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MITOGENOME REPORT

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The complete mitochondrial genome of *Periphyllus koelreuteriae* (Takahashi, 1919) (Hemiptera: Aphididae: Chaitophorinae)

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ABSTRACT

We sequenced the complete mitochondrial genome of the aphid *Periphyllus koelreuteriae*. It is 16,828 bp long and includes 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosome RNA genes (rRNAs), a control region, and a repeat region. The control region contains a 273-bp repeat motif repeated 2.3 times. The species-specific repeat region between *trnE* and *trnF* comprises two 340-bp repeat units. The maximum-likelihood tree based on all 37 mitochondrial genes indicated a close relationship between *P. koelreuteriae* and *Periphyllus diacerivorus*. This study provides a valuable mitogenomic resource for future research on Chaitophorinae phylogeny and *P. koelreuteriae* diversification.

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Mitogenome; aphid; control region; repeat region; phylogeny

Introduction

The aphid *Periphyllus koelreuteriae* (Takahashi, 1919) (Hemiptera: Aphididae: Chaitophorinae) is a pest species that causes serious damage to ornamental plants of *Koelreuteria* spp. (Sapindaceae) in China, South Korea, and Japan (Junkiert and Wieczorek 2019; Blackman and Eastop 2024). This species is monoecious and holocyclic. It feeds on the undersides of leaves, young shoots, and twigs of *Koelreuteria*. *P. koelreuteriae* not only induces leaf curling but also excretes substantial amounts of honeydew, causing sooty blotch disease and adversely affecting the growth of *Koelreuteria* plants (Jiang et al. 2011). Using multiple genes and morphological characteristics, Li et al. (2021) and Li, Wu, et al. (2022) indicated that *P. koelreuteriae* is a species complex, with two cryptic species adapted to different climate regions and host plants.

Mitochondrial genomes provide valuable genetic information in the phylogenetic and population genetic studies of aphids (De Jager et al. 2014; Chen et al. 2017; Zhang et al. 2024). To date, six mitogenome sequences of Chaitophorinae species have been published, among which only one is complete (Niu et al. 2016; Chen et al. 2017; Li, Chen, et al. 2022). Two partial mitogenome sequences obtained through Sanger sequencing are available for the mitogenome of *P. koelreuteriae* (Chen et al. 2017). One sequence, 11,469 bp long, contains 12 complete protein-coding genes (PCGs) and a partial *nad5* (GenBank accession number KP722572); another sequence of 2202 bp in length comprises complete *rrnS*, *trnV*, and *rrnL* (GenBank accession number KX507108). The other gene and non-coding sequences are missing. More importantly, it is uncertain whether the repeat region located between *trnE* and *trnF* exists in the mitogenome of *P. koelreuteriae*. The repeat region, which is unique in aphid mitogenomes and potentially serves as another origin for replication (Wang et al. 2013), is valuable in providing informative signals for investigating the intraspecific diversification of aphids (Zhang et al. 2024). Therefore, it is necessary to sequence and assemble the complete mitochondrial genome of *P. koelreuteriae*. Herein, we characterized the mitogenome architecture of *P. koelreuteriae* and discussed its phylogenetic position.

Materials and methods

Samples of *P. koelreuteriae* (Figure 1) were collected on *Koelreuteria paniculata* Laxm., 1772 from Beijing, China (40.00°N, 116.37°E) by Hao Liu in April 2021. Aphids were rapidly stored in 95% ethanol with a brush in the field. Apterous viviparous females were chosen to prepare the slide-mounted voucher specimens and were identified by Liyun Jiang according to taxonomic keys (Jiang et al. 2011). Aphid specimens were deposited in the National Animal Collection Resource Center, Institute of Zoology, Chinese Academy of Sciences, Beijing, China, and preserved in 95% ethanol at −30 °C (voucher no. 45960; Jing Chen, chenjing@ioz.ac.cn).

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Figure 1. Species reference image of *Periphyllus koelreuteriae*. This photo was taken by Hao Liu.

Total genomic DNA was extracted from aphid whole-body tissues using cetyltrimethylammonium bromide (CTAB) method. Sequencing was performed on an Illumina NovaSeq 6000 platform with 150 bp paired-end reads. A total of 11 Gb of raw data and 10.5 Gb of clean data were obtained. The sequencing depth of each genomic position was calculated using SAMtools version 1.3.1 (Li et al. 2009), and the read coverage depth map (Figure S1) shows a mean sequencing depth of 1621×. The mitogenome was assembled using clean data by NOVOPlasty version 4.2 (Dierckxsens et al. 2017) employing the *de novo* assembly method. Subsequently, the MITOS webserver (Bernt et al. 2013) and NCBI ORF Finder tool (https://www.ncbi.nlm.nih.gov/orffinder/) were utilized to annotate transfer RNA genes (tRNAs) and predict the positions of PCGs, respectively. The positions of ribosome RNA genes (rRNAs) were confirmed through aligning with closely related species. Repeat sequences were identified using the Tandem Repeats Finder webserver (Benson 1999). The sequence similarity between the repeat units in the control region and the repeat region was calculated using Sequence Manipulation Suite (Version 2) (Stothard 2000). The circular map of mitogenome was visualized through the Proksee webserver (Grant et al. 2023). Finally, the complete mitogenome sequence of *P. koelreuteriae* was submitted to GenBank under the accession number PP856044.

Phylogenetic analysis was conducted based on 22 aphid mitogenomes (Table S1), including the complete mitogenome of *P. koelreuteriae* generated in this study and the previously published incomplete one, as well as partial and complete mitogenome sequences from five other Chaitophorinae species. According to previous aphid phylogenetic research (Ortiz-Rivas and Martínez-Torres 2010; Du et al. 2021; Owen and Miller 2022), 14 species from the subfamilies Aphidinae and Calaphidinae, which are closely related to Chaitophorinae, were selected. *Daktulosphaira vitifoliae* (Fitch, 1855) from the family Phylloxeridae was employed as the outgroup. Each PCG was aligned using MAFFT in Phylosuite version 1.2.2 (Zhang et al. 2020). The RNA genes, including 22 tRNAs and two rRNAs, were each aligned with the MAFFT online server version 7 (Katoh and Standley 2013), and conserved sequences were then extracted using Gblocks version 0.91b (Castresana 2000). Subsequently, 37 mitochondrial gene alignments were concatenated into a single multiple sequence alignment totaling 14,331 bp using Phylosuite. Best-fit partitioning scheme and substitution models were estimated by PartitionFinder version 2.1.1 (Lanfear et al. 2017). All 37 genes were divided into five partitions, with GTR + I + G selected as the optimal model for each partition. Afterwards, a maximum-likelihood (ML) phylogenetic tree was constructed based on the concatenated 37 genes with RAxML version 8.2.12 (Stamatakis 2014). The reliability of each branch was evaluated using 1000 rapid bootstrapping replicates. Bootstrap (BS) values above 50% and 70% indicate moderate and strong support, respectively (Hillis and Bull 1993). The resulting ML tree was displayed by the tvBOT webserver (Xie et al. 2023).

Results

The complete mitogenome of *P. koelreuteriae* is 16,828 bp long and contains 13 PCGs, 22 tRNAs, two rRNAs, and two large non-coding regions known as the control region and the repeat region (Figure 2). The size of this mitogenome falls within the range of 14–20 kb reported for known Aphididae mitochondrial genomes (Zhang et al. 2024). There is no gene rearrangement in the *P. koelreuteriae* mitogenome, which maintains the consistent gene order as the inferred ancestral gene arrangement of insects (Clary and Wolstenholme 1985). The nucleotide composition of this mitogenome includes 45.0% A, 38.2% T, 5.9% G, 10.9% C, and a high A + T content of up to 83.2%. The majority strand transcribes 23 genes (nine PCGs and 14 tRNAs), while the minority strand transcribes 14 genes (four PCGs, eight tRNAs, and two rRNAs). All PCGs initiate with the standard ATN codon and terminate with TAA, except for *cox1* and *nad4*, in which a single T is considered an incomplete stop codon. The lengths of 22 tRNAs range from 60 to 73 bp. Loss of the dihydrouridine (DHU) arm occurs in *trnS* (AGN), while the other tRNAs form typical clover-leaf secondary structures. The lengths of *rrnL* and *rrnS* genes are 1352 bp and 767 bp, with A + T contents of 85.9% and 83.7%, respectively. The control region with an A + T content of 89.8% is 1136 bp long and is located between *rrnS* and *trnI*. It contains a 273-bp repeat unit that is repeated 2.3 times. Another large non-coding region, the repeat region situated between *trnE* and *trnF*, exhibits an A + T content of 90.1% and encompasses two 340-bp repeat units separated by a 241-bp spacer sequence.

Based on the concatenated 37 mitochondrial genes, the ML tree (Figure 3) supported the monophyly of Aphidinae (BS = 100%), two tribes within Aphidinae (Aphidini and Macrosiphini) (BS = 100%; BS = 89%), and Calaphidinae (BS = 100%). The subfamily Chaitophorinae was retrieved as monophyletic with strong support (BS = 100%) and was placed as the sister group to Aphidinae + Calaphidinae. Within the Chaitophorinae, the tribe Siphini was monophyletic (BS = 100%), while Chaitophorini was paraphyletic. The monophyly of the genus *Periphyllus* was supported, albeit with moderate support (BS = 58%). *P. koelreuteriae* was clustered with *Periphyllus diacerivorus* Zhang, 1982 (BS = 100%).

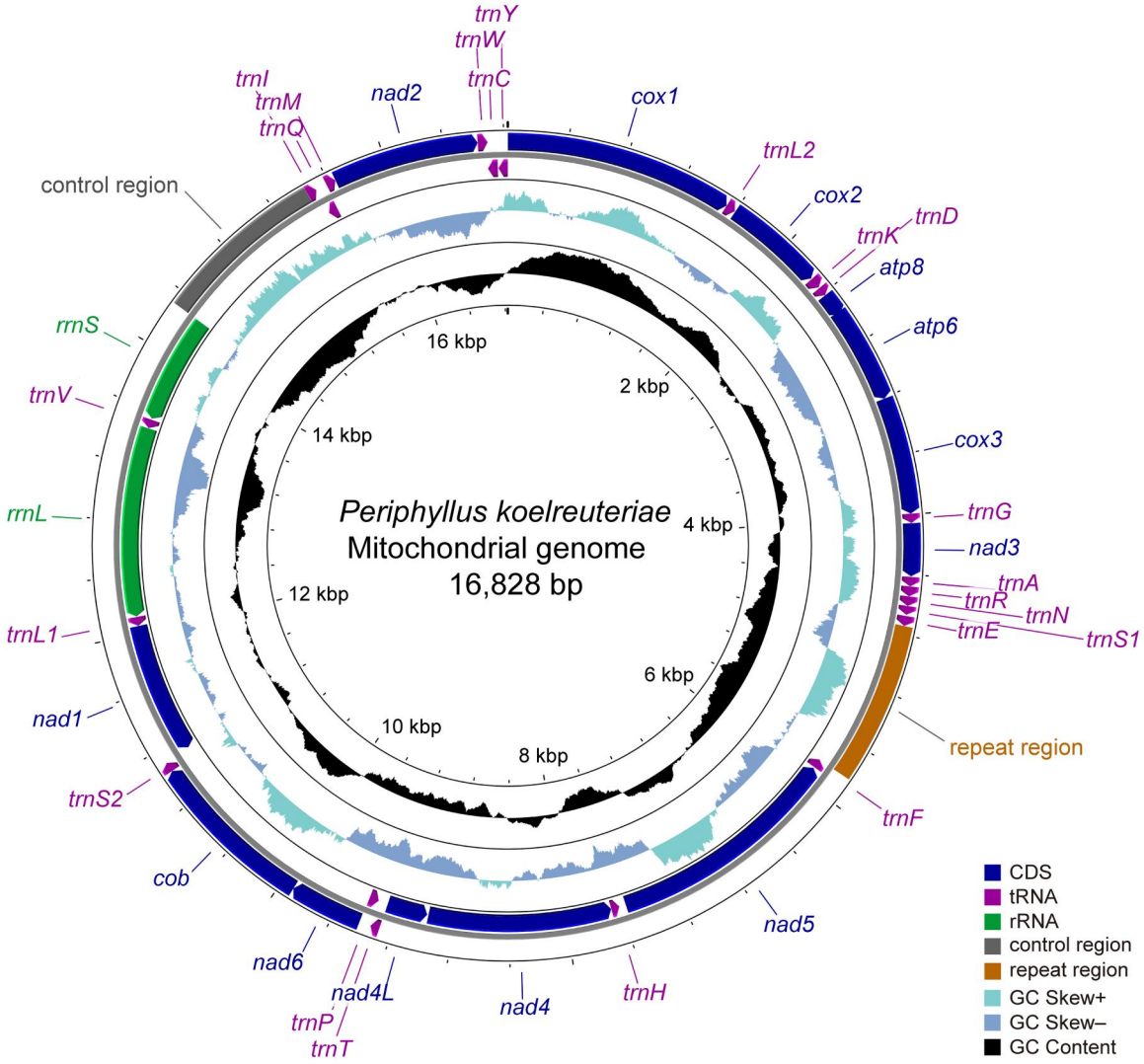

Figure 2. Circular map of the *Periphyllus koelreuteriae* mitochondrial genome. Arrows indicate the transcription directions of genes. The inner circles demonstrate GC skew and GC content.

Discussion and conclusions

In this study, we assembled and annotated the complete mitochondrial genome of *P. koelreuteriae*, addressing the deficiency of the previously incomplete mitogenome sequence for this species. The repeat region located between *trnE* and *trnF* exists in the *P. koelreuteriae* mitogenome. Compared with another complete mitogenome of the Chaitophorinae, *P. diacerivorus* (Li, Chen, et al. 2022), the repeat unit in *P. koelreuteriae* differs in nucleotide composition, length, and copy number. Tandem repeat sequences are also present in the control region of the *P. koelreuteriae* mitogenome, similar to the mitogenomes of *Periphyllus acerihabitans* Zhang, 1982, *P. diacerivorus*, and *Laingia psammae* Theobald, 1992 from Chaitophorinae (Niu et al. 2016; Li, Chen, et al. 2022). The sequence similarity between the repeat units within the control region and the repeat region of *P. koelreuteriae* is not high, at 47.08%, which is consistent with previous observations in Zhang et al. (2022).

The phylogenetic tree based on whole mitogenome sequence of *P. koelreuteriae* and 21 other aphid mitogenomes supported the monophyly of Chaitophorinae and its tribe Siphini. The monophyly of Chaitophorini was not recovered, which contradicts the results of Liu et al. (2022) and may be attributed to the extremely limited sampling. Therefore, more mitochondrial genomes are required to infer the phylogenetic relationships within Chaitophorinae from a mitogenomic perspective. In the present study, *P. koelreuteriae* was sister to *P. diacerivorus*, and *P. acerihabitans* was basal within the clade of *Periphyllus*. In Liu et al. (2022), *P. koelreuteriae* and *P. diacerivorus* were also clustered in one clade, and *P. acerihabitans* was placed in a distantly related position to these two species.

In conclusion, we present a valuable resource of mitogenomic data for Chaitophorinae aphids, which will be helpful in advancing our understanding of the mitogenome characteristics of this group, the phylogenetic relationships within Chaitophorinae, and the intraspecific differentiation of *P. koelreuteriae*.

Author contributions

JC, GQ, and LJ: conception and design; CL and HL: analysis and interpretation of the data; CL and HL: drafting of the paper; JC, GQ, and LJ:

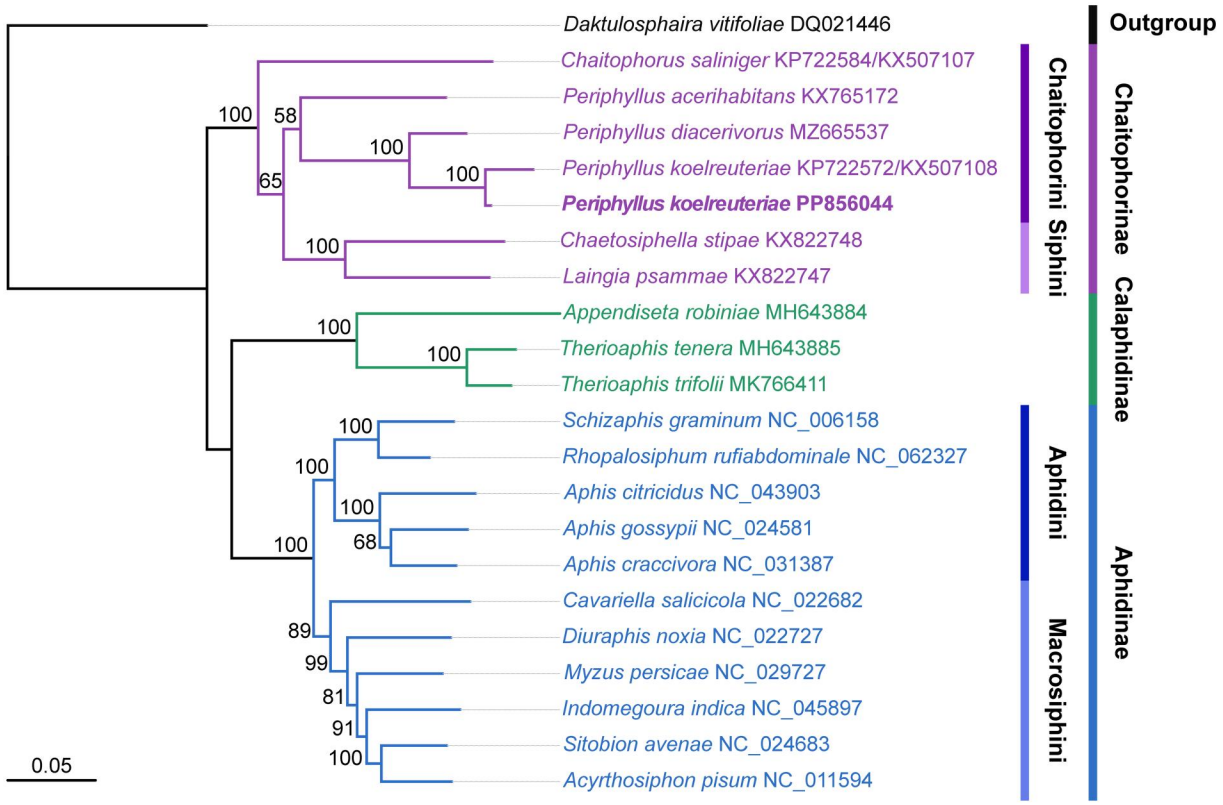

Figure 3. Maximum-likelihood phylogenetic tree based on whole mitogenomes of *Periphyllus koelreuteriae* and 20 other aphid species. Bootstrap values >50% are displayed above the branches. Detailed information on the aphid mitogenomes used in phylogenetic analysis is provided in Table S1.

critical revision of the paper. All authors approved the final version and agreed to be accountable for all aspects of the work.

Ethical approval

Ethical approval is not required for this study, because *Periphyllus koelreuteriae* is not a protected species and the specimens were not collected from a natural reserve.

Disclosure statement

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

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Data availability statement

The genome sequence data supporting this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov under the accession number PP856044. The associated BioProject, SRA, and Biosample numbers are PRJNA1139254, SRR29935274, and SAMN42768482, respectively.

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