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MITOGENOME REPORT

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The complete mitochondrial genome analysis of *Locastra muscosalis* (Walker, 1866) (Lepidoptera: Pyralidae) and phylogenetic implications

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ABSTRACT

The complete mitochondrial genome of *Locastra muscosalis* (Walker, 1866) was sequenced and characterized in this study, which was the first reported complete mitogenome of the genus *Locastra*. The mitogenome of *L. muscosalis* has a total length of 15,177 bp, encompassing 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), 2 ribosomal RNA genes (rRNAs), and an A-T rich region. Phylogenetic analysis revealed that *L. muscosalis* is closely associated with *Orthaga euadrusalis*. These data will serve as a valuable foundation for future investigations into the Epipaschiinae and Pyralidae evolutionary history.

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1. Introduction

Locastra muscosalis (Walker, 1866) belongs to Epipaschiinae of Pyralidae, it is a notorious forest pest widely distributed in China, Japan, Korea, India and Sri Lanka (Park et al. 1993; Rong and Li 2017). The larvae mainly feed on the leaves of *Ailanthus altissima*, *Carya illinoinensis*, *Choerospondias axillaris*, *Coriaria nepalensis*, *Euonymus alatus*, *Juglans mandshurica*, *Juglans regia*, *Liquidambar formosana*, *Padus racemose*, *Pistacia chinensis*, *Pterocarya stenoptera*, *Rhus chinensis* and *Toxicodendron vernicifluum* etc (Park et al. 1993; Bae and Paek 2006; Ajay 2018; Han et al. 2023). However, the molecular data and phylogenetic studies of this species haven't done so far. Here, the mitogenome analysis of *L. muscosalis* was performed for the first time to verify the phylogenetic relationships among species in the Pyralidae family, and the result may also provide a basis for scientific control of this insect.

2. Materials and methods

The specimen of *L. muscosalis* was collected by light trap in June 2023 from Mount Mao'er (25°51′N, 111°28′E), Guangxi Zhuang Autonomous Region, China. The specimen was deposited at the Entomological Museum in the College of Bioscience and Engineering, Jiangxi Agricultural University under the voucher number MT011 (contact person: Hua Rong, email: ronghua@jxau.edu.cn).

Permanent mounting methods of genitalia followed the techniques introduced by Li (2002). The specimen was identified by Hua Rong. *L. muscosalis* can be well differentiated from other species in the *Locastra* genus based on following characteristics (Figure 1): The forewing is yellowish brown, and has no longitudinal line below lower margin of cell. The male genitalia has inverted sub-trapezoidal uncus; the valva bears a triangular process at the base of the ventral margin (Rong and Li 2017). The photograph of *L. muscosalis* was taken by Hua Rong with the author's approval for use.

The whole specimen was preserved in absolute ethyl alcohol and stored at −20 °C for subsequent DNA extraction. Genomic DNA was extracted from the thoracic tissue of a single adult using the HiPure Insect DNA Kit (Magen, China) following the manufacturer's protocol. A total amount of

Figure 1. The Photograph of *L. muscosalis*.

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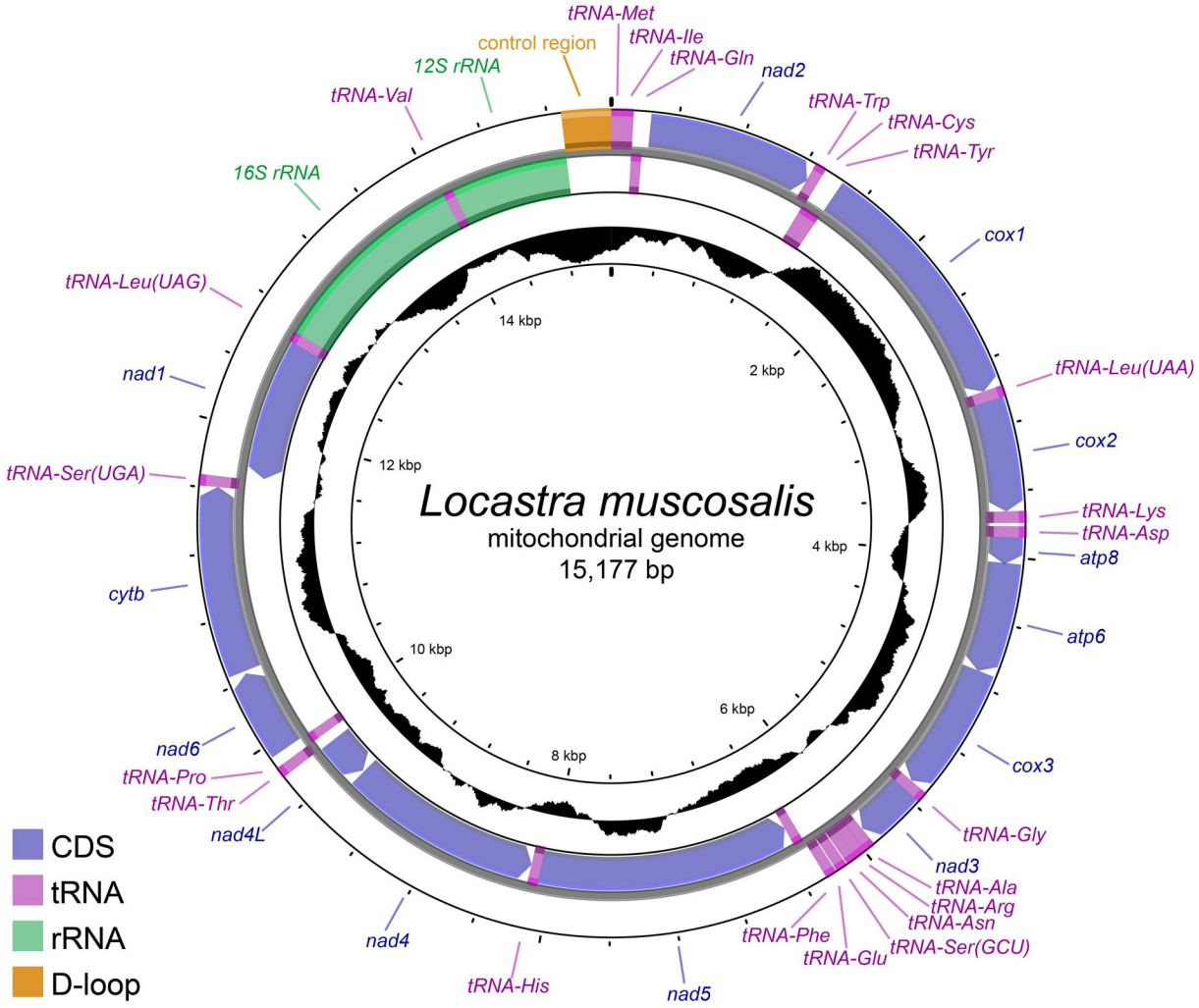

Figure 2. Circular maps of the newly sequenced complete mitochondrial genomes of *L. muscosalis* genes located outside the outer circle were transcribed clockwise, and those inside were transcribed counter-clockwise. The different colored legends in the bottom left corner represented genes with different functions. The black inner circle displayed the GC content of the mitochondrial genome.

0.2 μg genomic DNA was randomly fragmented by sonication into an average size of 350 bp, and then endpolished, A-tailed, and ligated with Illumina adapters. The resulting fragments were PCR-amplified, purified, quantified, and sequenced on Illumina NovaSeq 6000 platform with PE150 strategy. Finally, a total of 10 Gb raw data was obtained. After data quality control, the clean data was initially assembled and annotated employing SPAdes v. 3.14 (Bankevich et al. 2012), and then manual adjustment was performed using MITOS2 (Donath et al. 2019). In addition, to ensure the accuracy of the assembled mitochondrial genome sequence, Sanger sequencing was performed for the control region (see Table S1 for primer data). The circular genome map was drawn with the program proksee v.6.0.2 (https://proksee.ca/) (Grant et al. 2023).

To investigate the phylogenetic position of *L. muscosalis*, 21 mitogenomes of the closest species were downloaded based on the blasting results in GenBank, *Lymantria dispar* and *Hyphantria cunea* were selected as the outgroup. Each nucleotide sequence of the 13 PCGs were translated into amino acids, aligned separately with MAFFT implemented within PhyloSuite v1.2.2 (Zhang et al. 2020), and then toggled back into nucleotide alignments to avoid the influence of AT bias in the mitogenome nucleotide sequence. Intergeneric gaps and ambiguous sites were removed using Gblocks v0.91b (Castresana 2000), and individual alignments were concatenated using PhyloSuite. The maximum likelihood tree was generated by IQ-TREE v1.6.12 (Nguyen et al. 2015) and the Bayesian inference tree was generated by MrByes 3.2.7a (Ronquist et al. 2012). The resulting molecular phylogenetic tree is shown using iTOL (Letunic and Bork 2021).

3. Results

The complete mitogenome of *L. muscosalis* was a circular molecular with 15,177 bp in length (GenBank accession number: OR881395), with an average coverage depth of 1559.38× (Figure S1). The nucleotide compositions were 38.4% A, 40.6% T, 12.6% C, and 8.4% G, respectively, showing a marked AT bias. It consisted of 13 protein coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (*12S rRNA* and *16S rRNA*), and a control region (A-T rich region/D-loop) (Figure 2). In all the 37 genes, 23 genes were encoded on the heavy strand, and 14 were encoded on

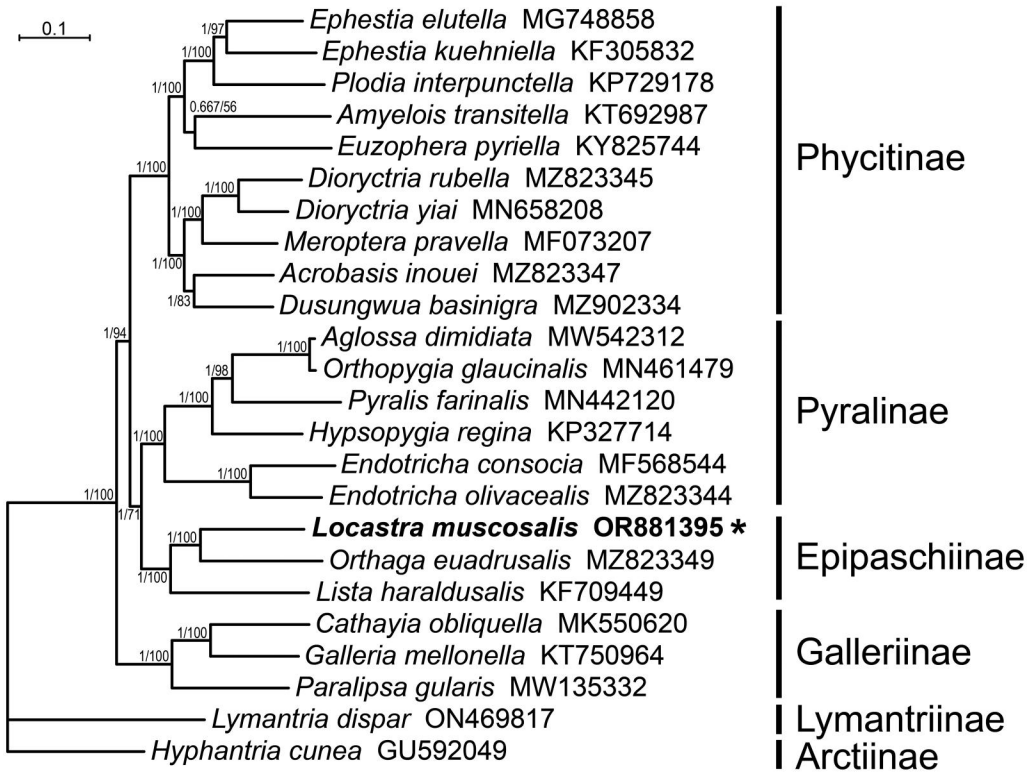

Figure 3. Phylogenetic trees using both ML and BI analyses based on 13 PCGs. The numbers above the branches were Bayesian posterior probabilities and bootstrap support values (BSV). Alphanumeric terms indicated the GenBank accession numbers. The following sequences were used to infer the tree: *Orthaga euadrusalis* MZ823349, *Acrobasis inouei* MZ823347, *Dioryctria rubella* MZ823345, *Dusungwua basinigra* MZ902334, *Endotricha olivacealis* MZ823344 (Liu et al. 2021), *Lista haraldusalis* KF709449 (Ye et al. 2015), *Cathayia obliquella* MK550620 (Roh et al. 2020), *Paralipsa gularis* MW135332 (Guo et al. 2021), *Galleria mellonella* KT750964 (Park et al. 2017), *Dioryctria yiai* MN658208 (Wu et al. 2020), *Euzophera pyriella* KY825744 (Yang et al. 2017), *Meroptera pravella* MF073207 (Ali et al. 2017), *Ephestia kuehniella* KF305832 (Traut et al. 2013), *Ephestia elutella* MG748858 (Liu et al. 2018), *Plodia interpunctella* KP729178 (Liu et al. 2015), *Amyelois transitella* KT692987 (Chang & Shen 2016), *Endotricha consocia* MF568544, *Hypsopygia regina* KP327714 (Zhu et al. 2018), *Aglossa dimidiata* MW542312 (Hu et al. 2021), *Orthopygia glaucinalis* MN461479, *Pyralis farinalis* MN442120 (Mao et al. 2019), *Lymantria dispar* ON469817 (Jeong & Lee 2022) (outgroup), *Hyphantria cunea* GU592049 (Liao et al. 2010) (outgroup).

the light strand. The total length of the 13 PCGs was 11,184 bp, accounting for 73.4% of the whole genome. Most PCGs initiated with typical ATN codons except for *cox1* with CGA and *nad4* with GTG. Eight of the 13 PCGs terminated with complete stop codon TAA, whereas *nad1* ended with TAG, four (*cox1*, *cox2*, *nad4* and *nad5*) used single T nucleotide as an incomplete stop codon. A total of 24 bp overlap between genes in 11 locations, the longest 7 bp overlap located between *atp8* and *atp6*. And the total length of the intergenic spacer was 104 bp, ranging in size from 1 to 40 bp. The longest spacer sequence is 40 bp, located between the *tRNA-Gln* and *nad2* genes.

The phylogenetic relationships among subfamilies in Pyralidae was as follows: (Galleriinae + (Phycitinae + (Epipaschiinae + Pyralinae))) (Figure 3). The newly sequenced species *L. muscosalis* belongs to the subfamily Epipaschiinae which was clustered together with high bayesian posterior probabilities (BP $\geq$ 1) and high bootstrap support values (BSV $\geq$ 100). And *L. muscosalis* is closely associated with *Orthaga euadrusalis*.

4. Discussion and conclusion

The mitogenome of *L. muscosalis* was structurally consistent with other reported snout moth (Zhu et al. 2018; Liu et al. 2021). The A + T content of *L. muscosalis* was 79%, which was accord with the characteristic of highly A/T bias as commonly present in insect mitogenomes (Boore 1999), but the A + T content was somewhat lower than that of other reported Epipaschiinae species (Ye et al. 2015; Liu et al. 2021). The initiation codon CGA for *cox1* was commonly found in lepidopteran (Sun et al. 2017; Cheng et al. 2022; Xiao et al. 2022), while the GTG utilized by *nad4* was extremely rare in the reported lepidopteran mitogenomes, which was only partially present in *cox1* of Micropterioidea and *cox2* of Zygaenoidea (Chen et al, 2022). The complete stop codon TAA was employed by *nad1* in most pyralid mitogenome, while in *L. muscosalis*, the *nad1* ended with TAG, which was also found in *Plodia interpunctella* and *Orybina plangonalis* (Liu et al. 2015; Zhu et al. 2018).

Both the ML and BI phylogenetic trees indicated that *L. muscosalis* belongs to the subfamily Epipaschiinae. Further analysis of a larger number of mitogenomes was required to accurately support the relationship among species in Epipaschiinae.

Ethical approval

There is no ethical research involved in this study. We obtained permission from Jiangxi Agricultural University and the Scenic Area Management Committee of Mount Mao'er to collect the reported sample species.

Author's contributions

YW and HR collected the samples; YW and HR conceived and designed the experiments; LYW, JLL and HSL performed the experiments; LYW and YW analyzed and interpreted the data; LYW drafted the manuscript; YW and HR revised the manuscript. All authors read 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 OR881395. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1046083, SRR26969505, and SAMN38471705, respectively.

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