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Genetic Insights and Phylogenetic Relationships of *Lycorma delicatula* (Hemiptera: Fulgoridae, 1845) Revealed Through Mitochondrial Genome Sequence Analysis

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ABSTRACT

Lycorma delicatula, a phytophagous agricultural pest, is invading various regions across the globe. We employed the mitochondrial genome and mitochondrial marker NADH dehydrogenase subunit 2 (*ND2*) to elucidate the mitochondrial genome characterization and phylogenetic relationships. The mitochondrial genome revealed a circular mitochondrial structure with a length of 16 138 bp encompassing 37 genes and a GC% of 23.41%. Codon usage preferences exhibited significant variation, with A or U being the most preferred bases in the third codon position. Most tRNAs displayed a typical cloverleaf structure, while a few exhibited base mismatches, primarily in G-U pairs. Phylogenetic analysis using the *ND2* genes indicated that the population in northwest China could be tightly near together, with a genetic distance ranging from 0 to 0.01. Speculations arising from the mitochondrial genome analysis suggest that species from northern China were closer relatives to invaders from other regions, *Lycorma meliae* emerged as a close relative of *Lycorma delicatula* among the Hemipterans. Collectively, our findings enhance the understanding of phylogenetic relationships and complement the mitochondrial genomic information of *Lycorma delicatula*, thereby contributing valuable data for subsequent studies on this pest species.

Keywords: Spotted lanternfly, Genetic analysis, Evolutionary relationship, Genetic distance, Species dispersal.

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INTRODUCTION

Lycorma delicatula, commonly known as the spotted lanternfly, belongs to the Hemiptera: Fulgoridae family. This invasive insect, originally from China, has now established itself in Japan, Europe, and America, posing a significant threat (Jung, Kho, Gook, Lee, & Lee, 2022). Its ability to infest both forests and agricultural trees is particularly alarming. This pest prefers forested habitats, feeds on phloem sap, and secretes honeydew, leading to extensive ecological devastation and significant economic losses (Urban & Leach, 2023). What's more, its potential for continuous spread poses a major risk to other regions. Human activities and the distribution of host plants are among the many factors that influence the spotted lanternfly's migration patterns (Ladin, Eggen, Trammell, & D'Amico, 2023). The widespread presence of *L. delicatula* across the globe has led to strict trade barriers for exports, significantly impeding global economic development and globalization efforts. Effective management of these invasive pests requires the application of diverse control measures tailored to specific genetic structures and sources. Therefore, accurate knowledge of the phylogeographic pattern is crucial for the successful containment and elimination of these pests.

Mitochondrial DNA (mtDNA) is ubiquitous in all aerobic eukaryotes, and mitochondrial reads have comprised a significant portion of Next-Generation whole genome sequencing data (Desalle, Schierwater, & Hadrys, 2017). Given its unique attributes, including brevity and rapid evolution, the mitochondrial genome has been extensively leveraged in insect species evolution, phylogenetics, and species identification research (Wang et al., 2022). The insect mitochondrial genome is a covalently closed circular double-stranded DNA molecule that encompasses protein-coding genes (PCGs), rRNA, tRNA, and a non-coding region (Milián-García et al., 2022). Sequencing and elucidating the phylogenetic relationship of the complete mitochondrial genome serve as a crucial tool to understand the evolution and migration patterns of *L. delicatula*. Mitochondrial markers have been developed, including fragments encoding *ND2*, *ND6*, and *cox* proteins of *L. delicatula* (Zhang, Zhao, Wang, & Qin, 2019). In this context, the *ND2* gene was selected for population genetic analysis due to its well-documented variability and suitability for such purposes. However, it is important to acknowledge that the use of a single gene marker, such as *ND2*, has limitations. Relying solely on *ND2* may introduce biases and may not fully capture the complexity of population genetic structures. This limitation should be considered when interpreting the results and should be addressed in future studies by incorporating additional markers to enhance the robustness and comprehensiveness of the genetic analyses. The sequencing of mitochondrial genomes and their subsequent deposition in NCBI GenBank facilitate the detection of genetic structures and the assessment of genetic diversity among populations of this pest, providing valuable insights into its management and control strategies.

MATERIALS AND METHODS

Sample collection and DNA extraction

A comprehensive sampling effort was undertaken in October 2021 in Yinchuan City, located in Ningxia Province, China (geographical coordinates: 38°29′06″N,

106°13′30″E). A total of 44 adult specimens were meticulously collected during this expedition. The sampling was conducted using a systematic approach, where potential habitats were identified based on ecological preferences of the species. At each sampling site, individuals were captured using appropriate techniques, ensuring minimal disturbance to the natural population. Morphological identification of the specimens was facilitated by the use of a high-resolution camera (EOS 700D, Canon, Japan) to capture detailed images of diagnostic features (White, 1845)

Following collection, DNA was extracted from the legs of *L. delicatula* using an optimized alkaline lysis process (Danaeifar, 2022) due to its efficiency and reliability in isolating high-quality DNA. The leg tissue was carefully dissected and subjected to alkaline solutions to disrupt cell membranes and release DNA.

Gene amplification and sequencing

In a total reaction volume of 50 μL, the PCRs were run with 1 μL of DNA template, 1 μL of each 10 μM forward and reverse primers, 25 μL of 2 × Taq plus Master Mix (Biosharp, Anhui Province, China), and 22 μL of ddH_2O. The target gene amplification was performed on a thermal cycler with the following parameters: an initial pre-denaturation step at 94°C for 5 min, followed by 35 cycles of three-step PCR amplification. Each cycle comprised denaturation at 94°C for 30 s, annealing at 46°C for 30 s, and extension at 72°C for 1 min. The reaction was terminated with a final extension step at 72°C for 5 min. Subsequently, the PCR products were purified using the Agarose Gel DNA Recovery Kit (Bioteke, Jiangsu Province, China) and then sent to IGE Biotechnology Ltd (Guangzhou, China) for sequencing. The forward primer *ND2*-238F (5'-AATTGCCCCATTAATGAAAGA-3') and the reverse primer *ND2*-866R (5'-TTTGATTTGGTTATTGTAGGGATT-3') were specifically designed to amplify protein-coding genes *ND2* (Nakashita, Wang, Lu, Shimada, & Tsuchida, 2022).

Mitochondrial genome assemble and annotation

The genomic DNA of *L. delicatula* was fragmented for the purpose of constructing of a paired-end library, with an insert size ranging from 300 to 500 base pairs. This fragmentation was achieved using the TruSeqTMNano DNA Sample Prep Kit (Illumina, San Diego, USA). Subsequently, the library was sequenced on the NovaSeq 6000 platform, generating paired-end reads of 2 × 150 bases each. NOVOPlasty was employed to reconstruct the complete mitochondrial genome, while the Pilon software version 1.22 was to utilized to verify the accuracy of the assembly (Dierckxsens, Mardulyn, & Smits, 2017). Gene annotation was performed using the Geneious 2019.2 software and MITOS Web Server (http://mitos.bioinf.uni-leipzig.de/index.py), referencing the previously published mitochondrial genome with GenBank accession number NC012835. The complete mitochondrial genome sequence of *L. delicatula* was submitted to the BioProject database at NCBI (BioProject number: PRJNA933954) accessible https://www.ncbi.nlm.nih.gov/bioproject.

The Genebank file obtained was visualized in the CHLOROBOX server (https://chlorobox.mpimp-golm.mpg.de/OGDRAW.html) and ChiPlot (https://www.chiplot.online/circos.html). The software tRNAScan-SE was employed to identify the 22 tRNA genes (Chan, Lin, Mak, & Lowe, 2021; Greiner, Lehwark, & Bock, 2019). Additionally, EMBOSS

(https://www.bioinformatics.nl/emboss-explorer/) was utilized to calculate the relative synonymous codon usage (RSCU) value, enabling the investigation of putative codon usage bias in *L. delicatula*. A positive usage bias for a codon was indicated when the RSCU exceeded 1 (Li et al., 2022).

Phylogenetic analysis

The mitochondrial *ND2* gene sequences of *L. delicatula* were scrutinized for any ambiguously alignments using MEGA11 software and cross-referenced with sequences in the NCBI BLAST database before being officially deposited in GenBank (GenBank accession number: OR640256 - OR640299) (Tamura, Stecher, & Kumar, 2021). For phylogenetic analyses, *Ahamus yushuensis* (Lepidoptera, Hepialidae) and *Cyriopagopus hainanus* (Araneae, Theraphosidae) were selected as outgroups. Using MAGE11 for sequence alignment, a phylogenetic tree encompassing 50 sequences was constructed employing the Neighbor-Joining (NJ) method, with a consensus of 1000 bootstrap repeats, to calculate interspecific genetic distances (Saitou & Nei, 1987).

The phylogenetic analysis encompassed a comprehensive dataset consisting of the entire mitochondrial genomes of 44 species. This dataset included the complete sequences of mitochondrial DNA, encompassing all 37 mitochondrial genes arranged in tandem, as well as the non-coding control regions. It comprised one sequence reported in this study and 42 previously sequenced genomes, representing a diverse array of genera, including Fulgoridae, Cercopidae, Aphrophoridae, Ricaniidae, Flatidae, Delphacinae, and Reduviidae (Table 1). As the outgroup, *Ahamus yushuensis*, belonging to the Hepialida family of Lepidoptera order, was chosen. Prior to alignment, the sequence start positions were meticulously calibrated to guarantee precision in the alignment process. Subsequently, a direct alignment of the entire mitochondrial genome was performed for all 44 species using MAFFT, without individually extracting and aligning the genes. This approach maintained the integrity of the genomic sequence and captured potential regulatory regions and intergenic spaces (Katoh, Rozewicki, & Yamada, 2019). To evaluate the phylogenetic evolution of Hemiptera, a maximum likelihood (ML) phylogenetic tree was reconstructed using the RAxML software version 8.2.12, providing a comprehensive understanding of the evolutionary relationships within this insect group (Stamatakis, 2014).

Table 1. General information of Hemiptera species employed in this study.

Family	Genus	Species	Accession Number
Fulgoridae	*Lycorma*	*L. delicatula* (China)	PRJNA933954
	Lycorma	*L. delicatula* (Korea)	MT079523
	Lycorma	*L. delicatula* (Korea)	MT079561
	Lycorma	*L. delicatula* (USA)	MT079712
	Lycorma	*L. delicatula* (USA)	MT079706
	Lycorma	*L. delicatula* (China NX)	MT079612
	Lycorma	*L. delicatula* (China)	NC012835
	Lycorma	*L. delicatula* (China ZJ)	MT079724
	Lycorma	*L. delicatula* (China SXI)	MT079702
	Lycorma	*L. delicatula* (China SHX)	MT079672
	Lycorma	*L. delicatula* (China SH)	MT079661
	Lycorma	*L. delicatula* (China Sd)	MT079650

table continued

Family	Genus	Species	Accession Number
Fulgoridae	*Lycorma*	*L. delicatula* (China SC)	MT079628
	Lycorma	*L. delicatula* (China QH)	MT079618
	Lycorma	*L. delicatula* (China LN)	MT079598
	Lycorma	*L. delicatula* (China JX)	MT079511
	Lycorma	*L. delicatula* (China JS)	MT079500
	Lycorma	*L. delicatula* (China HUN)	MT079461
	Lycorma	*L. delicatula* (China HEN)	MT079450
	Lycorma	*L. delicatula* (China HUB)	MT079442
	Lycorma	*L. delicatula* (China HEB)	MT079420
	Lycorma	*L. delicatula* (China GZ)	MT079412
	Lycorma	*L. delicatula* (China GS)	MT079392
	Lycorma	*L. delicatula* (China CQ)	MT079378
	Lycorma	*L. delicatula* (China BJ)	MT079362
	Lycorma	*L. delicatula* (China AH)	MT079350
	Lycorma	*L. delicatula* (Japan)	MT079468
	Lycorma	*L. delicatula* (Japan)	MT079465
	Lycorma	*Lycorma meliae*	NC056249
	Aphaena	*Aphaena amabilis*	NC045075
	Aphaena	*Aphaena discolor*	MN025523
Cercopidae	*Callitettix*	*Callitettix braconoides*	NC025497
	Callitettix	*Callitettix biformis*	NC025496
Aphrophoridae	*Philagra*	*Philagra albinotata*	NC079668
	Aphrophora	*Aphrophora memorabilis*	NC071833
Ricaniidae	*Pochazia*	*Pochazia discreta*	NC060730
	Pochazia	*Pochazia guttifera*	NC060806
	Ricania	*Ricania fumosa*	NC060809
	Ricania	*Ricania speculum*	NC031369
Flatidae	*Geisha*	*Geisha distinctissima*	NC012617
	Metcalfa	*Metcalfa pruinosa*	NC070019
Delphacinae	*Sogatella*	*Sogatella furcifera*	OR491281
Reduviidae	*Valentia*	*Valentia hoffmanni*	NC012823
Hepialida	*Ahamus*	*Ahamus yushuensis*	NC060512

RESULTS

Morphological identification

L. delicatula can be readily distinguished by its distinctive features, which include the characteristic shape of black spots and red wing pads. In accordance with the alignment results of the *ND2* gene and mitochondrial genome, all populations utilized in this study were initially classified as *L. delicatula* on the basis of morphological characteristics. Upon full wing expansion, the specimens examined demonstrated a body length that ranged between 3.80 and 4.90 cm (Fig. 1).

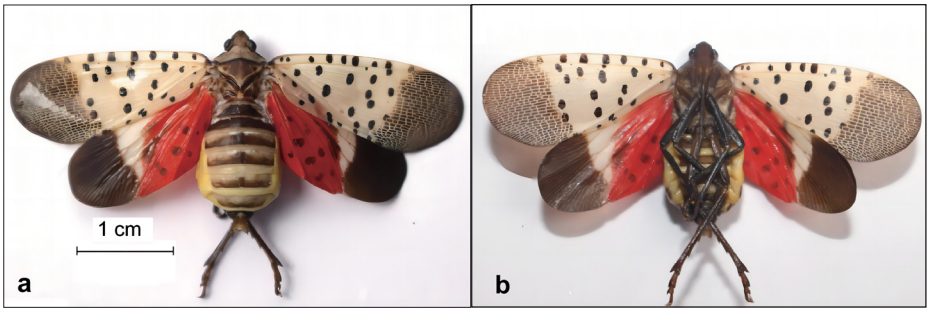

Figure 1. Photographic documentation of the adult *L. delicatula*. a) dorsal view, b) ventral view.

Genome features

The mitochondrial genome of *L. delicatula* was assembled at a length of 16138 bp, comprising 22 tRNA genes, 2 rRNA genes, and 13 PCGs, and displayed as a single, contiguous circular molecule (Fig. 2). The GC content was 23.41%, while the AT content was 76.59%, exhibiting a strong preference for AT pairs, similar to other species within *L. delicatula* group. The majority of genes, including 9 PCGs, 14 tRNAs, were encoded on the heavy strand in the forward orientation. The remaining genes, consisting of 4 PCGs, 8 tRNAs, and both of rRNAs (12S rRNA and 16S rRNA), were encoded on the light strand in the reverse orientation.

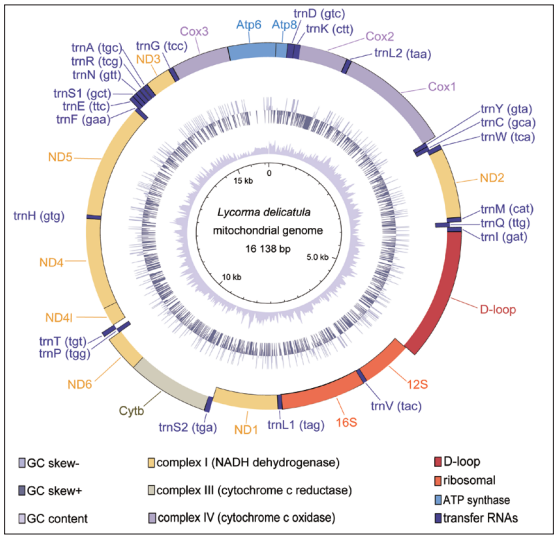

Figure 2. Presentation of the mitochondrial genome map of *L. delicatula*: exterior transcription occurs clockwise on the heavy strand, with interior transcription reversed on the light strand.

The PCG region spaned 11018 bp in length, accounting for 68.27% of the total mitochondrial genome of *L. delicatula*. The sizes of the 13 PCGs ranged from 162 bp (*Atp8*) to 1716 bp (*ND5*). Within the PCGs of *L. delicatula*, the AT content of almost all genes was in the largest proportion. Notably, the base composition of *ND1*, *ND4*, *ND4l*,

and *ND5* was characterized by a high percentage of T and a minimal percentage of C, with varying degrees of consistency. For the remaining PCGs, the trend of component content from high to low is A, T, C, and G (Table 2).

To assess codon usage bias, the RSCU (relative synonymous codon usage) value for *L. delicatula* was calculated, accounting for differences in amino acid frequencies. This allowed for the determination of the ratio of observed to expected codon frequencies under conditions of equal codon usage (Fig. 3).

Table 2. The nucleotide composition of mitochondrial genome.

Nucleotide	A%	T%	G%	C%	G+C%	A+T%
Atp6	44.80	30.89	8.10	16.21	24.31	75.69
Atp8	58.02	25.93	3.09	12.96	16.05	83.95
Cox1	37.04	31.45	12.93	18.58	31.51	68.49
Cox2	45.09	27.98	8.92	18.01	26.93	73.07
Cox3	42.02	29.50	10.22	18.26	28.48	71.52
Cytb	38.96	31.28	10.99	18.77	29.76	70.24
ND1	20.11	56.30	16.08	7.51	23.59	76.41
ND2	48.14	32.19	4.45	15.22	19.67	80.33
ND3	45.11	30.75	8.05	16.09	24.14	75.86
ND4	18.97	59.38	14.06	7.59	21.65	78.35
ND4l	20.29	58.70	15.58	5.43	21.01	78.99
ND5	18.82	60.43	13.64	7.11	20.75	79.25
ND6	46.91	31.14	7.39	14.57	21.95	78.05

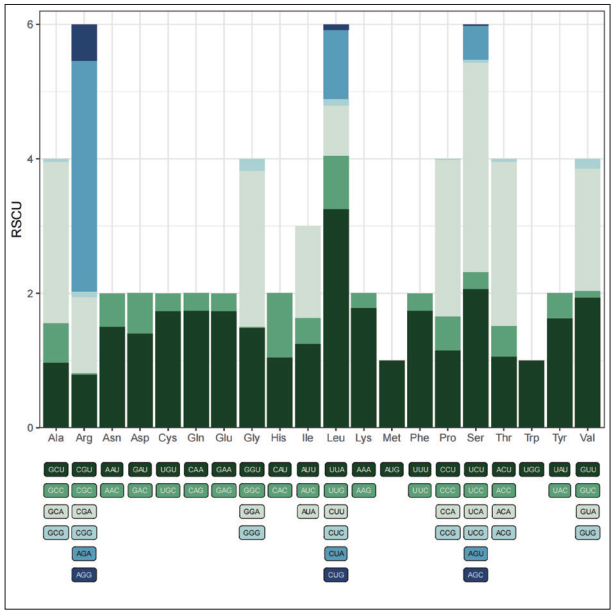

Figure 3. Analysis of codon usage bias in the mitochondrial genomes of *L. delicatula*: the x-axis represents codon families, with the y-axis reflecting relative synonymous codon usage (RSCU) values. RSCU values above 1 indicate a preference for a specific codon, while values below 1 suggest a preference against it. an rscu value of 1 implies no preference.

Codons with RSCU values below 1 indicate negative codon usage bias, whereas codons with RSCU values above 1 exhibit a preference (Andargie and Congyi 2022). The results showed that AGA-Arg (3.43), UUA-Leu (3.26), UCA-Ser (3.12) were the most frequently used codons in the mitochondrial PCGs of *L. delicatula*. Conversely, CCG-Pro (0.01), CGC-Arg (0.02), and AGC-Ser (0.02) were less frequently used codons. Furthermore, nucleotides A or U were preferentially used in the third codon position, compared to other nucleotides. Among the 20 amino acids, three types (Arg, Leu, and Ser) possessed six codons, five types (Ala, Gly, Pro, Thr, and Val) had four codons, and Ile had three codons.

Of the 19 tRNA genes, all displayed the characteristic cloverleaf secondary structure, except for *trnS1* and *trnV*, which were devoid of dihydrouridine (DHU) arms, and *trnH*, which lacked a TΨC arm (Fig. 4). Additionally, the secondary structure of the tRNA genes in *L. delicatula* contained 16 unmatched base pairs, including weak G-U pairs present in *trnA*, *trnC*, *trnF*, *trnG*, *trnH*, *trnL1*, *trnL2*, *trnP*, *trnQ*, *trnV*, and *trnY*.

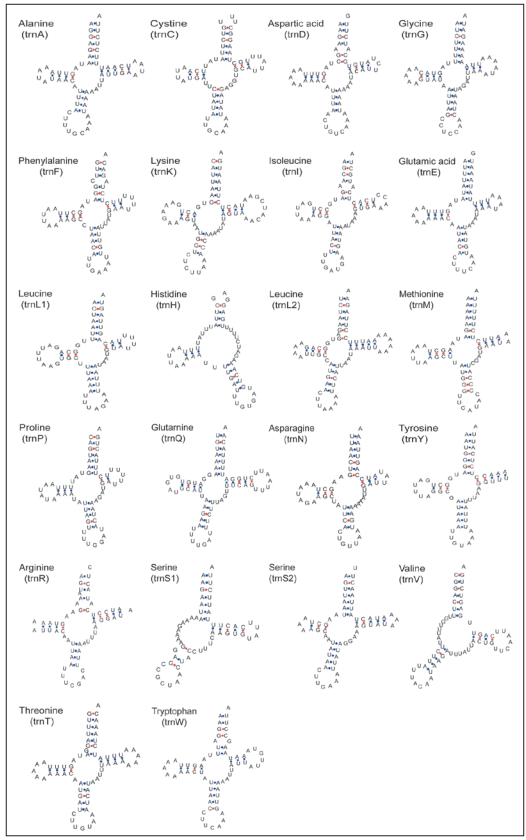

Figure 4. The secondary structure of tRNA in the mitochondrial genome of *L. delicatula* comprises an acceptor stem, a DHU arm, an anticodon stem-loop, and a TΨC arm. The blue dot signifies A-U and G-U pairs, whereas the red dot represents C-G pairs. The amino acids specified by the codon are displayed in the upper left corner of the tRNA secondary structure.

Phylogenetic relationship of the *ND2* genes

A total of 44 samples utilized in the experiment were unequivocally identified as *L. delicatula* through alignment with sequences sourced from the GenBank. The highest query cover observed was 99%, accompanied by an E-value of 0 and a Per.Ident of 99%, whereas the lowest query cover was 97%, also with an E-value of 0 but a slightly lower Per.Ident of 98.99%. All the sequences converged in a single cluster, clearly distinguishing them from the outgroups. Notably, specimens of *L. delicatula* from the United States, Japan, China, and Korea appeared to be a tight cluster, with genetic distances ranging from 0 to 0.01, indicating a strong phylogenetic affinity among these populations (Fig. 5). The accession numbers for the sequences specimens of *L. delicatula* from the United States, Japan, China, and Korea as follows: USA (MT079711), JPN (LC649256), CHN (MK450271), and KOR (KC422361).

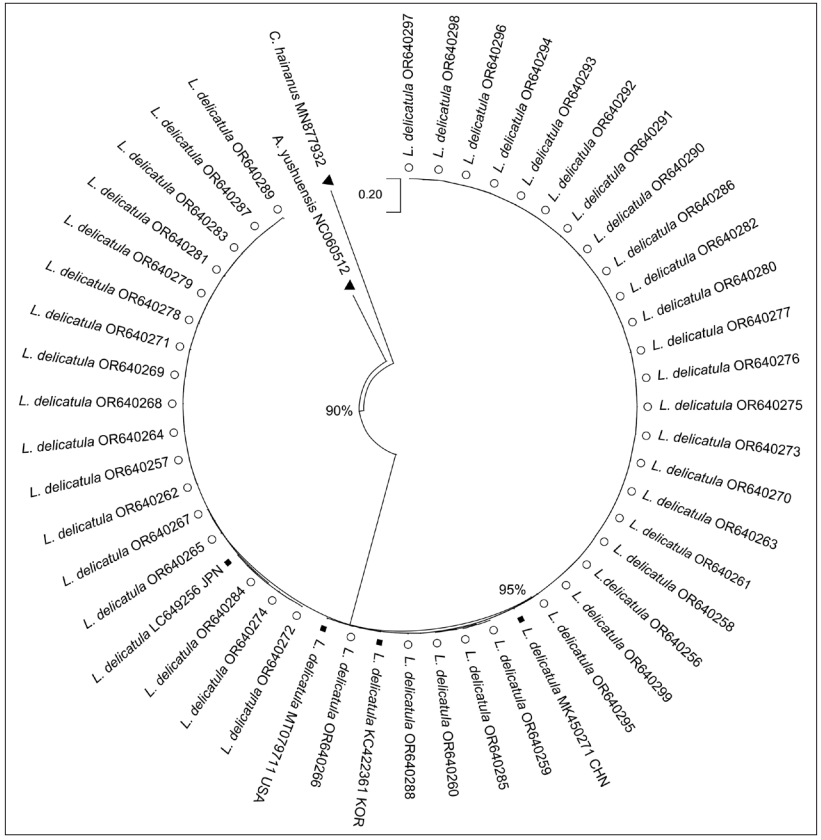

Figure 5. A phylogenetic tree was constructed utilizing the ND2 gene sequences of *L. delicatula.* Comprising 44 isolates obtained from this study and 6 referenced sequences, the tree was confidently generated using the Neighbor-Joining (N-J) method with 1000 bootstrap replicates. The isolates from this study are represented by circles, the reference sequences by squares, and the outgroups by triangles. The abbreviations CHN, KOR, USA, and JPN represent South China, Korea, the United States, and Japan, respectively.

Phylogenetic relationship of the mitochondrial genomes

The seven familys of Hemiptera employed in the study were organized into two distinct clades. Aphrophoridae and Cercopidae comprised one clade, in which *Philagra albinotata* (Hemiptera: Aphrophoridae) emerged as a monophyletic group (Fig. 6). *Valentia hoffmanni* (Hemiptera: Reduviidae) occupied a separate branch, forming a complex clade alongside Ricaniidae, Flatidae, Delphacinae and Fulgoridae. The mitochondrial genomes identified in this study aligned with a clade that was closely associated with previously published mitochondrial genomes of *L. delicatula*. Simultaneously, the branch further bifurcated into two clusters. The cluster containing the genomes obtained in this study encompassed samples from the United States, China, Japan and Korea.

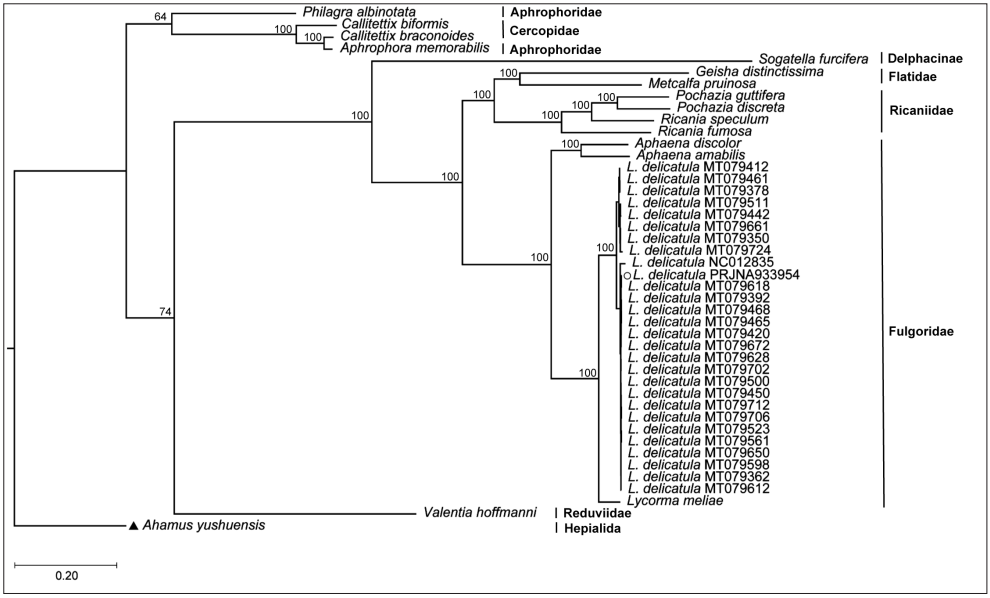

Figure 6. The phylogenetic tree of *L. delicatula* within the Hemiptera family was constructed based on the mitochondrial genome, utilizing the ML method with 1000 bootstrap replicates. The mitochondrial genome from this study was graphically represented as a circle, while *Ahamus yushuensis*, serving as the outgroup, was depicted by triangles.

Lycorma meliae (Hemiptera: Fulgoridae) emerged as the closest relative of *L. delicatula* among the hemipterans, followed by *Aphaena amabilis* (Hemiptera: Fulgoridae) and *Aphaena discolor* (Hemiptera: Fulgoridae). In contrast, Cercopidae and Aphrophoridae were the most distantly related hemipteran families to *L. delicatula*. The ML phylogenetic tree also revealed Cercopidae and Aphrophoridae as the families least related to *L. delicatula*.

DISCUSSION

The mitochondrial genome of *L. delicatula* was analyzed and sequenced, revealing a circular sequence of 16138 bp circle with a GC content of 23.41%. This genome exhibited the significant AT bias commonly observed in insects. When compared to other published mitochondrial genomes, ranging from 15798 to 15946 bp, the sequence length obtained in this study was longer. This difference is speculated to be due to an increase in nonfunctional ORF sequences (Jeong, Kim, Lee, Lee, & Kim, 2020). To further complement the existing characterization of the L mitochondrial genome, we analyzed the codon usage and secondary structure of tRNA.

With the exception of tryptophan and methionine, which are encoded by a single codon, most amino acids are encoded by multiple synonymous codons with preferential selection (Arella, Dilucca, & Giansanti, 2021). The most frequently used codons in *L. delicatula* were AGA-Arg, UUA-Leu, and UCA-Ser, while CCG-Pro, CGC-Arg, and AGC-Ser were less common. At the third codon position, A or U nucleotides predominated, potentially enhancing translation efficiency through selective mutations in coding regions without altering the peptide sequence. Most tRNAs in *L. delicatula* exhibited a complete secondary structure, including five parts forming a conserved cloverleaf (de Jesus, Biedenbänder, Vögele, Wöhnert, & Fürtig, 2022). However, *trnS1* and *trnV* lacked the entire DHU arms, and *trnH*, the shortest tRNA, lacked a TΨC arm. Comparable findings were observed in other species, indicating that not all tRNAs fold into the typical cloverleaf structure (Huang, Sun, Li, Zhao, & Yao, 2022; Huang et al., 2023). While the DHU arm and the TΨC arm are crucial for maintaining a stable fold, it remains unclear how incomplete tRNAs adapt their functional 3D structure (Lorenz, Lünse, & Mörl, 2017).

The *ND2* genes exhibit a remarkable rate of molecular evolution, rendering them an exceptional candidate as DNA barcode marker (Zhang et al., 2019). The phylogenetic analysis of these genes indicated that populations of *L. delicatula* found in the USA, Japan, South China, and Korea belonged to a sister clade to that of northwest China, sharing a sharing a comparable timeline of evolution. It is plausible that species originating from northwest China were involved in biological invasions in other countries, likely through both natural and human-mediated dispersal mechanisms. Samples collected from various surveys and applications exhibited highly similar sequences, differing only by a handful of bases. This observation suggests a relatively low level of genetic diversity polymorphism in *L. delicatula*, with genetic distances ranging from 0 to 0.01, well below the 2% criterion typically used for insect species differentiation (Zhang et al., 2019). However, it's worth noting that these conclusions are primarily based on a limited subset of *ND2* gene populations. To gain a more comprehensive understanding of the dispersal patterns and evolutionary processes of *L. delicatula*, it is imperative to consider multiple DNA markers and species in future studies.

The phylogenetic analysis of mitochondrial genomes revealed that 43 Hemiptera species clustered into a distinct clade, clearly separated from Lepidoptera. Within

this clade, 28 mtDNA sequences of *L. delicatula* formed a single clade, further divided into two distinct branches. Notably, one lineage comprised solely of samples from southeast China, while the other encompassed species from other invasive countries as well as northwest China. A comparable phylogeographic investigation has partially shed light on the invasion process of *L. delicatula*, revealing that Korean populations originated from multiple invasions emanating from eastern China and Japan, whereas populations in the United States resulted from a singular invasion event originating in South Korea (Du et al., 2020). This observation suggests that, to some extent, species from the northern regions may possess a stronger dispersal advantage compared to those from the south, potentially influenced by climatic factors and host availability in these regions. Given that mitochondrial genomes are predominantly inherited through the maternal line, they fail to mirror occurrences like interbreeding and genomic introgression throughout the species' evolutionary history. Consequently, this has impeded precise analysis of the factors contributing to the emergence of two distinct genetic lineages in China. Hemiptera constitutes a highly diverse order of insects, encompassing more than 100 000 described species. A significant proportion of these species are recognized as agricultural pests and vectors, underscoring their importance in both ecological and economic contexts. Additionally, the ML phylogenetic root tree constructed using mtDNAs illuminated the family-level relationships among various groups, with strong support for the following arrangement: (((((Flatidae + Ricaniidae) + Fulgoridae) + Delphacinae) + Reduviidae) + (Aphrophoridae + Cercopidae). The observation that closely related species share a more recent common ancestor implies that they have diverged relatively recently in their evolutionary history and may have been subjected to comparable natural selection pressures. This shared ancestry could have resulted in more similar traits among them, as they exhibit analogous adaptive evolutions in specific characteristics (Song et al., 2024). In the evolutionary tree, Flatidae, Ricaniidae, and Delphacinae are positioned more closely together, indicative of their phylogenetic proximity. These groups share similar habits, including enhanced jumping abilities, and they are all phytophagous insects, with the majority exhibiting phototropism (Zou et al., 2024).

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The complete mitochondrial genome sequence of *L. delicatula* was submitted to the BioProject database at NCBI (BioProject number: PRJNA933954) accessible at https://www.ncbi.nlm.nih.gov/bioproject. The mitochondrial *ND2* gene sequences of *L. delicatula* have been deposited in GenBank at NCBI (GenBank accession numbers: OR640256 - OR640299), and they are publicly accessible via the following URL: https://www.ncbi.nlm.nih.gov/genbank/.

AUTHOR CONTRIBUTIONS

Shi Cheng (Data curation, Formal analysis, Visualization, Validation, Writing—original draft), Mingyu Li (Investigation, Data curation, Formal analysis, Validation), Yuni

Wang (Investigation, Data curation, Formal analysis), and Yajun Lu (Conceptualization, Methodology, Project administration, Funding acquisition, Writing-original draft, Writing-review & editing).

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REFERENCES

Andargie, M. & Congyi, Z. (2021). Genome-wide analysis of codon usage in sesame (*Sesamum indicum* L.). *Heliyon*, 8(1), e08687. https://doi.org/10.1016/j.heliyon.2021.e08687

Arella, D., Dilucca, M., & Giansanti, A. (2021). Codon usage bias and environmental adaptation in microbial organisms. *Molecular genetics and genomics : MGG*, 296(3), 751-762. https://doi.org/10.1007/s00438-021-01771-4

Chan, P. P., Lin, B. Y., Mak, A. J., & Lowe, T. M. (2021). tRNAscan-SE 2.0: improved detection and functional classification of transfer RNA genes. *Nucleic acids research*, 49(16), 9077-9096. https://doi.org/10.1093/nar/gkab688

Danaeifar, M. (2022). New horizons in developing cell lysis methods: A review. *Biotechnology and bioengineering*, 119(11), 3007-3021. https://doi.org/10.1002/bit.28198

de Jesus, V., Biedenbänder, T., Vögele, J., Wöhnert, J., & Fürtig, B. (2022). NMR assignment of non-modified tRNA from Escherichia coli. *Biomolecular NMR assignments*, 16(1), 165-170. https://doi.org/10.1007/s12104-022-10075-6Ile

Desalle, R., Schierwater, B., & Hadrys, H. (2017). MtDNA: The small workhorse of evolutionary studies. *Frontiers in bioscience (Landmark edition)*, 22(5), 873-887. https://doi.org/10.2741/4522

Dierckxsens, N., Mardulyn, P., & Smits, G. (2017). NOVOPlasty: de novo assembly of organelle genomes from whole genome data. *Nucleic acids research*, 45(4), e18. https://doi.org/10.1093/nar/gkw955

Du, Z., Wu, Y., Chen, Z., Cao, L., Ishikawa, T., Kamitani, S., Sota, T., Song, F., Tian, L., Cai, W., & Li, H. (2020). Global phylogeography and invasion history of the spotted lanternfly revealed by mitochondrial phylogenomics. *Evolutionary applications*, 14(4), 915-930. https://doi.org/10.1111/eva.13170

Greiner, S., Lehwark, P., & Bock, R. (2019). OrganellarGenomeDRAW (OGDRAW) version 1.3.1: expanded toolkit for the graphical visualization of organellar genomes. *Nucleic acids research*, 47(W1), W59-W64. https://doi.org/10.1093/nar/gkz238

Huang, L., Sun, H. Q., Li, C. J., Zhao, W. X., & Yao, Y. X. (2022). The complete mitochondrial genome of *Platygaster robiniae* (Hymenoptera: Platygastridae): A novel tRNA secondary structure, gene rearrangements and phylogenetic implications. *International journal for parasitology. Parasites and wildlife*, 18, 249-259. https://doi.org/10.1016/j.ijppaw.2022.06.007

Huang, Y., Yang, Y., Qi, L., Hu, H., Rasplus, J. Y., & Wang, X. (2023). Novel Gene Rearrangement Pattern in *Pachycrepoideus vindemmiae* Mitochondrial Genome: New Gene Order in Pteromalidae (Hymenoptera: Chalcidoidea). *Animals : an open access journal from MDPI*, 13(12), 1985. https://doi.org/10.3390/ani13121985

Jeong, N. R., Kim, M. J., Lee, W., Lee, G. S., & Kim, I. (2020). Complete mitochondrial genome of the spotted lanternfly, *Lycorma delicatula* White, 1845 (Hemiptera: Fulgoridae). *Mitochondrial DNA. Part B, Resources*, 5(1), 370-372. https://doi.org/10.1080/23802359.2019.1703577

Jung, M., Kho, J. W., Gook, D. H., Lee, Y. S., & Lee, D. H. (2022). Dispersal and oviposition patterns of *Lycorma delicatula* (Hemiptera: Fulgoridae) during the oviposition period in Ailanthus altissima (Simaroubaceae). *Scientific reports*, 12(1), 9972. https://doi.org/10.1038/s41598-022-14264-0

Katoh, K., Rozewicki, J., & Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics*, 20(4), 1160-1166. https://doi.org/10.1093/bib/bbx108

Ladin, Z. S., Eggen, D. A., Trammell, T. L. E., & D'Amico, V. (2023). Human-mediated dispersal drives the spread of the spotted lanternfly (*Lycorma delicatula*). *Scientific reports*, 13(1), 1098. https://doi.org/10.1038/s41598-022-25989-3

Li, Y., Khandia, R., Papadakis, M., Alexiou, A., Simonov, A. N., & Khan, A. A. (2022). An investigation of codon usage pattern analysis in pancreatitis associated genes. *BMC genomic data*, 23(1), 81. https://doi.org/10.1186/s12863-022-01089-z

Lorenz, C., Lünse, C. E., & Mörl, M. (2017). tRNA Modifications: Impact on Structure and Thermal Adaptation. *Biomolecules*, 7(2), 35. https://doi.org/10.3390/biom7020035

Milián-García, Y., Hempel, C. A., Janke, L. A. A., Young, R. G., Furukawa-Stoffer, T., Ambagala, A., Steinke, D., & Hanner, R. H. (2022). Mitochondrial genome sequencing, mapping, and assembly benchmarking for Culicoides species (Diptera: Ceratopogonidae). *BMC genomics*, 23(1), 584. https://doi.org/10.1186/s12864-022-08743-x

Nakashita, A., Wang, Y., Lu, S., Shimada, K., & Tsuchida, T. (2022). Ecology and genetic structure of the invasive spotted lanternfly *Lycorma delicatula* in Japan where its distribution is slowly expanding. *Scientific reports*, 12(1), 1543. https://doi.org/10.1038/s41598-022-05541-z

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, 4(4), 406-425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Song, N., Wang, MM., Huang, WC. et al. (2024). Phylogeny and evolution of hemipteran insects based on expanded genomic and transcriptomic data. *BMC Biol 22*, 190. https://doi.org/10.1186/s12915-024-01991-1

Stamatakis A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England)*, 30(9), 1312-1313. https://doi.org/10.1093/bioinformatics/btu033

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular biology and evolution*, 38(7), 3022-3027. https://doi.org/10.1093/molbev/msab120

Urban, J. M., & Leach, H. (2023). Biology and Management of the Spotted Lanternfly, *Lycorma delicatula* (Hemiptera: Fulgoridae), in the United States. *Annual review of entomology*, 68, 151-167. https://doi.org/10.1146/annurev-ento-120220-111140

Wang, H., Wen, Q., Wang, T., Ran, F., Wang, M., Fan, X., Wei, S., Li, Z., & Tan, J. (2022). Next-Generation Sequencing of Four Mitochondrial Genomes of *Dolichovespula* (Hymenoptera: Vespidae) with a Phylogenetic Analysis and Divergence Time Estimation of Vespidae. *Animals : an open access journal from MDPI*, 12(21), 3004. https://doi.org/10.3390/ani12213004

Zhang, L., Zhao, W., Wang, F., & Qin, D. (2019). Genetic Diversity and Population Structure of Natural *Lycorma delicatula* (White) (Hemiptera: Fulgoridea) Populations in China as Revealed by Microsatellite and Mitochondrial Markers. *Insects*, 10(10), 312. https://doi.org/10.3390/insects10100312

Zou, Y., Zhang, Z., Zeng, Y., Hu, H., Hao, Y., Huang, S., & Li, B. (2024). Common Methods for Phylogenetic Tree Construction and Their Implementation in R. *Bioengineering (Basel, Switzerland)*, 11(5), 480. https://doi.org/10.3390/bioengineering11050480