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MITOGENOME ANNOUNCEMENT

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The complete mitochondrial genome and phylogenetic analysis of *Polythlipta liquidalis* Leech, 1889 (Crambidae: spilomelinae)

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ABSTRACT

The complete mitochondrial genome of *Polythlipta liquidalis* Leech, 1889 was sequenced and annotated in this study, which was the first reported complete mitogenome of the genus *Polythlipta*. The mitogenome of *P. liquidalis* is 15,305 bp in length and was predicted to encode 37 typical mitochondrial genes including 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs), and one major non-coding A-T rich region. The maximum likelihood phylogenetic analysis based on the 13 PCGs was constructed, including *P. liquidalis* and 15 related Spilomelinae species, using *Ostrinia furnacalis* as the outgroup. The result showed that *P. liquidalis* is grouped with *Sinomphisa plagialis*. These data will serve as a molecular resource for species identification of *P. liquidalis* and become a valuable resource for a range of genetic, functional, evolutionary and comparative genomic studies on members of Spilomelinae.

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1. Introduction

Polythlipta liquidalis Leech, 1889 is widely distributed in China, Japan and North Korea. This species is subordinate to the subfamily Spilomelinae of Crambidae, which contains a variety of species that can bring about great economic loss to agriculture and forestry. The genetics structure and phylogenetic status of the genus *Polythlipta* have not been investigated before, therefore the complete mitochondrial genome of *P. liquidalis* was characterized for the first time in this study, which would provide useful genomic data for future phylogenetic and taxonomic classification of the Spilomelinae.

2. Materials and methods

The specimen of *P. liquidalis* was collected by light trap in July 2018 from Mount Emei (29.59°N, 103.38°E), Sichuan Province, China. The specimen was deposited at the Entomological Museum in the College of Bioscience and Engineering, Jiangxi Agricultural University under the voucher number MT001 (https://www.jxau.edu.cn/, Hua Rong, ronghua@jxau.edu.cn).

The species *P. liquidalis* can be well diagnosed by the remarkable wing pattern as follows (Figure 1): Both the forewing and hindwing are white. The basal 1/3 of the forewing has a yellow-brown triangle patch which is surrounded by fuscous line; a large black patch is located at upper 2/3 of the distal part. The hind wing has a blackish brown ribbon-like discocellular spot (Li et al. 2012).

Total genomic DNA was extracted from thoracic muscle using the HiPure Insect DNA Kit (Magen, China). Libraries with an average fragment length of 350 bp were constructed. Then, the whole-genome sequencing was performed using the Illumina NovaSeq 6000 platform, and approximately 6 Gb raw data was obtained. After data quality control, the clean data was assembled into the complete mitogenome and annotated employing MitoZ v2.4-alpha (Meng et al. 2019). Furthermore, manual correction was performed to ensure annotation accuracy using MITOS2 (Donath et al. 2019). Finally, the mitochondrial genome cycle map of *P. liquidalis*

Figure 1. External features of *P. liquidalis*. This photo was taken by Hua Rong with the author's approval for use.

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was drawn with the program proksee v.6.0.2 (https://proksee.ca/) (Grant et al. 2023).

To analyze the phylogenetic position of *P. liquidalis*, the complete sequences of mitochondrial genomes of the fifteen closest species were downloaded based on the blasting results in GenBank, and *Ostrinia furnacalis* was selected as the outgroup (Table 1). Nucleotide sequences for each of the 13 PCGs were translated into amino acids, aligned separately with MAFFT implemented in PhyloSuite v1.2.2, and then toggled back into nucleotide alignments. The individual nucleotide alignments were concatenated using PhyloSuite v1.2.2. Based on the concatenated alignment, the phylogenetic tree was generated using the maximum likelihood (ML) analyses with 1000 bootstrap replicates in PhyloSuite v1.2.2 (Zhang et al. 2020).

Table 1. Species and GenBank accession number of mitogenomes used in this study.

NO.	Species	Accession ID	References
1	*Polythlipta liquidalis*	OQ439905	This study
2	*Pygospila tyres*	ON939556	Unpublished
3	*Botyodes diniasalis*	OQ354975	In press
4	*Glyphodes quadrimaculalis*	KF234079	Park et al. (2015)
5	*Palpita nigropunctalis*	KX150458	Unpublished
6	*Cydalima perspectalis*	MH602288	Que et al. (2019)
7	*Patania balteata*	OQ472989	Unpublished
8	*Omiodes indicata*	MG770232	Yang et al. (2018)
9	*Botyodes principalis*	MZ823351	Liu et al. (2021)
10	*Pycnarmon lactiferalis*	KX426346	Chen et al. (2016)
11	*Palpita hypohomalia*	MH013483	Yang et al. (2018)
12	*Pycnarmon cribrata*	OQ632459	Unpublished
13	*Haritalodes derogata*	KR233479	Zhao et al. (2016)
14	*Glyphodes pyloalis*	KM576860	Unpublished
15	*Maruca vitrata*	KJ466365	Unpublished
16	*Sinomphisa plagialis*	MZ823346	Liu et al. (2021)
17	*Ostrinia furnacalis*	MN793323	Zhou et al. (2020)

3. Results

The complete mitochondrial genome sequence of *P. liquidalis* was deposited in GenBank under accession number OQ439905. It was a closed circular molecular structure of 15,305 bp in length, with an average depth of 605.2× (Supplementary Figure S1). For the low coverage depth of the AT-rich region around 15,000 bp, Sanger sequencing was further performed, and the result was consistent with that of NGS assembly, indicating the correctness of this mitochondrial genome assembly data (Supplementary Figure S2). It contains 13 protein coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (*12S rRNA* and *16S rRNA*), and a non-coding control region (A-T rich region) (Figure 2). The order and orientation of the mitochondrial genes are identical to the inferred ancestral arrangement of Lepidoptera. The nucleotide composition is A 40.3%, C 11.5%, G 7.5%, and T 40.7%, showing a highly A/T bias as commonly present in insects (Boore 1999).

The total length of the 13 PCGs is 11,199 bp, accounting for 73.2% of the whole genome. Most PCGs initiate with

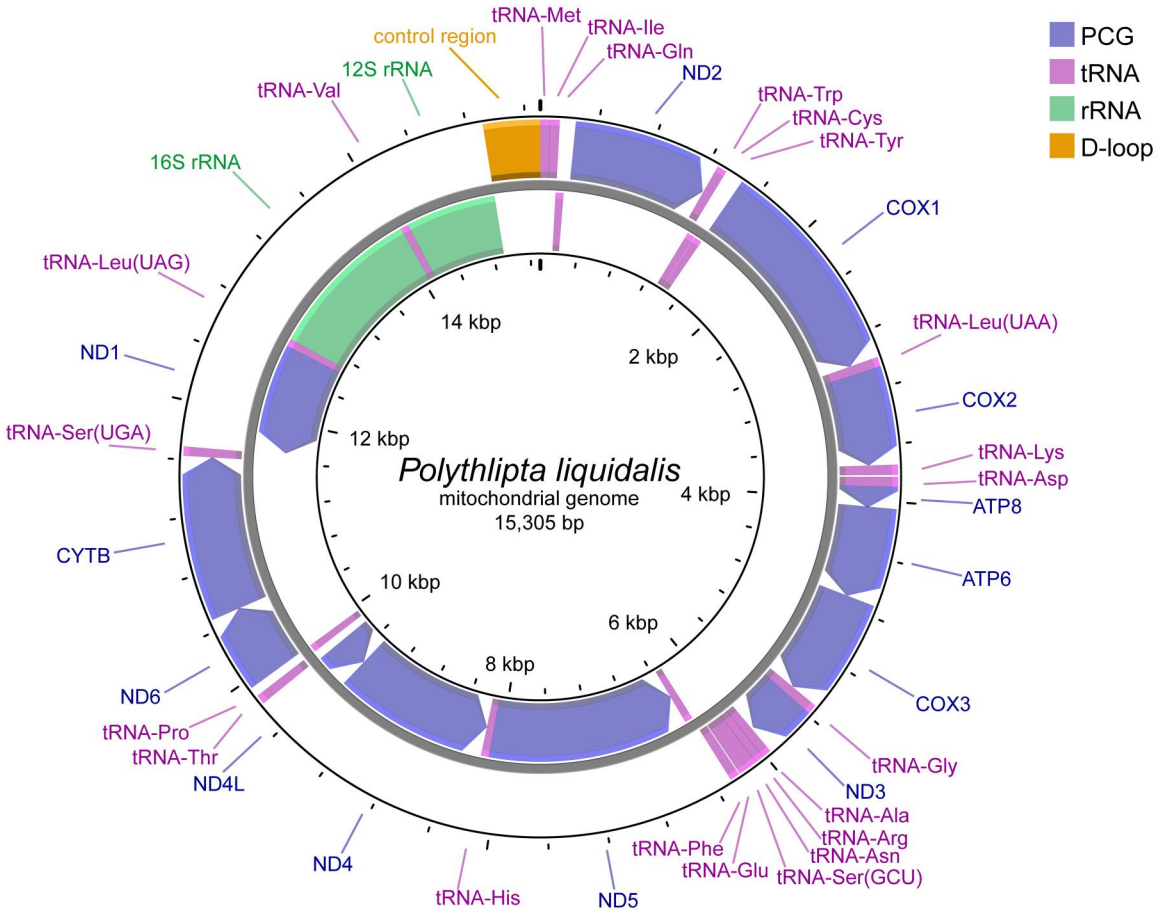

Figure 2. Mitochondrial genome map of *P. liquidalis*. Represented with arrows, the transcription directions for the outer and inner genes are listed clockwise and anticlockwise, respectively.

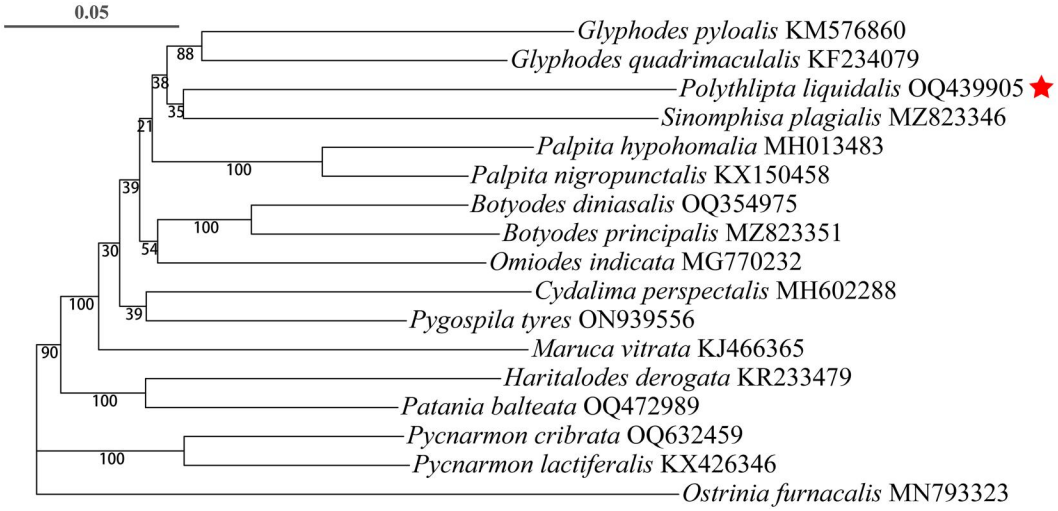

Figure 3. Phylogenetic trees using ML analyses based on 13 PCGs. The numbers above the branches are bootstrap support values (BS). alphanumeric terms indicate the GenBank accession numbers.

typical mitochondrial start codon ATN except for *COX1* with TTG, as four PCGs (*ND2*, *ND3*, *ND5* and *ND4L*) with ATT, six (*COX2*, *ATP6*, *COX3*, *ND4*, *CYTB* and *ND1*) with ATG, and two (*ATP8* and *ND6*) with ATC. Ten of the 13 PCGs terminated with complete stop codon TAA, whereas *COX1* and *COX2* used single T nucleotide, and *ND5* used TA nucleotides as an incomplete stop codon. The lengths of 22 tRNAs ranged from 63 bp (*tRNA-Arg*) to 71 bp (*tRNA-Lys* and *tRNA-Asp*). Two rRNA genes (*12S rRNA* and *16S rRNA*), which are divided by *tRNA-Val*, are 1348 bp and 782 bp, respectively.

The ML phylogenetic tree showed that the *P. liquidalis* was firstly clustered with *Sinomphisa plagialis*, then together with *Glyphodes pyloalis* and *Glyphodes quadrimaculalis* (Figure 3).

4. Discussion and conclusion

By this work, the complete mitogenome of *P. liquidalis* was identified for the first time. It was similar to that of other Lepidoptera members in relation to gene organization and composition (Liu et al. 2021). In the ML phylogenetic tree, *P. liquidalis* is grouped with *S. plagialis*. These results will be helpful for understanding the systematics among members of the subfamily Spilomelinae.

We noticed that the read counts were extremely low in the high AT-content control region of the mitogenome. This phenomenon has also been reported in other studies (Oyola et al. 2012; Gan et al. 2019), suggesting a bias of Illumina sequencing toward high AT-content regions. One possible explanation may be that the DNA fragment with high AT-content inhibited the amplification of DNA polymerase in sequencing reactions. For sequencing of the AT-rich region, it might be a feasible strategy to improve the representation of extremely high AT-content DNA fragments by lowering the annealing and extension temperature during library preparation. Alternatively, a PCR-free library preparation is also an option to obtain uniform coverage depth of genome with high AT-content (Williams et al. 2012). We hope this finding will be instructive for the subsequent Illumina sequencing and assembly of mitochondrial genomes.

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Ethical approval

The permission for collecting the sample was received from the Scenic Area Management Committee of Mount Emei. And the experiments were performed in accordance with the recommendations of the Ethics Committee for Animal Experiments of Jiangxi Agricultural University. These policies were enacted according to the Chinese Association for the Laboratory Animal Sciences and the Institutional Animal Care and Use Committee (IACUC) protocols.

Author contributions

HR and YW collected the samples; HR and LDY conceived and designed the experiments; LDY, RHW and YYZ performed the experiments; YW analyzed and interpreted the data; LDY wrote the manuscript; YW and HR revised the manuscript. All authors read, revised, and approved the final manuscript and agreed to be accountable for the work.

Disclosure statement

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

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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 number OQ439905. The associated BioProject, SRA,

and Bio-Sample numbers are PRJNA935109, SRR23461129, and SAMN33298022, respectively.

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