

MITOGENOME REPORT



Nanopore sequencing of complete mitochondrial genome of the cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Coccoidea: Pseudococcidae)

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ABSTRACT

Mealybugs are aggressive pests worldwide, but few complete mitochondrial genomes have been reported. Here, we got the complete mitochondrial genome of the cotton mealybug, *Phenacoccus solenopsis* Tinsley, using Oxford Nanopore Technology. It is 14743 bp in length, encodes 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and 2 ribosomal RNAs (rRNAs), and exhibits a high AT content of 90.33%. Maximum likelihood phylogenetic tree revealed that *P. solenopsis* is closely related to *P. manihoti*. This study presents the first complete mitogenome of a mealybug obtained through long-read sequencing, providing a valuable genomic resource for further studies of the mealybug family.

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Introduction

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Tinsley, 1898) (Hemiptera: Pseudococcidae), is native to North America, and has spread to all continents except Antarctica (Tong et al. 2019). Adult females are wingless, dorsoventrally flattened, and typically exhibit an ovoid body form, which is often concealed by a dense covering of powdery wax (Figure 1). In contrast, adult males are elongated, possess a single pair of functional wings, and lack any waxy coating. Like other mealybugs, the cotton mealybug demonstrates a broad host range, high reproductive capacity, and strong adaptability to local environments, causing serious economic losses on over 150 crop and horticultural plant species (Nagrare et al. 2009; Tong et al. 2019).

In mealybugs, the chromosome-level genome data of the cotton mealybug has been published (Li et al. 2020), along with multiple omics data, including genome-wide microsatellites (Ma et al. 2019), transcriptome (Omar et al. 2020; Tong et al. 2024), proteome and metabolome (Tong et al. 2022), however, no chromosome-level genomes were reported for others. Mitochondrial genome is small, it exhibits several unique characteristics, including maternally inherited, highly conserved, low rate of sequence recombination and evolve rapidly (Sun et al. 2020). Therefore, acquiring more mitochondrial genome is particularly valuable for phylogenetic research of mealybugs.

Comparing to the second-generation sequencing technology, third-generation sequencing, characterized by single-molecule real-time (SMRT) sequencing methods such as Oxford Nanopore Technology (ONT) and PacBio SMRT, operated without PCR amplification and produced longer read length, which has an absolute advantage in assembling complex sequences (e.g. high AT repetitive region) (Nakano et al. 2017; Athanasopoulou et al. 2021). The mitochondrial genomes in Coccoidea generally exhibit significantly higher AT content, it was 89.3% in *P. manihoti* (NC_066716), 84.7% in *Diuraphis noxia* (KF636758) (Zhang et al. 2014), 85.3% in *Aphis glycines* (MK111111) (Song et al. 2019), 91.3% in *Matsucoccus matsumurae* (PP103290) (Lee et al. 2024), 84.5% in *Aclerda takahashii* (NC_063660) (Deng et al. 2022), 82.5% in *Didesmococcus koreanus* (NC_057479) (Xu et al. 2021). Therefore, we used the ONT in this study, and successfully assembled and annotated the whole mitochondrial genome of *P. solenopsis* for the first time, moreover, its phylogenetic relationships about Coccoidea were investigated and discussed. Our results will provide valuable genetic information for further studies on mealybugs.

Materials and methods

The specimens of *P. solenopsis* used here were collected from our laboratory population, which was originally collected from a population from Jinhua (29.13°N, 119.44°E), China, in

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Figure 1. Morphological characteristics of *P. solenopsis*, photographed by Qianjin Lin. Adult females are wingless, dorsoventrally flattened, and typically exhibit an ovoid body form, which is often concealed by a dense covering of powdery wax.

2016. The specimen was deposited at the College of Life Sciences, China Jiliang University under the voucher number CJLU JHLX03. Contact person: Qianjin Lin, lylqjzc@gmail.com. Detailed overview of insect growth and tomato plant cultivation methods are provided in Tong et al. (2019).

Total genomic DNA was extracted from the whole insects using the Qiagen DNeasy Blood and Tissue kit following the manufacturer's protocols. DNA quantitation was estimated by Nanodrop (OD260/280) and Qubit (Thermo Fisher Scientific). To generate Nanopore long reads, ~15 μg of genomic DNA was size-selected (>20 Kb) with a Blue Pippin System (Sage Science). According to *1D Genomic DNA by Ligation* protocol (Oxford Nanopore Technologies, ONT), ONT SQK-LSK108 library preparation kit was used to construct PCR-free libraries. Shanghai Biozeron Biotechnology Corporation performed the Nanopore sequencing of prepared libraries using the GridION X5 sequencer according to manufacturer's instructions (Oxford Nanopore, Oxford, UK). Guppy 3.2.10 allowed for successful high-accuracy base-calling and the mitochondrial genome was assembled with Canu (Koren et al. 2017) with average depth of 411.62X (Figure S1). MITOS2 (Bernt et al. 2013) software was used to predict the mitochondrial genome to identify coding proteins, tRNA and rRNA genes. Then the initial genes predicted by MITOS2 were de-redundant, and the start and stop codon positions were

manually corrected through BLASTn search against NCBI nucleotide database of closely related taxa. The circular map was created using CGView (Grant and Stothard 2008).

To reveal the phylogenetic position of *P. solenopsis*, we downloaded nucleotide sequences of the 13 PCGs from 12 species of Coccoidea insects from the NCBI. The phylogenetic tree was constructed based on Maximum likelihood (ML) method with RAXML-NG v1.2.0 (Kozlov et al. 2019). Two aphids, *Aphis glycines* (MK111111) and *Diuraphis noxia* (KF636758) were used as the outgroups. The GTR+I+G4 model was selected based on the initial analysis with Modeltest-NG v0.1.7 (Darriba et al. 2020). Heuristic searches were performed 10 times to identify optimal trees, with 1000 bootstrap replicates used to evaluate node support. The final phylogenetic tree visualization was accomplished using iTOL (Letunic and Bork 2019).

Results

The mitochondrial genome of the cotton mealybug (Genbank: PV100887) is 14743 bp in length and comprises a total of 37 genes, including 13 PCGs, 2 rRNA genes, and 22 tRNA genes (Figure 2). Among them, 20 genes were identified on the majority strand, while the remaining 17 are encoded on the minority strand. The nucleotide composition of the mitochondrial genome of the cotton mealybug is 47.45% for A, 42.89% for T, 6.16% for C, and 3.50% for G, with an A+T content of 90.33%. All PCGs start with an ATN (ATA, ATT, ATC and ATG) codeword, with only COX3 and ND4 terminating an individual (T), while the remaining PCGs terminate with the TAA codeword. The two rRNAs (*rrnL* and *rrnS*) are 1,069 bp and 615 bp long, respectively. Among these 22 tRNAs, with the shortest being 55 bp (*tRNA-Phe*) and the longest being 71 bp (*tRNA-Leu2(UUR)*).

The phylogenetic tree based on 13 PCGs showed that all species of each family were clustered into one branch with high bootstrap (most of them is 100%), except the *Didesmococcus koreanus* with lowest bootstrap support (63%) (Figure 3). For mealybugs, *P. solenopsis* and *P. manihoti* were robustly clustered within the family Pseudococcidae branch (bootstrap support = 100%), indicating a closest phylogenetic relationship between them (Figure 3).

Discussion and conclusion

At least 32 genera and nearly 105 mealybug species have been documented to date (Tong et al. 2022). Currently, the complete annotation for mitochondrial genome of one mealybug species, *P. manihoti* (NC_066716), has been released in public database. In this study, we provided the complete mitochondrial genome of the cotton mealybug. To our knowledge, this is the first report on mealybug's mitochondrial genome by using ONT technology, especially given the exceptionally high AT content of the genome. Our results successfully showed that the mitochondrial genome of *P. solenopsis* is a typical circular DNA with 37 coding genes. Several structural rearrangements were identified compared to *P. manihoti*, including the transcription directions of *ND6*

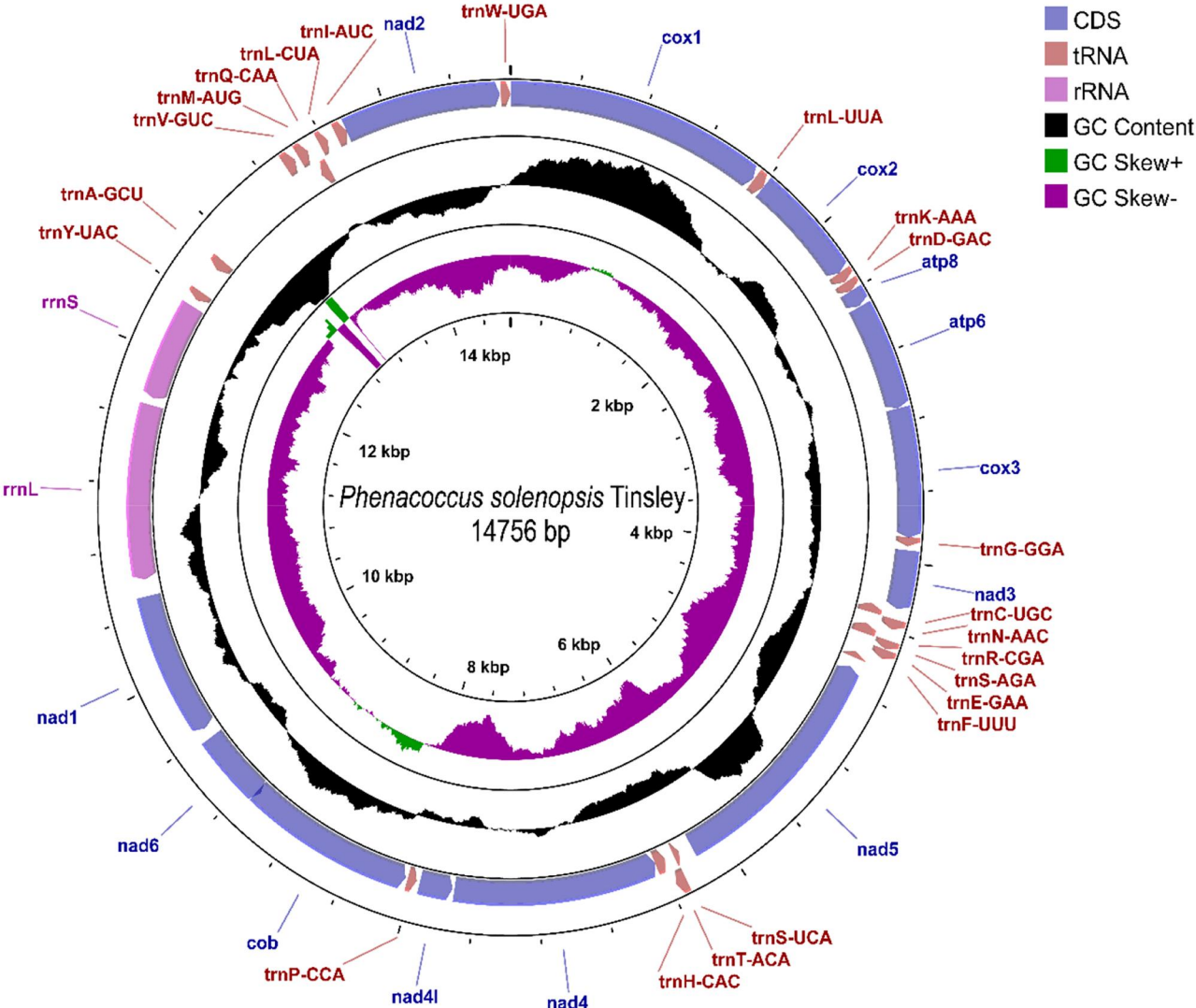


Figure 2. The organization of the mitogenome of *P. solenopsis*. Genes for proteins and rRNAs are shown with standard abbreviations. Genes for tRNAs are represented by a single letter for the corresponding amino acid and by codon. From outside in: the first circle represents gene arrangement, the second circle represents GC skew, and the third circle represents GC content.

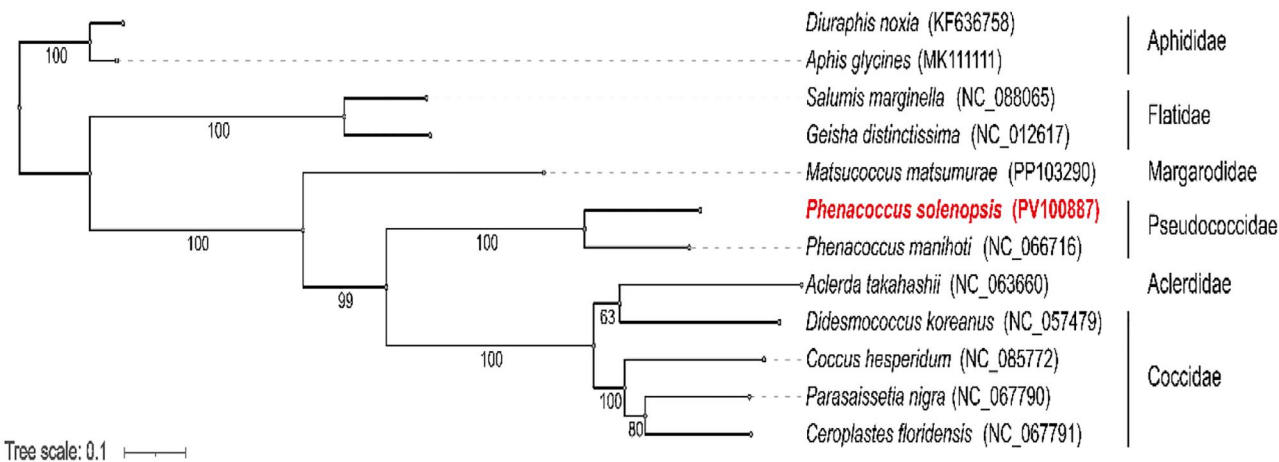


Figure 3. The maximum-likelihood (ML) phylogenetic tree for *P. solenopsis* and the other species based on the concatenated nucleotide sequences of 13 mitochondrial PCGs. The sequences used for phylogenetic analysis include *Diuraphis noxia* (KF636758) (Zhang et al. 2014), *Aphis glycines* (MK111111) (Song et al. 2019), *Salurnis marginella* (NC_088065) (Kim et al. 2021), *Geisha distinctissima* (NC_012617) (Song & Liang 2009), *Matsucoccus matsumurae* (PP103290) (Lee et al. 2024), *Phenacoccus manihoti* (NC_066716) (unpublished), *Aclerda takahashii* (NC_063660) (Deng et al. 2022), *didesmococcus koreanus* (NC_057479) (Xu et al. 2021), *Coccus hesperidum* (NC_085772) (unpublished), *parasaissetia nigra* (NC_067790) (Unpublished), *Ceroplastes floridensis* (NC_067791) (unpublished).

and *CYTB* genes, and the tRNA genes arranges between *ND3* and *ND5*.

Phylogenetic tree based on 13 PCGs showed strong nodal support across most clades, with bootstrap values largely exceeded 95%, indicating high phylogenetic reliability. All included species clustered consistently within their respective families, confirming the monophyly of these taxonomic units at the mitochondrial genome level. Within the Pseudococcidae, *P. solenopsis* and *P. manihoti* formed a highly supported clade (bootstrap support = 100%), further validating their close phylogenetic affinity within the genus *Phenacoccus* (Deng et al. 2024; Zhao et al. 2025). Moreover, close relationship between Aclerdidae and Coccidae was consistent with the view proposed by Deng et al. (2022, 2024). Low bootstrap support may cause the cluster of *Didesmococcus koreanus* and *Aclerda takahashii*. In the future, more genomic data and broader taxon sampling are needed to fully resolve the relationships among these families.

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Author contributions

CRediT: **Qianjin Lin**: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing; **Chengcheng Ye**: Formal analysis, Investigation, Writing – review & editing; **Zicheng Li**: Formal analysis, Investigation, Writing – review & editing; **Kaixin Wang**: Formal analysis, Validation, Formal analysis, Visualization; **Kaijie Wang**: Formal analysis, Validation, Formal analysis, Visualization; **Lilu Sheng**: Formal analysis, Visualization; **Haojie Tong**: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Ethical approval

The insect specimens collected in this study were not regulated invertebrates and no special permit was required. The sequenced insect species were common chloroform insects in China and were not included in the list of protected animals in China. This study did not involve endangered species, and the research procedures were in accordance with the national experimental guidelines of China.

Disclosure statement

No potential conflict of interest was reported by the authors.

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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/nuccore/PV100887.1/>. The associated BioSample, BioProject, and SRA numbers are SAMN46077434, PRJNA1206340 and SRR3189289-SRR31892899, respectively.

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