MITOCHONDRIAL DNA PART B: RESOURCES
2025, VOL. 10, NO. 4, 320–324
https://doi.org/10.1080/23802359.2025.2482262

Taylor & Francis
Taylor & Francis Group

MITOGENOME REPORT

OPEN ACCESS Check for updates

Complete mitochondrial genome of *Paraisaria gracilioides* (Hypocreales: Ophiocordycipitaceae) and phylogenetic analysis

Minghao Tang[a]*, Wei Zheng[a]*, Yixi Zhang[a], Zihao Wang[a], Wei Huang[a], Qiaosun Huang[a], Feifan Wang[a], Yanan Wang[b] and Luodong Huang[a]

[a]College of Life Science and Technology, Guangxi University, Nanning, China; [b]State Key Laboratory for Quality Ensurance and Sustainable Use of Dao-Di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China

ABSTRACT

Paraisaria gracilioides (Kobayasi) Luangsa-ard, Mongkolsamrit & Samson, originally described as *Ophiocordyceps gracilioides*, is a member of the Ophiocordycipitaceae, within the order Hypocreales. This study, presented the mitochondrial genome of *P. gracilioides* structured as a circular molecule spanning 181,140 bp, was larger than those of most Ophiocordycipitaceae species. Despite this size variation, encoding 49 genes, including 15 PCGs, 26 tRNAs, two rRNAs, and six ORFs. Phylogenetic analysis based on nucleotide sequences of 14 PCGs revealed that *P. gracilioides* clustered with *Paraisaria gracilis*, forming a subgroup within *Ophiocordyceps* species. The results contribute to refining the taxonomic framework of Ophiocordycipitaceae.

ARTICLE HISTORY
Received 12 September 2024
Accepted 14 March 2025

KEYWORDS
Paraisaria gracilioides; Ophiocordycipitaceae; mitochondrial genome; phylogenetic analysis

1. Introduction

Paraisaria was introduced as asexual stage of *Ophiocordyceps* by Samson and Brady (1983) and Sung et al. (2007). It is formally recognized as an independent taxon within the Ophiocordycipitaceae by Mongkolsamrit et al. (2019), based on the morph of stroma it is solitary, usually dark red to brown to grayish yellow, with an enlarged Fertile part at the end of the stroma, spherical to subspherical in shape. However, its taxonomic status remains a subject of considerable debate.

The genus *Paraisaria* encompasses 22 formally documented species, distributed across six continents (Mongkolsamrit et al. 2019; Tehan et al. 2023). Among these, *Paraisaria dubia* (syn. *P. gracilis*), the type species of the genus (previously referred to as *Ophiocordyceps gracilis*), has garnered attention for its medicinal potential (Abuduaini et al. 2021). This species is notably abundant in nucleoside compounds and intracellular polysaccharides, exhibiting antioxidative properties and inhibitory effects on the proliferation of AGS gastric cancer cells (Huang et al. 2019; Manzilamu et al. 2019; Wang et al. 2019). As an alternative resource to *Ophiocordyceps sinensis* for medicinal applications, it presents substantial prospects for pharmaceutical development and practical utilization.

Paraisaria gracilioides (Kobayasi) Luangsa-ard, Mongkolsamrit et al. (2019), the anamorph of *Ophiocordyceps gracilioides* (syn. *Cordyceps gracilioides*), was initially characterized by Kobayasi on larvae of Cossidae (Coleoptera) (Shimizu 1994). Currently, *P. gracilioides* is documented in China, Japan, and the United States (Fan et al. 2001; Tehan et al. 2023). Limited research on its systematic classification has hindered the exploration of its potential applications. To clarify phylogenetic relationships within *Paraisaria*, the mitogenome of *P. gracilioides* was sequenced and analyzed. This study constitutes the first report of its mitogenome and establishes a foundational framework for advancing research on phylogenetic dynamics within the Ophiocordycipitaceae family.

2. Materials and methods

2.1. Fungal materials

The specimen of *P. gracilioides* (NBRC 111628) employed in this study was originally isolated from Naka-gun (N 36.58°, E 138.14°), Tokushima, Japan. Acquired from the NITE Biological Resource Center (NBRC), it was cultured on potato dextrose agar (PDA) medium and subsequently deposited in the Herbarium of the College of Life Science and Technology, Guangxi University, Nanning, Guangxi, China, where it was

CONTACT Luodong Huang ynhuangld@gxu.edu.cn College of Life Science and Technology, Guangxi University, No. 100 Daxue East Road, Nanning, Guangxi 530004, China; Yanan Wang wangyn@nrc.ac.cn State Key Laboratory for Quality Ensurance and Sustainable Use of Dao-Di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China

Supplemental data for this article can be accessed online at https://doi.org/10.1080/23802359.2025.2482262.

*Both authors contributed equally to this work.

cataloged under voucher number GXU-FOGI628. The designated contact for this material was Luodong Huang (ynhuangld@gxu.edu.cn).

2.2. DNA sequencing, mitogenome assembly, and annotation

The cultivation of *P. gracilioides* was conducted on PDA medium at 25 °C for 30 days (Figure 1), followed by the collection of mycelia for DNA extraction. Genomic DNA was isolated using the MiniBEST Universal Genomic DNA Extraction Kit (TaKaRa, Beijing, China) in strict adherence to the manufacturer's protocol. High-throughput paired-end 150 bp sequencing was performed using an Illumina HiSeq 4000 platform by BGI Biotechnology Co. (Shenzhen, China). *De novo* assembly of the mitogenome and ITS sequences was executed using GetOrganelle software (Jin et al. 2020).

The mitogenome annotation adhered to the methodology outlined by Abuduaini et al. (2021), utilizing tools such as MFannot, MITOS, MITOFY, GeSeq, tRNAscan-SE, RNAmmer v1.2, and the UGENE open reading frames (ORFs) finder. Subsequent manual refinements were executed via the Apollo software (Dunn et al. 2019) and the UGENE toolkit (Okonechnikov et al. 2012). Intron identification was conducted using RNAweasel (http://megasun.bch.umontreal.ca/cgi-bin/RNAweasel/RNAweaselInterface.pl). Circular genome maps and cis-splicing gene visualization were constructed with PMGmap (http://www.1kmpg.cn/pmgview) (Li et al. 2025). Sequencing depth and coverage analyses followed an online protocol provided by Ni et al. (2023).

2.3. Phylogenetic analysis

To determine the phylogenetic position of *P. gracilioides*, 12 mitochondrial genomes from species within the Ophiocordycipitaceae were analyzed. *Cordyceps cicadae* (MH922223) and *Cordyceps militaris* (KF432176) served as out-group species. Shared mitochondrial genes were identified and aligned using HomBlocks (Bi et al. 2018), while ITS sequences corresponding to the 14 species were aligned with MEGA v.12 (Kumar et al. 2024). Phylogenetic relationships were inferred through the maximum-likelihood approach implemented in MEGA v.12, employing the GTR + G + I model with 1000 bootstrap replicates to ensure statistical robustness (Kumar et al. 2024).

3. Results

3.1. Basic features of the P. gracilioides mitogenome

The complete mitogenome of *P. gracilioides* is a circular DNA molecule spanning 181,140 bp, now available in the NCBI database under GenBank accession number: OP832231. Sequencing coverage depth across the assembled mitogenome ranged from 172× to 8028×, with an average depth of 7641.15× (Supplementary Figure S1). This mitogenome encoded 49 genes, comprising 15 protein-coding genes (PCGs), 26 tRNA genes, two rRNA genes, and six ORFs (Figure 2). Its nucleotide composition included 39.2% A, 15.5% C, 11.8% G, and 33.5% T, resulting in an elevated AT content of 72.7%. The AT content in rRNA and tRNA sequences was 65.8% and 62%, respectively.

The 15 PCGs comprised three *atp* genes, three *cox* genes, seven *nad* genes, one ribosomal protein gene, and one *apocytochrome* b gene. Six ORFs were predicted, among which four (orf108, orf118, orf211, and orf529) showed homology with genes from *Ophiocordyceps sinensis* (KY622006) (Li et al. 2015), while the remaining two unique to *P. gracilioides*, were designated Ogs01 and Ogs2 (Figure 2). The total length of the PCGs was 19,567 bp (10.8%), aligning with previously reported mitogenomes of *P. gracilis* (MT371080). Notably, intergenic regions constituted 17.8% (32,254 bp) of the mitogenome, reflecting a substantial proportion. With the exception of nad6, which has TAG as its stop codon, all PCGs

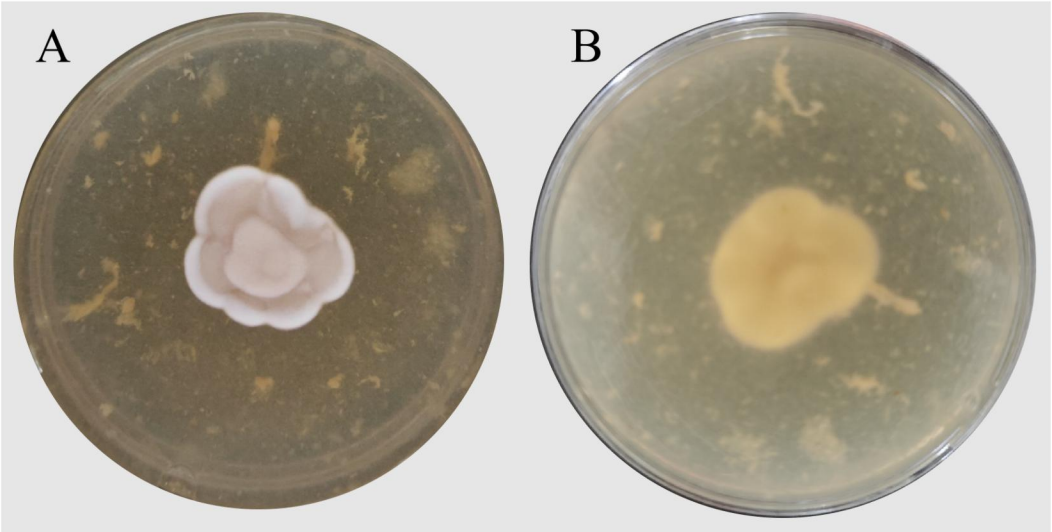

Figure 1. Colony characteristics of *P. gracilioides* grown on PDA medium, with panel A depicting the obverse and panel B displaying the reverse. The photographs were taken by Luodong Huang on 1 September 2023, in the Biological Resources and Environmental Microbiology Laboratory, College of Life Science and Technology, Guangxi University.

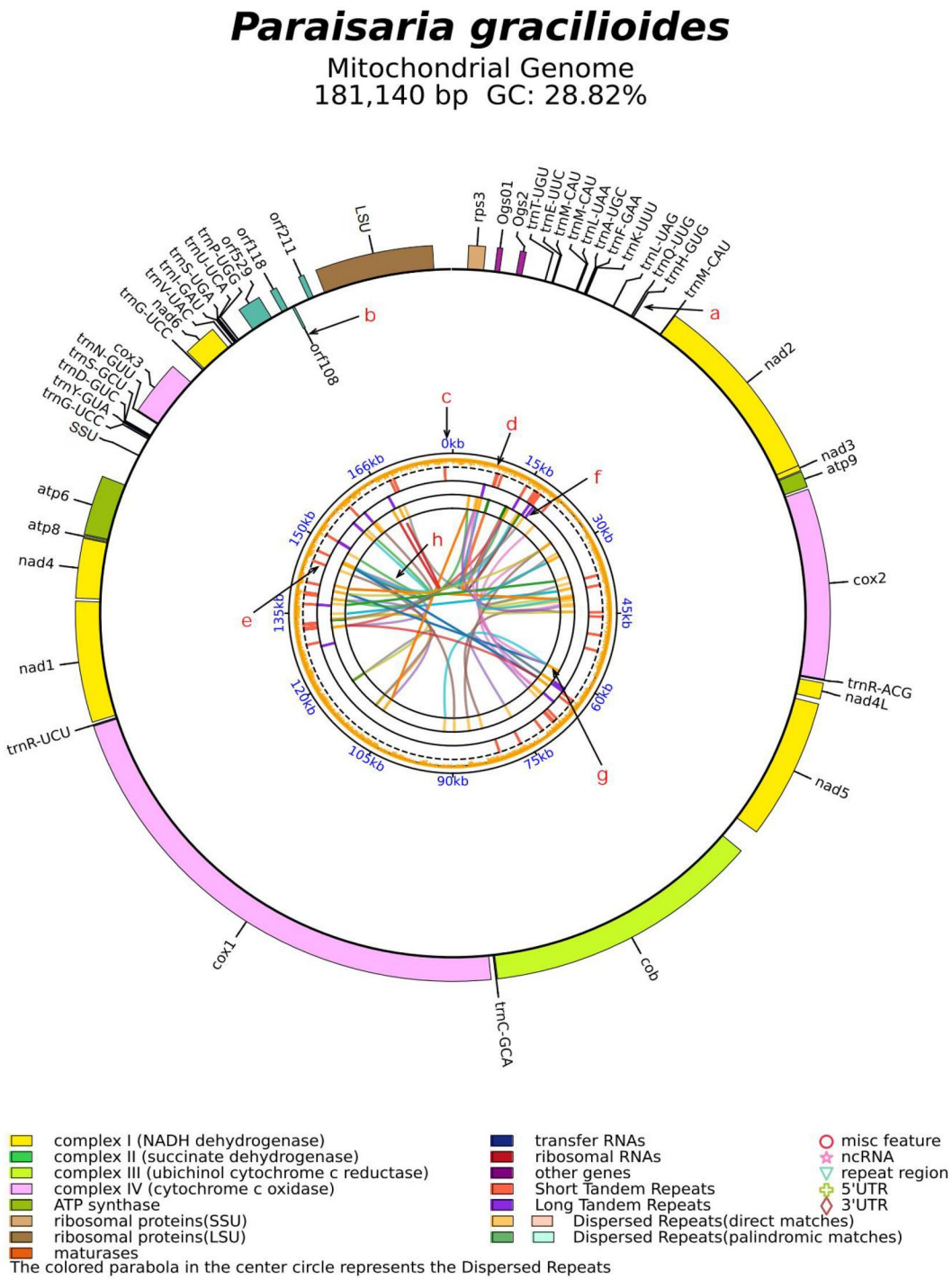

Figure 2. The circular mitochondrial genome map of *P. gracilioides*, with genes categorized by functional groups and distinguished through specific color coding. Variations in color intensity and hue signify different gene types. Ogs01 and Ogs2 are identified as newly predicted ORFs within the genome. The map, constructed using PMGmap, highlights key structural features of the circular mitogenome. Components include (a) genes located on the positive strand, (b) genes on the negative strand, (c) the scale coordinate axis, (d) GC content distribution along the chromosome, (e) microsatellite repeat sequence distribution, (f) tandem repeat sequence distribution, (g) dispersed repeat sequence distribution (yellow representing direct repeats, green indicating inverted repeats), and (h) interconnections between dispersed repeat sequences.

initiated with ATG codon and terminated with TAA codon. The mitogenome included 74 introns, with 57 classified under group I, and 17 under group II (Supplementary Table S1). Interestingly, 12 genes (*atp6*, *atp9*, *cob*, *cox1*, *cox2*, *cox3*, *nad1*, *nad2*, *nad4*, *nad5*, *nad6*, and *rnl*) identified as a cis-splicing introns using PMGmap (http://www.1kmpg.cn/pmgview) (Li et al. 2025), as depicted in Supplementary Figure S2.

3.2. Phylogenetic position of P. gracilioides in Ophiocordycipitaceae

The mitochondrial genomes of 12 Ophiocordycipitaceae species were analyzed by aligning 10,931 bp collinear block, which included 14 shared genes (*cox1*, *cox2*, *cox3*, *cob*, *atp6*, *atp8*, *atp9*, *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, and *nad6*).

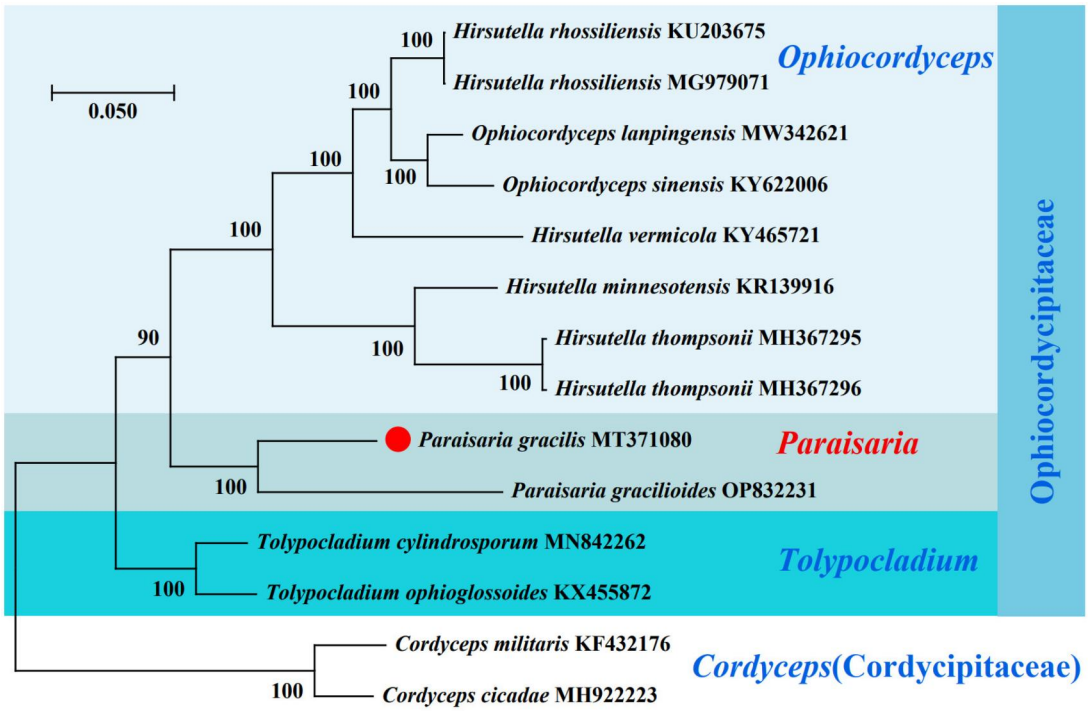

Figure 3. Phylogenetic relationships among 14 mitochondrial genomes within *Ophiocordycipitaceae*. Bootstrap values are provided at the nodes, while the scale bar represents 0.20 substitutions per nucleotide position. GenBank accession numbers for the sequences are enclosed in parentheses: *Hirsutella rhossiliensis* KU203675 (Wang et al. 2016), *Hirsutella rhossiliensis* MG979071 (Yan et al. 2019), *Ophiocordyceps lanpingensis* MW342621 (Shabana et al. 2023), *Ophiocordyceps sinensis* KY622006 (Li et al. 2015), *Hirsutella vermicola* KY465721 (Zhang et al. 2017), *Hirsutella minnesotensis* KR139916 (Zhang et al. 2016), *Hirsutella thompsonii* MH367295 (Wang et al. 2018), *Hirsutella thompsonii* MH367296 (Wang et al. 2018), *Paraisaria gracilis* MT371080 (Abuduaini et al. 2021), *Tolypocladium cylindrosporum* MN842262 (Zhang and Zhang 2020), *Tolypocladium ophioglossoides* KX455872 (Huang et al. 2017), *Cordyceps cicadae* MH922223 (Fan et al. 2019), and *Cordyceps militaris* KF432176 (Sung 2015).

Phylogenetic trees were constructed from these consensus sequences.

The mitogenome phylogenetic analysis revealed that *P. gracilioides* clustered with *P. gracilis*, collectively forming the genus *Paraisaria* with strong support values (Figure 3). *Paraisaria* further groups with the *Ophiocordyceps* and *Tolypocladium*, defining the Ophiocordycipitaceae within the order Hypocreales. These mitogenome phylogenetic structures introduced a revised classification of *Paraisaria* and its relationship to *Ophiocordyceps*. However, ITS and mitochondrial phylogenetic trees were inconsistent (Supplementary Figure S3), and *Parasaria* belongs to *Ophiocordyceps*. Notably, Mongkolsamrit et al. (2019) resurrected *Paraisaria* as a new and separate genus, which is also confirmed by the mitochondrial genome results in this study. Paraisaria is a separate taxonomic group in Ophiocordycipitaceae (Tehan et al. 2023). Thus, the phylogeny of the mitochondrial genome may be a better representation of the evolution of Ophiocordycipitaceae. However, the large-scale *Paraisaria* mitochondrial genome data are lacking at present. These results indicate that the study is of great value as the second complete mitochondrial genome of *Parasaria*.

4. Discussion and conclusions

The study presents the first mitogenome of *Paraisaria gracilioides*, revealing a genome size of 181,140 bp, significantly larger than those of *P. gracilis* (Abuduaini et al. 2021) and *O. sinensis* (Li et al. 2015). It was found that the change of *P. gracilioides* mitochondrial genome size were mainly caused by the increase of introns, the expansion of non-coding regions and more abundant repeat sequences. Phylogenetic analysis of mitochondrial sequences confirmed the placement of *Paraisaria* within the Ophiocordycipitaceae (Figure 3). Comparative analysis of mitogenomes from *Paraisaria* and *Ophiocordyceps* highlighted a strong genetic association (Abuduaini et al. 2021), substantiating the distinction of *Paraisaria* as a separate taxonomic group. These findings support the reinstatement of *Paraisaria* as independent taxa from the Ophiocordyceps family (Mongkolsamrit et al. 2019). Expanding mitogenomic research is essential for elucidating phylogenetic relationships within *Paraisaria*. Future investigations should consider collinearity assessments, ancestral structural analysis, homologous recombination events, and divergence time estimation to deepen understanding of the *Paraisaria*.

Acknowledgements

The authors express their gratitude to Bullet Edits Limited for providing linguistic editing and proofreading services for this manuscript.

Author contributions

CRediT: **Minghao Tang**: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Wei Zheng**: Conceptualization, Formal analysis, Methodology, Resources, Software, Validation, Visualization, Writing – original draft; **Yixi Zhang**: Data curation, Funding acquisition, Investigation, Validation, Writing – original draft; **Zihao Wang**: Data curation, Methodology, Software, Validation, Writing – original draft; **Wei Huang**: Data curation, Investigation; **Qiaosun Huang**: Data curation, Visualization; **Feifan Wang**: Data curation, Methodology; **Yanan Wang**:

Conceptualization, Formal analysis, Funding acquisition, Project administration, Supervision, Writing – review & editing; **Luodong Huang**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Ethical approval

The study did not involve humans or animals, and the fungus specimen is not designated as endangered species. It requires no specific permissions or licenses.

Disclosure statement

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

Funding

This work was supported by the Key Project at Central Government Level: The Ability Establishment of Sustainable Use for Valuable Chinese Medicine Resources (2060302), China Academy of Chinese Medical Sciences Innovation Fund (CI2023E002), Yunnan Key Laboratory for Fungal Diversity and Green Development, Kunming Institute of Botany, Chinese Academy of Sciences (202205AG070092), and Guangxi College Students Innovation and Entrepreneurship Training Program (202310593023, 202410593298).

Data availability statement

The genome sequence data that support the findings of this study are openly available in GenBank of NCBI under accession no. OP832231. The associated BioProject, BioSample, and SRA numbers are PRJNA1154461, SAMN43417214, and SRR30482961, respectively.

References

Abuduaini A, Wang YB, Zhou HY, Kang RP, Ding ML, Jiang Y, Suo FY, Huang LD. 2021. The complete mitochondrial genome of *Ophiocordyceps gracilis* and its comparison with related species. IMA Fungus. 12(1):31. doi:10.1186/s43008-021-00081-z.

Bi G, Mao Y, Xing Q, Cao M. 2018. HomBlocks: a multiple-alignment construction pipeline for organelle phylogenomics based on locally collinear block searching. Genomics. 110(1):18–22. doi:10.1016/j.ygeno.2017.08.001.

Dunn NA, Unni DR, Diesh C, Munoz-Torres M, Harris NL, Yao E, Rasche H, Holmes IH, Elsik CG, Lewis SE. 2019. Apollo: democratizing genome annotation. PLoS Comput Biol. 15(2):e1006790. doi:10.1371/journal.pcbi.1006790.

Fan MZ, Li CR, Chen YY, Li ZZ. 2001. *Cordyceps gracilioides*, a new Chinese record. Mycosystema. 20:273–274.

Fan WW, Zhang S, Zhang YJ. 2019. The complete mitochondrial genome of the Chan-hua fungus *Isaria cicadae*: a tale of intron evolution in Cordycipitaceae. Environ Microbiol. 21(2):864–879. doi:10.1111/1462-2920.14522.

Huang FL, Li YQ, Chen XA. 2017. The complete mitochondrial genome of a medicinal fungus, *Tolypocladium ophioglossoides*. Mitochondrial DNA B Resour. 2(1):95–96.

Huang LD, Yf MA, Wang Y, Manzilamu Z, Suo FY. 2019. Research status and utilization progress of *Ophiocordyceps gracilis*. Acta Edulis Fungi. 26(2):141–151.

Jin JJ, Yu WB, Yang JB, Song Y, dePamphilis CW, Yi TS, Li DZ. 2020. GetOrganelle: a fast and versatile toolkit for accurate de novo assembly of organelle genomes. Genome Biol. 21(1):241. doi:10.1186/s13059-020-02154-5.

Kumar S, Stecher G, Suleski M, Sanderford M, Sharma S, Tamura K. 2024. MEGA12: molecular evolutionary genetic analysis version 12 for adaptive and green computing. Mol Biol Evol. 41(12):msae263. doi:10.1093/molbev/msae263.

Li J, Ni Y, Lu Q, Chen H, Liu C. 2025. PMGA: a plant mitochondrial genome annotator. Plant Commun. 6(3):101191. doi:10.1016/j.xplc.2024.101191.

Li Y, Hu XD, Yang RH, Hsiang T, Wang K, Liang DQ, Liang F, Cao DM, Zhou F, Wen G, et al. 2015. Complete mitochondrial genome of the medicinal fungus *Ophiocordyceps sinensis*. Sci Rep. 5(1):13892. doi:10.1038/srep13892.

Manzilamu Z, Huang LD, Ye X, Abuduaini A, Zhou HY, Yf MA, Suo FY. 2019. Antioxidant and anti-lung cancer activity of ethanol extract from *Ophiocordyceps gracilis* in Xinjiang. J Central China Normal Univ (Nat Sci). 53(4):542–550.

Mongkolsamrit S, Noisripoom W, Arnamnart N, Lamlertthon S, Himaman W, Jangsantear P, Samson RA, Luangsa-Ard JJ. 2019. Resurrection of *Paraisaria* in the Ophiocordycipitaceae with three new species from Thailand. Mycol Progress. 18(9):1213–1230. doi:10.1007/s11557-019-01518-x.

Ni Y, Li JL, Zhang C, Liu C. 2023. Generating sequencing depth and coverage map for organelle genomes. protocols.io. doi:10.17504/protocols.io.4r3l27jkxg1y/v1.

Okonechnikov K, Golosova O, Fursov M. 2012. Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics. 28(8):1166–1167. doi:10.1093/bioinformatics/bts091.

Samson RA, Brady BL. 1983. *Paraisaria*, a new genus for *Isaria dubia*, the anamorph of *Cordyceps gracilis*. Trans Br Mycol Soc. 81(2):285–290. doi:10.1016/S0007-1536(83)80081-3.

Shabana B, Wang D, Wang YB, Mustafa G, Yu H. 2023. Mitogenomic and phylogenetic analysis of the entomopathogenic fungus *Ophiocordyceps lanpingensis* and comparative analysis with other *Ophiocordyceps* species. Genes. 14(3):710. doi:10.3390/genes14030710.

Shimizu D. 1994. Color iconography of vegetable wasps and plant worms. Tokyo: Seibundo Shinkosha; p. 1–381.

Sung GH, Nigel LHJ, Sung JM, Shrestha B, Spatafora JW, Luangsa-Ard JJ. 2007. Phylogenetic classification of *Cordyceps* and the Clavicipitaceous fungi. Stud Mycol. 57:5–59. doi:10.3114/sim.2007.57.01.

Sung GH. 2015. Complete mitochondrial DNA genome of the medicinal mushroom *Cordyceps militaris* (Ascomycota, Cordycipitaceae). Mitochondrial DNA. 26(5):789–790. doi:10.3109/19401736.2013.855754.

Tehan RM, Dooley CB, Barge EG, McPhail KL, Spatafora JW. 2023. New species and new combinations in the genus *Paraisaria* (Hypocreales, Ophiocordycipitaceae) from the U.S.A., supported by polyphasic analysis. MycoKeys. 100:69–94. 13 doi:10.3897/mycokeys.100.110959.

Wang L, Zhang S, Li JH, Zhang YJ. 2018. Mitochondrial genome, comparative analysis and evolutionary insights into the entomopathogenic fungus *Hirsutella thompsonii*. Environ Microbiol. 20(9):3393–3405. doi:10.1111/1462-2920.14379.

Wang N, Zhang Y, Hussain M, Li K, Xiang M, Liu X. 2016. The mitochondrial genome of the nematode endoparasitic fungus *Hirsutella rhossiliensis*. Mitochondrial DNA B Resour. 1(1):114–115. doi:10.1080/23802359.2016.1143336.

Wang Y, Li ZL, Suo FY, Sun DP. 2019. Study of mycelial polysaccharide from *Paraisaria dubia* of *Ophiocordyceps gracilis* asexual. Zhongguo Zhong Yao Za Zhi. 44(8):1704–1709. doi:10.19540/j.cnki.cjcmm.20190318.201.

Yan QQ, Liu XJ, Zhang YJ. 2019. Reanalysis of the mitochondrial genome of the nematophagous fungus *Hirsutella rhossiliensis*. Acta Microbiol Sin. 59(2):280–290.

Zhang S, Zhang YJ. 2020. Complete mitogenome of the entomopathogenic fungus *Tolypocladium cylindrosporum*. Mitochondrial DNA B Resour. 5(1):680–682. doi:10.1080/23802359.2020.1714495.

Zhang YJ, Zhang HY, Liu XZ, Zhang S. 2017. Mitochondrial genome of the nematode endoparasitic fungus *Hirsutella vermicola* reveals a high level of synteny in the family Ophiocordycipitaceae. Appl Microbiol Biotechnol. 101(8):3295–3304. doi:10.1007/s00253-017-8257-x.

Zhang YJ, Zhang S, Liu XZ. 2016. The complete mitochondrial genome of the nematode endoparasitic fungus *Hirsutella minnesotensis*. Mitochondrial DNA A DNA Mapp Seq Anal. 27(4):2693–2694. doi:10.3109/19401736.2015.1046126.