrial genome of *T. liani* was assembled de novo using NOVOPlasty version 4.3.3, employing a k-mer size of 28 [25]. The annotation of the mitochondrial genome was conducted following previously established protocols [26–28], which utilized the MFannot tool (https://megasun.bch.umontreal.ca/apps/mfannot/) [29] and MITOS2 [30]. The NCBI Open Reading Frame Finder enables the anticipation or modification of PCGs or ORFs that surpass 100 amino acids in length [31]. The functions of protein-coding genes (PCGs) or open reading frames (ORFs) were annotated *via* BLASTP searches against the NCBI non-redundant protein sequence database [32]. The accurate identification of exon and intron boundaries in protein-coding genes was facilitated by the use of exonerate version 2.2 [33]. The presence of tRNA genes in the mitochondrial genome of *T. liani* was established and validated through the use of tRNAscan-SE v1.3.1 [34]. The PMGmap online web tool, accessible at http://www.1 kmpg.cn/pmgmap, was employed to visualize the structures of intron-containing genes and the graphical representation of the mitochondrial genome [35].

2.3. Phylogenetic analysis

The phylogenetic tree was constructed using previously described methods, commonly employed as an inference technique for mitochondrial genomic phylogeny [36–38]. The alignment of individual mitochondrial genes (excluding intron regions) was carried out using MAFFT v7.037 software [39]. We utilized SequenceMatrix v1.7.8 to merge the aligned mitochondrial genes, resulting in a consolidated mitochondrial dataset [40]. To ascertain potential phylogenetic disparities among various mitochondrial genes, an initial partition homogeneity test was carried out utilizing PAUP v 4.0b10 [41], in line with established literature [42]. PartitionFinder 2.1.1 was employed to determine the optimal partitioning schemes and evolutionary models for the combined mitochondrial dataset [43]. The software MrBayes v3.2.6 was used for the construction of phylogenetic trees *via* the Bayesian inference method [44]. When conducted BI analysis, two independent runs with four chains (three heated and one cold) each were conducted simultaneously for 2×10^6 generations. Each run was sampled every 1000 generations. We assumed that stationarity had been reached when the estimated sample size (ESS) was greater than 100, and the potential scale reduction factor (PSRF) approached 1.0. The first 25% of samples were discarded as burn-in, and the remaining trees were used to calculate Bayesian posterior probabilities (BPP) in a 50% majority-rule consensus tree.

3. Results

The average depth of the coverage-depth map was 3701.69× (Supplementary Figure 1). The mitochondrial genome of *T. liani* spans 38,000 bp with a GC content of 24.61%. Gene structures containing introns are illustrated in Supplementary Figure 2. In *T. liani*, the mitochondrial genome comprises 36.52% adenine, 14.02% guanine, 38.87% thymine, and 10.59% cytosine. Examination of the *T. liani* mitochondrial genome revealed 25 open-reading frames encompassing 15 core PCGs (cox1, cox2, cox3, atp6, atp8, atp9, cob, nad1, nad2, nad3, nad4, nad4L, nad5, nad6, and rps3), 4 free-standing ORFs, and 6 intronic ORFs (Figure 2). Notably, the

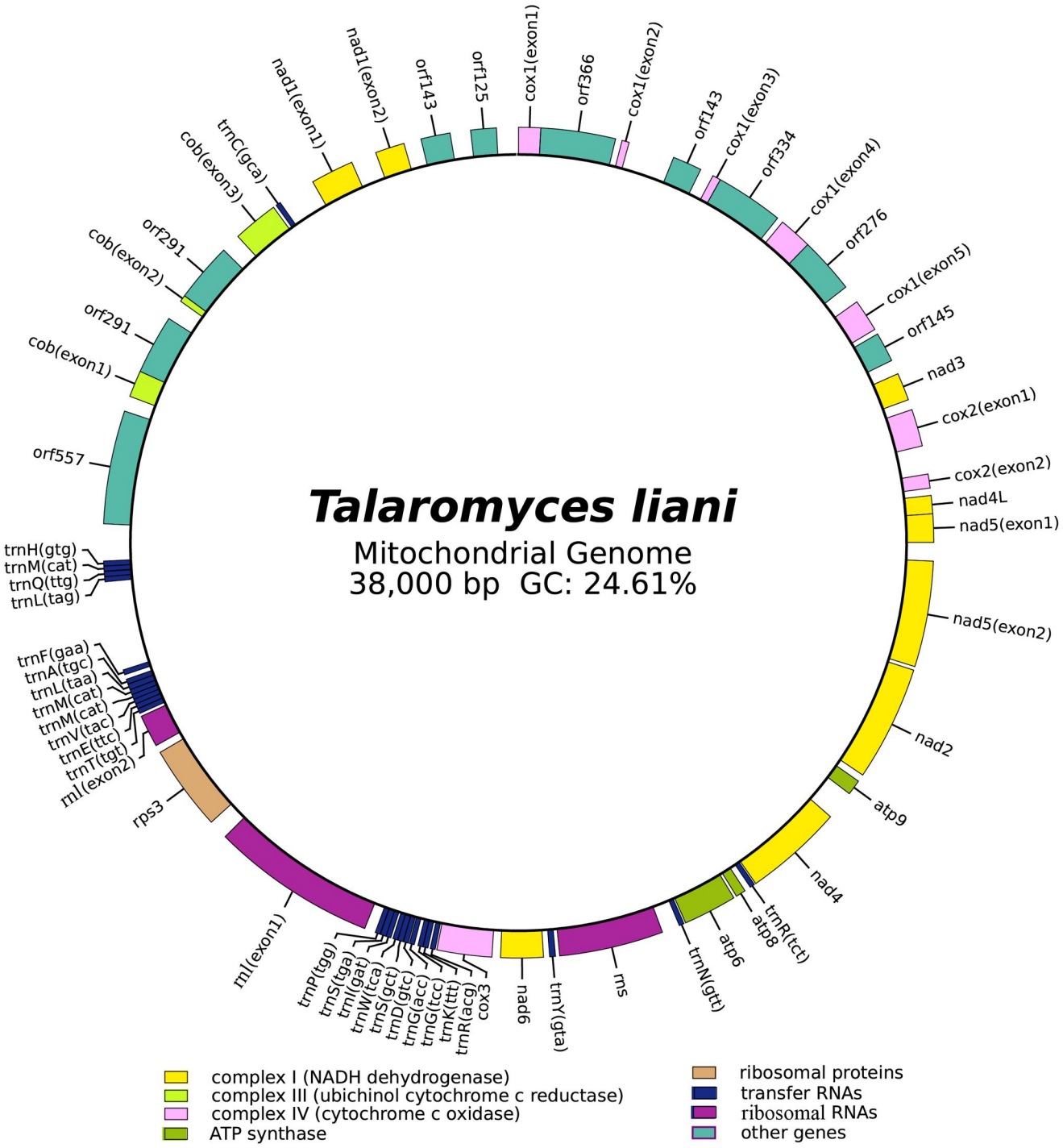

Figure 2. The circular mitochondrial genome map of *Talaromyces liani*. Different color blocks represent different genes. Genes containing introns are labeled. All genes are on the direct strand.

functions of proteins encoded by free-standing ORFs remain unknown. The mitochondrial genome of *T. liani* harbors 10 introns, categorized as 4 Group IB, 2 Group IA, 2 Group I (derived), 1 Group IC2, and 1 Group ID. Some introns contain intronic ORFs encoding LAGLIDADG homing endonucleases or GIY-YIG homing endonucleases. Additionally, the mitochondrial genome of *T. liani* contains two ribosomal RNA genes, the small subunit (rns) and the large subunit (rnl), and 26 transfer RNA genes. Phylogenetic analysis indicated that *T. liani* is phylogenetically closest to *T. pinophilus*, as shown in Figure 3.

4. Discussion and conclusion

The phylogenetic relationships between species can be better understood by utilizing the mitochondrial genome [50–54]. The lack of a mitochondrial reference genome for *T. liani* hinders the utilization of mitochondrial genomes for the classification and exploration of the phylogenetic relationships among Eurotiales fungi [54]. In this study, we sequenced the complete mitochondrial genome of a *Talaromyces* species, revealing a length of 38,000 bp and a GC content of 24.61%. The genome comprised 15 core protein-coding genes (PCGs),

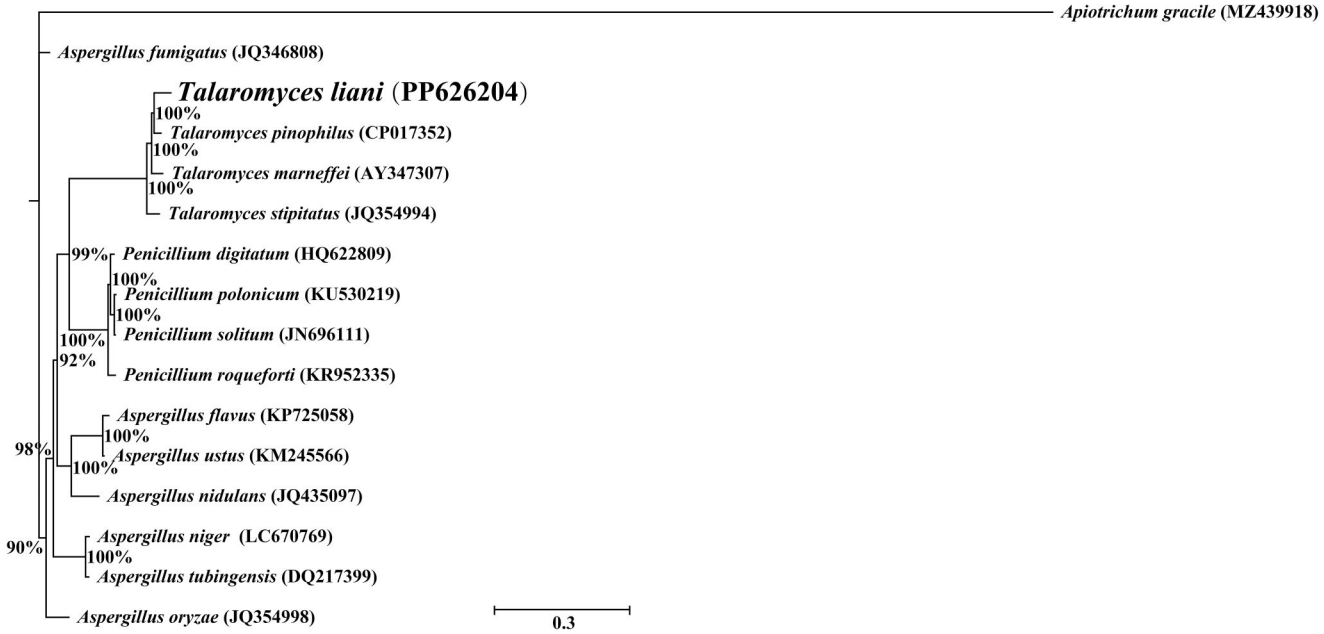

Figure 3. Bayesian inference (BI) tree generated using 14 concatenated mitochondrial protein-coding genes (*atp6*, *atp8*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*) from *Talaromyces liani* and 15 other fungal species. *Apiotrichum gracile* (MZ439918) was used as the outgroup [28]. The accession numbers of the sequences were as follows: *Talaromyces pinophilus* (CP017352) [17], *Talaromyces marneffei* (AY347307) [18], *Talaromyces stipitatus* (JQ354994) [19], *Penicillium digitatum* (HQ622809) [45], *Penicillium polonicum* (KU530219) [46], *Penicillium solitum* (JN696111) [47], *Penicillium roqueforti* (KR952335), *Aspergillus flavus* (KP725058), *Aspergillus ustus* (KM245566) [48], *Aspergillus nidulans* (JQ435097), *Aspergillus Niger* (LC670769), *Aspergillus tubingensis* (DQ217399) [49], *Aspergillus oryzae* (JQ354998) [19], and *Aspergillus fumigatus* (JQ346808) [19].

4 independent ORFs, 6 intronic ORFs, 26 tRNAs, and 2 rRNA genes. The *rps3* gene was found to be located in the intron region of the *rnl* gene, which is a Group IA intron. Notably, the mitochondrial genome of *T. liani* is the largest among the four *Talaromyces* species [17–19]. Phylogenetic analysis using the BI method placed *T. liani* closest to *T. pinophilus* among 15 fungal species from Eurotiales, with robust support for major clades. This research enhances our understanding of *Talaromyces* species differentiation, mitochondrial evolution, and diversity within this important fungal group.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Author contributions

J H and J-W H planned and designed the research. J-W H collected the materials, J H performed the experiments, and H-J Q and Y-Q Y analyzed the data and review the manuscript. J H and J-W H wrote the manuscript.

Ethics statement

The study did not involve humans or animals. In this study, samples were collected without ethical approval or permission.

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Data availability statement

The genome sequence data that support the findings of this study are openly available in the NCBI GenBank at https://www.ncbi.nlm.nih.gov/ under accession no. PP626204. The associated BioProject, SRA, and BioSample numbers are PRJNA1098444, SRR28606097 and SAMN40907818, respectively.

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