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MITOGENOME REPORT

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The complete mitogenome of *Alternaria tenuissima* (Kunze) Wiltshire 1933 (Pleosporaceae), a fungus causing apple leaf blotch disease

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ABSTRACT

Alternaria tenuissima (Kunze) Wiltshire 1933 is a plant pathogenic fungus mainly causing leaf blotch disease. Here, we de novo assembled mitochondrial genome of *A. tenuissima* isolate AT-1224. The total mitogenome size is 57,475 bp with 29.00% G + C content. The genome contained 12 coding genes and 15 hypothetical proteins, 34 transfer RNA (tRNA) genes and 2 ribosomal RNA (rRNA). There are 227 SSR repeats, range from 2 to 4 base pairs, most five repeats were AT (144), AAT (54), AG (33), AC (13) and AAG (5). The results also found 13 tandem repeats (>100 bp), the largest repeat were forward 2 times located from 13,405 to 20,024 bp and 25,549 to 32,168 bp. Phylogenetic analysis based on 17 species complete mitogenomes indicated that *A. tenuissima* mitogenome was closest to 2 species, *A. solani* and *A. alternata,* sister clade to 6 species, representing *Curvularia clavate*, *Exserohilum rostratum*, *Exserohilum turcicum*, *Bipolaris cookie*, *Bipolaris oryzae* and *Bipolaris sorokiniana*. Further analysis among common fungus in local apple orchards using mitochondrial protein-coding genes revealed *A. tenuissima* were closing to 2 Alternaria fungi and a fungus representing *Phoma* sp. These results provide a basic reference for identification and evolution studies of *A. tenuissima* on apple trees.

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Alternaria tenuissima; apple leaf blotch; mitogenome; phylogenetic

Introduction

Alternaria tenuissima is a specie of fungus belonging to the genus of Alternaria, which is known for containing many plant pathogens (Logrieco et al. 2009). This fungus like other species in the genus of Alternaria, can cause disease in a wide range of host plants, leading to various forms of leaf blotch, rots, and stem blights (Kumar et al. 2022). *A. tenuissima* causing disease in apples can lead to a condition commonly known as Alternaria leaf blotch and postharvest diseases. This fungal pathogen affects the leaves, fruits, and occasionally the twigs, leading to reduced fruit quality and yield (Elfar et al. 2019; Cao et al. 2024). Various apple cultivars are susceptible to Alternaria species, the disease typically manifests as small, dark, circular spots on the leaves, which can expand and merge to form larger necrotic areas (Elfar et al. 2018a; Madhu et al. 2020). On the fruit, the spots may appear as sunken, dark lesions that affect the apple's appearance and marketability. Although multiple *Alternaria* species have been described as causing leaf blotch of apple (Harteveld et al. 2013), such as *A. mali* (Shahzad et al. 2002; Gur et al. 2017), *A. alternata, A. arborescens* (Toome-Heller et al. 2018), *A. infectoria* and *A. longipes* (Elfar et al. 2018b), *A. tenuissima* have also been commonly species (Elfar et al. 2023). During late summer of 2020–2023, an outbreak of apple leaf blotch was observed in Chenggong district in Kunming city in China, thus in this study, we collected samples, sequenced and assembled the mitogenome of *A. tenuissima*. In addition, we compared the mitogenomes using various species by phylogenetic analysis. To the best of our knowledge, this is the first report on the assembled mitogenome of *A. tenuissima*, which will provide insight to understand the fungal adaptation and evolution in local orchards.

Materials and methods

The foliage with typical blotch was collected from *Malus domestica* 'Fuji' in the chenggong district (E 102.54 and N 24.82), Kunming City, China, on 5 May 2023. Subsequently, pure cultures of *A. tenuissima* were obtained by single spore isolation on PDA medium. The fungal morphology was identified as described in preious study (Tymon et al. 2016), *A. tenuissima* deposited in the School of Life Sciences at Yunnan Normal University (Chen Tan, tanchen1632022@163.com) under the voucher number AT-1224 (Figure 1). The total genomic DNA of AT-1224 was extracted using a modified CTAB method (Cota-Sánchez et al. 2006). Library construction and DNA sequencing were conducted on the Illumina Novoseq 6000 platform (Illumina, San Diego, CA); for sequencing,

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libraries with an average fragment length of 150 bp were created using high-quality DNA and the NexteraXT DNA Library Preparation Kit. Sequencing proceeded using Pair-End 150 on the generating 647.3 Mb of raw sequence data. Adapter sequences and pairing data were trimmed using CLC Genomic Workbench 20.0.3 with default parameters, yielding 628.7 Mb of data. The data were mapped to the mitogenomes of *A. alternata* and *A. solani* (NCBI Reference Sequence:

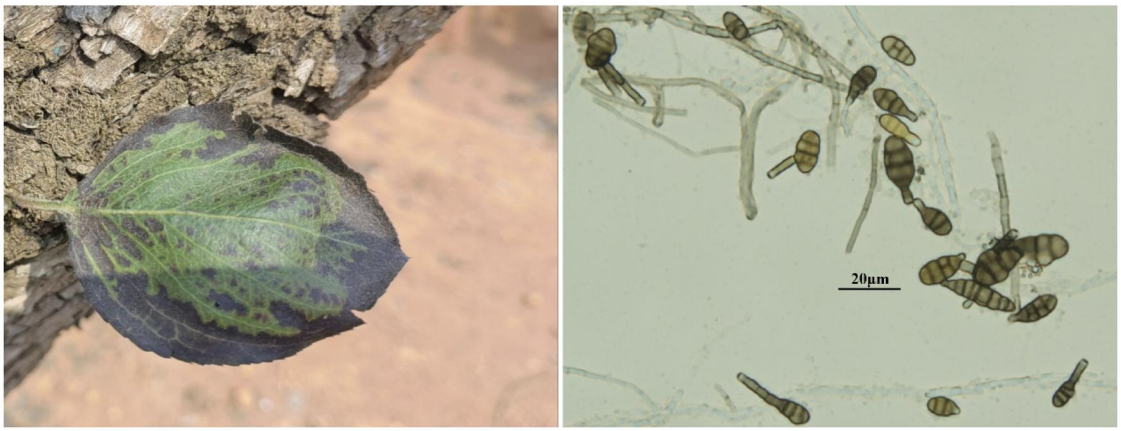

Figure 1. The apple leaf blotch symptom and spore morphological features of *A. tenuissima* at-1224 (Photographed by Chen Tan).

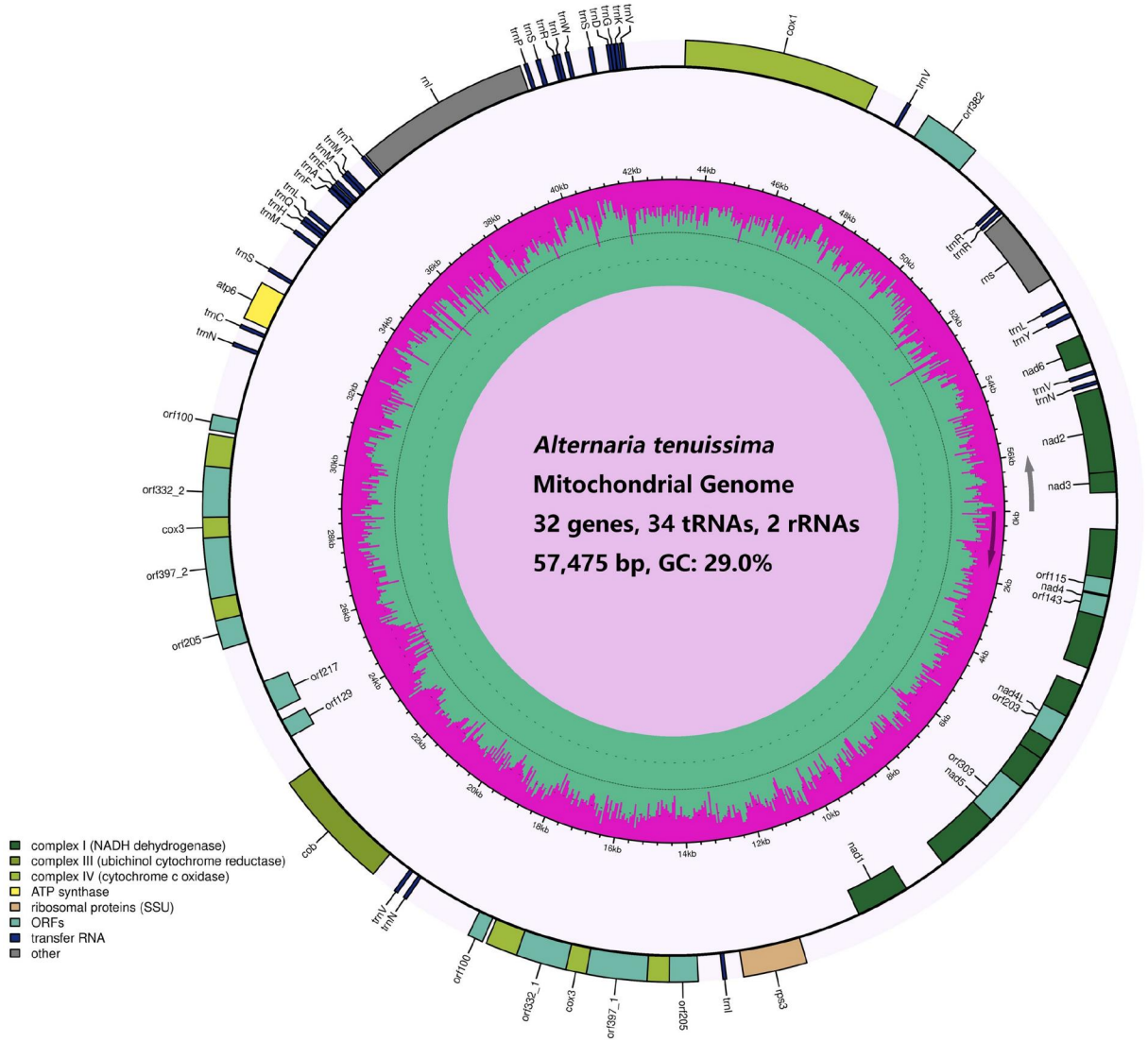

Figure 2. The mitogenome map of *A. tenuissima* at-1224. Genes drawn outside the outer circle are transcribed counterclockwise, otherwise genes are transcribed clockwise. The differently legends in the left bottom representing various gene functions. The purple inner circle indicates the GC content of the whole mitogenome.

Figure 3. Cis-splicing genes in *A. tenuissima* at-1224 mitogenome.

MF669499, PP091967, respectively) using Bandage (Wick et al. 2015). The aligned BAM files were then transfer to fasta read files by CLC Genomics Workbench 20.0.3 and de novo assembled with GetOrganelle v1.7.7.0 (Jin et al. 2020). From these assembly procedures, we obtained the final mitogenome sequence of *A. tenuissima*. The genome was annotated using MFannot web platform (https://megasun.bch.umontreal.ca/apps/mfannot/). The genome map was finally visualized online using chloroplot(https://irscope.shinyapps.io/chloroplot/) (Zheng et al. 2020). Cis and trans splicing genes were analyzed by CPGView 0.07 (Liu et al. 2023). A Maximum-Likelihood tree was selected to conduct from 18 mitogenomes using GTR+G+I model with 1000 bootstrap replicates. Meanwhile, all mitochondrial coding-protein genes from 9 common fungi, including *Botryotinia fuckeliana* (KC832409), *Fusarium oxysporum* (LT906347), *Colletotrichum acutatum* (NC_027280), *Monilinia fructicola* (NC_056195), *Cladosporium anthropophilum* (NC_061970), *Penicillium expansum* (NC_079663), *Phoma* sp.(OM236666), *Alternaria alternata* (MF669499) and *Alternaria solani* (PP091967) were multi-aligned to *A. tenuissima* (PP484585) by Geneious Tree Builder with Tamura-Nei model and Neighbor-joining method in Geneious Prime 2021.1.1 (https://www.geneious.com).

Results and discussion

The result obtained 10,772,940 raw reads were generated by Illumina sequencing, and 10,479,164 clean reads after trim procedure. In total, a length of 57,475 bp circular assembled mitochondrial DNA was submitted to GenBank, and the accession number PP484585.3 was obtained. The sequencing coverage figure is detailed in the supplementary materials (Figure S1). The genome sequence contained 8 introns, 12 coding genes included nad2, cox1, cox3, nad1, nad5, nad4L, nad4, nad3, nad6, atp6, rps3, cob and 15 hypothetical proteins), 34 transfer RNA (tRNA) genes and 2 ribosomal RNA (rRNA) gene contained 1 small ribosomal RNA and 1 large ribosomal RNA (Figure 2). The GC content of mitogenome was 29.00%. Furthermore, the genome contained 227 SSR repeats, range from 2-4 base pairs, most repeats were range from 2 to 4 base pairs, most five repeats were AT (144), AAT (54), AG (33), AAT (24), AC (13) and AAG (5) (supplementary Table S1). The results also found 13 tandem repeats (>100 bp) in mitogenome (supplementary Table S2). The longest repeat unit representing 2 times forward transcription direction 6,620 bp was located in 13,405–20,024 and 25,549–32,168. In addition, six Cis splicing genes were found in the mitogenome (nad4, nad4L, nad5, cox3, cob and cox1) (Figure 3).

To analysis mitogenome phylogenetic position, 17 fungal mitogenomes were collected from NCBI with PP484585 were genome-wide alignment by MAFFT v7.49 (Katoh and Standley 2013), a 1000 replicates bootstrap consensus tree were generated by MEGA 11 (Tamura et al. 2021). Neighbor joining with best model by hierarchical likelihood ratio tests was used to construct tree. The results indicated that best models (GTR+G+I) with the lowest BIC scores (1583895.098) are considered to describe the substitution pattern. *A. tenuissima* PP484585 is closest to *A. solani* PP091967 and *A. alternata* MF669499. Besides, the phylogenetic status exhibits six species, *Curvularia clavate* NC_062622.1, *Exserohilum rostratum* NC_063082.1, *Exserohilum turcicum* NC_062883.1 *Bipolaris cookie* NC_036417.1, *Bipolaris oryzae* NC_057095 and *Bipolaris sorokiniana* NC_047242.1 representing a sister clade to the genus of Alternaria (Figure 4). Further analysis among common fungus in local apple orchards using all mitochondrial protein-coding genes in each species revealed *A. tenuissima* were closing to two Alternaria fungi and a fungus representing *Phoma* sp., which causing apple fruit spot (Supplementary Figure S2).

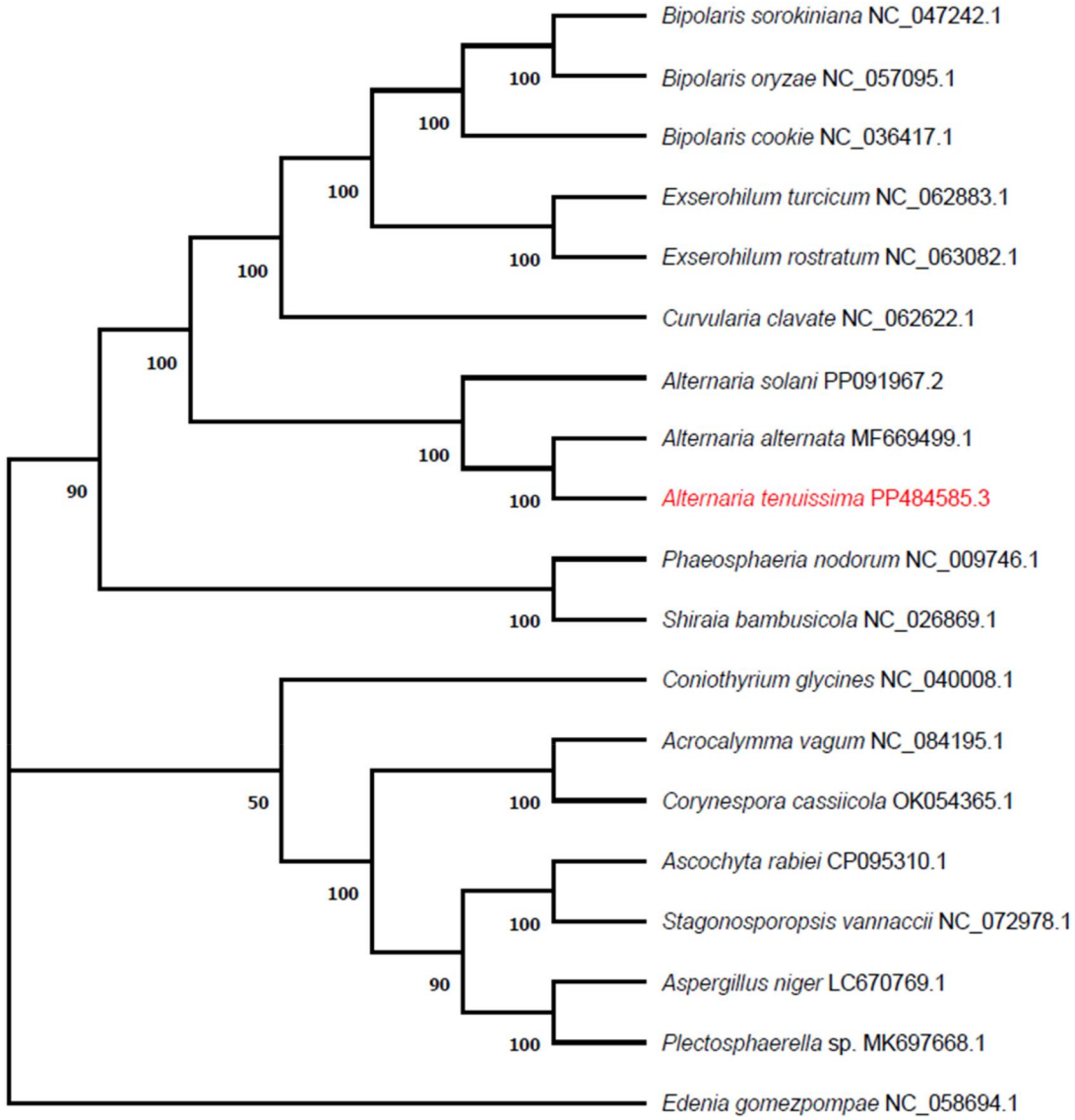

Figure 4. Mitogenome phylogenetic analysis of 18 whole fungal mitogenomes. The following sequences obtained from GenBank were used: *Alternaria solani* (PP091967.2) (Unpublished), *Alternaria alternata* (MF669499.1) (Liao et al. 2017), *Curvularia clavate* (NC_062622.1) (Unpublished), *Exserohilum rostratum* (NC_063082.1) and *Exserohilum turcicum* (NC_062883.1) (Ma et al. 2022), *Bipolaris cookie* (NC_036417.1) (Unpublished), *Bipolaris oryzae* (NC_057095.1) (Deng et al. 2019), *Bipolaris sorokiniana* (NC_047242.1) (Unpublished), *Aspergillus Niger* (LC670769.1) (Unpublished), *Acrocalymma vagum* (NC_084195.1) (Unpublished), *Edenia gomezpompae* (NC_058694.1) (Unpublished), *Phaeosphaeria nodorum* (NC_009746.1) (Unpublished), *Shiraia bambusicola* (NC_026869.1) (Shen et al. 2015), *Stagonosporopsis vannaccii* (NC_072978) (Unpublished), *Ascochyta rabiei* (CP095310.1) (Shah et al. 2020), *Coniothyrium glycines* (NC_040008.1) (Unpublished), *Plectosphaerella* sp. (MK697668.1) (Unpublished) and *Corynespora cassiicola* (OK054365.1) (Ma et al. 2021). The bootstrap values were shown on the nodes.

Conclusions

In this study, the complete mitogenome of *A. tenuissima* was sequenced for the first time and had a total length of 57,475 bp. A total of 12 coding genes, 15 hypothetical proteins, 34 tRNA and 2 rRNA were also annotated. In conclusion, our results provide genetic resources to species identification and phylogenetic relationships in the genus of Alternaria. This research also provides the reliable mitogenomic data for further studies on the adoption of the *A. tenuissima* from apple trees in south west China

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Ethical approval

The material covered in the article does not involve any ethical conflict. *A. tenuissima* is not a quarantine fungus and wide host range fungus in China, thus it did not need any specific permissions. All acquisition and sequencing works were carried out in strict compliance with relevant local laws and laboratory regulations in order to preserve wild resources. This research is complete comply with nation and international legislation.

Author contributions

Wei Tang conceived and designed this research. Chen Tan and Jianmei Li collected samples, conducted experiments and wrote the draft, Jing Peng, Xiangdong Zhang and Aihua Fu assembled the mitogenome. Wei Tang and Yan Feng revised the manuscript. Yan Feng conducted genomic annotation and phylogenetic analysis. All authors read and approved the final manuscript and agreed to submit.

Disclosure statement

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

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Data availability statement

The mitogenome sequence data that support the findings of this study are openly available in GenBank of the NCBI at https://www.ncbi.nlm.nih.gov/ under the accession No. PP484585.3. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1088303, SRR28390632 and SAMN40466408, respectively.

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