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MITOGENOME REPORT

Complete mitochondrial genome of *Rhopalosiphum maidis* (Hemiptera: Aphididae) and its phylogenetic implications

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ABSTRACT

Rhopalosiphum maidis Fitch, 1856 is widespread in tropical and temperate regions. *R. maidis* can spread viral diseases in maize and harm various important crops. In the present study, we report the first complete mitochondrial genome of *R. maidis*. The circular genome is found to be 17,021 bp in length, includes a standard set of 22 transfer RNAs, two ribosomal RNAs, 13 protein-coding genes, and two non-coding control regions. The base composition is 84.32% AT and 15.79% GC. The phylogenetic tree of the 17 Aphidini families constructed based on the nucleotide sequences of complete mitochondrial genomes strongly supports the conclusion that *R. maidis* is closely related to *R. rufiabdominalis*.

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Introduction

At present, *Rhopalosiphum maidis* Fitch, 1856 is widely distributed in tropical and temperate regions and cause damage to various gramineous crops, including corn, sorghum, wheat, and millet (Al-Eryan and El-Tabbakh 2020). In recent years, the damage caused by *R. maidis* has gradually increased. *R. maidis* primarily resides in the heart leaves and male flowers, and can remove photosynthates, further reducing crop growth and yield. Additionally, *R. maidis* transmits a variety of viruses, such as maize yellow dwarf, barley yellow dwarf, cucumber mosaic, and sugarcane mosaic viruses (Klein and Smith 2002). There have been reports that the potential harm caused by their viral transmission *via R. maidis* may be far greater than the loss of maize yield (Kuo et al. 2006). To the best of our knowledge, this study is the first to characterize the complete mitochondrial genome of *R. maidis* using Illumina Novaseq and PacBio Sequel techniques to reconstruct the phylogenetic relationships based on the published genome sequences of the Aphididae family. Our findings provide molecular information for the phylogenetic and evolutionary study of *R. maidis*.

Materials and methods

On February 4, 2023, 30 adult specimens of *R. maidis* were collected from sorghum fields in Hakjia village (109.58E, 18.28 N) in Jiyang Town, Hainan Province, China. Five specimens were stored in 95% ethanol under voucher number MgPL2023022312 at the College of Plant Protection, Shanxi Agricultural University, Taiyuan, China (X.K., xingkun1215@126.com).

The taxonomic status of the *R. maidis* was determined following the morphological identification reported in the literature. The wingless adult was oval, soft-bodied, 2.5 mm long and bottle green with black antennae, legs, and cornicles. The 7th segment of the abdomen was black, 8th segment had a dorsal transverse band, and body surface had a mesh. The antennae had six segments. The imbricated ventral tube was long, cylindrical, and had a contracted end. The tail was conical, with four to five hairs (Razmjou and Golizadeh 2010). The morphology of *R. maidis* is shown in Figure 1.

Total DNA was extracted using a tissue genomic DNA extraction kit (Tiangen Biochemical Technology Co. Ltd., Beijing, China). The mitochondrial genome was sequenced by Shanghai Personalbio Biotechnology Co., Ltd. (Shanghai, China) using Illumina Novaseq PE250 (Illumina, San Diego, CA, USA) and PacBio Sequel system. The Illumina data were assembled using A5-miseq v20150522 (Coil et al. 2015) and SPAdesv3.9.0 (Bankevich et al. 2012). Contigs with a high coverage depth were annotated by BLAST against the Nucleotide database in National Center of Biotechnology Information (NCBI) using blastn (v2.2.31) to extract the mitochondrial sequences (Chen et al. 2015). The collinearity of the assembly results from different software was calculated using MUMmer v3.1 (Kurtz et al. 2004) to determine the position

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Figure 1. Species reference image captured by Kun Xing.

relationships among contigs. The gaps among contigs were filled, and the results were corrected using Pilon v1.18 (Walker et al. 2014). The repeat regions of the Illumina data were further corrected and confirmed by the PacBio Sequel system. The PacBio Sequel data were assembled using Flye v2.9.1-b1781 (Kolmogorov et al. 2019). The assembly results were annotated by mitos2 (Bernt et al. 2013), and the boundary was adjusted by referring to the genome sequence of *Rhopalosiphum nymphaeae* (MN943499) and *Rhopalosiphum rufiabdominalis* (MN876840) to obtain the complete mitochondrial genome. The CGView visualization software was used to generate a whole-genome circular map (Stothard and Wishart 2005). The PacBio Sequel data were further mapped to the complete mitochondrial genome to obtain a sam assembly file and the sam assembly file was transformed to a bam assembly file. The coverage depth of each base on the genome was also calculated using SAMtools v1.16.1 (Li et al. 2009) and the sequencing depth and coverage map was draw by ggplot2 (Ito and Murphy 2013) in R (Figure S1).

The complete mitochondrial sequences in "Aphidini" were searched from NCBI database after excluding incomplete and repeating records. The nucleotide sequences of complete mitochondrial genomes from Aphidini species and outgroup from *Greenidea psidii* were downloaded from NCBI for the phylogenetic analysis. The nucleotide sequences of the complete mitogenome were aligned using ClustalW (Larkin et al.

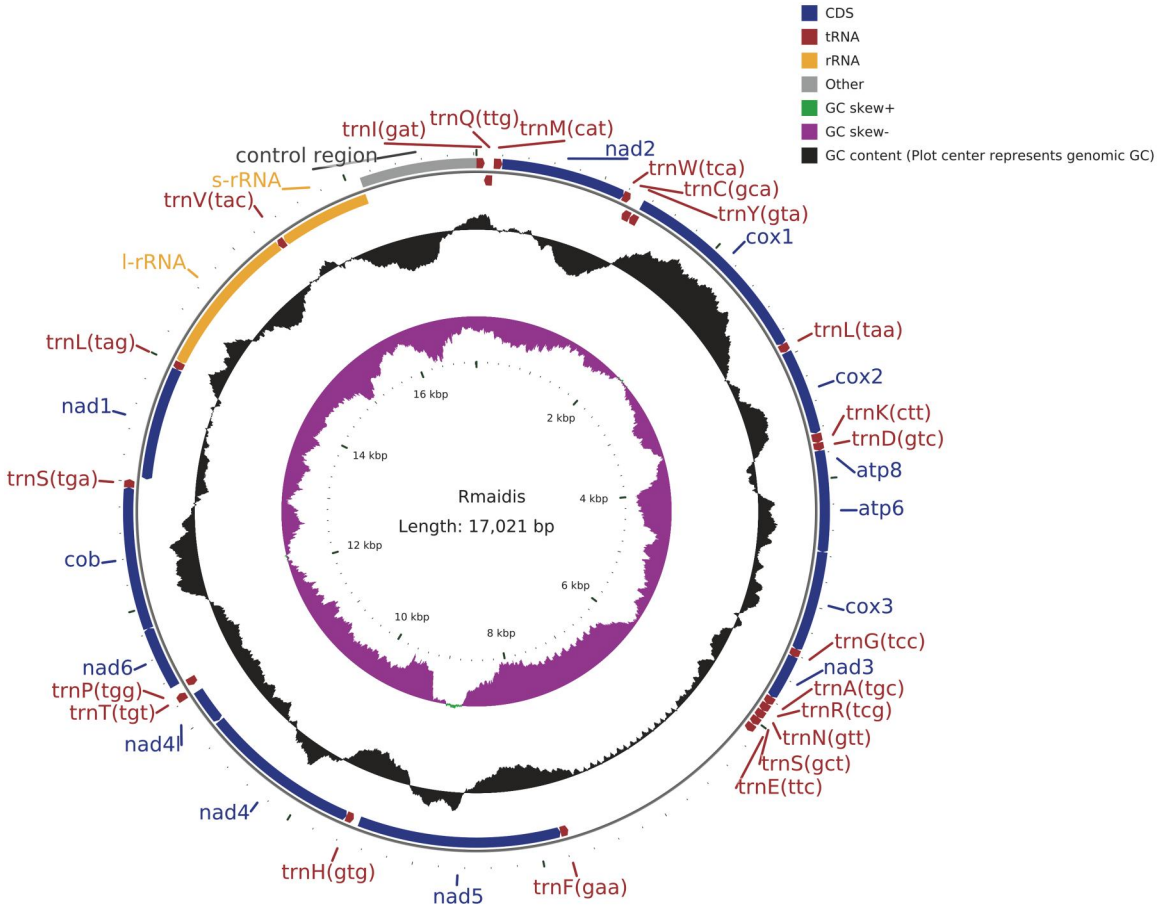

Figure 2. Circular genome feature map of *Rhopalosiphum maidis* drawn by CGView. The CDS, tRNAs, rRNAs, and other are denoted by the color blocks. Genes outside the map are transcribed clockwise, whereas those inside are transcribed counterclockwise. The window size is 5 bp and step is 1 bp.

Table 1. Accession number and reference information for 18 species.

GenBank accession No.	Species	Genus	Tribe	Length (bp)	References
MN871977	*Aphis aurantii*	Aphis	Aphidini	15296	Pu et al. (2020)
MK540501	*Aphis citricidus*	Aphis	Aphidini	16763	Wei et al. (2019)
OM894972	*Aphis coreopsidis*	Aphis	Aphidini	15623	Unpublished
MT095075	*Aphis craccivora*	Aphis	Aphidini	15478	Voronova et al. (2020)
MG897128	*Aphis fabae mordvilkoi*	Aphis	Aphidini	15346	Voronova et al. (2020)
KJ669654	*Aphis gossypii*	Aphis	Aphidini	15869	Zhang et al. (2016)
OM894973	*Aphis solanella*	Aphis	Aphidini	15331	Unpublished
MN316642	*Aphis spiraecola*	Aphis	Aphidini	15465	Du et al. (2019)
MK111111	*Aphis glycines*	Aphis	Aphidini	17954	Unpublished
OK641613	*Hyalopterus amygdali*	Hyaloptera	Aphidini	15306	Unpublished
OK274075	*Hyalopterus arundiniformis*	Hyaloptera	Aphidini	15408	Unpublished
MT898422	*Hyalopterus pruni*	Hyaloptera	Aphidini	15410	Unpublished
OR148359	***Rhopalosiphum maidi***	Rhopalosiphum	Aphidini	17021	Unpublished
MN943499	*Rhopalosiphum nymphaeae*	Rhopalosiphum	Aphidini	15594	Unpublished
MN876840	*Rhopalosiphum rufiabdominalis*	Rhopalosiphum	Aphidini	15289	Thao et al. (2004)
AY531391	*Schizaphis graminum*	Schizaphis	Aphidini	15721	Unpublished
MW811104	*Melanaphis sacchari*	Melanaphis	Aphidini	15111	Unpublished
MH844624	*Greenidea psidii*	Greenidea	Greenideinae	16202	Unpublished

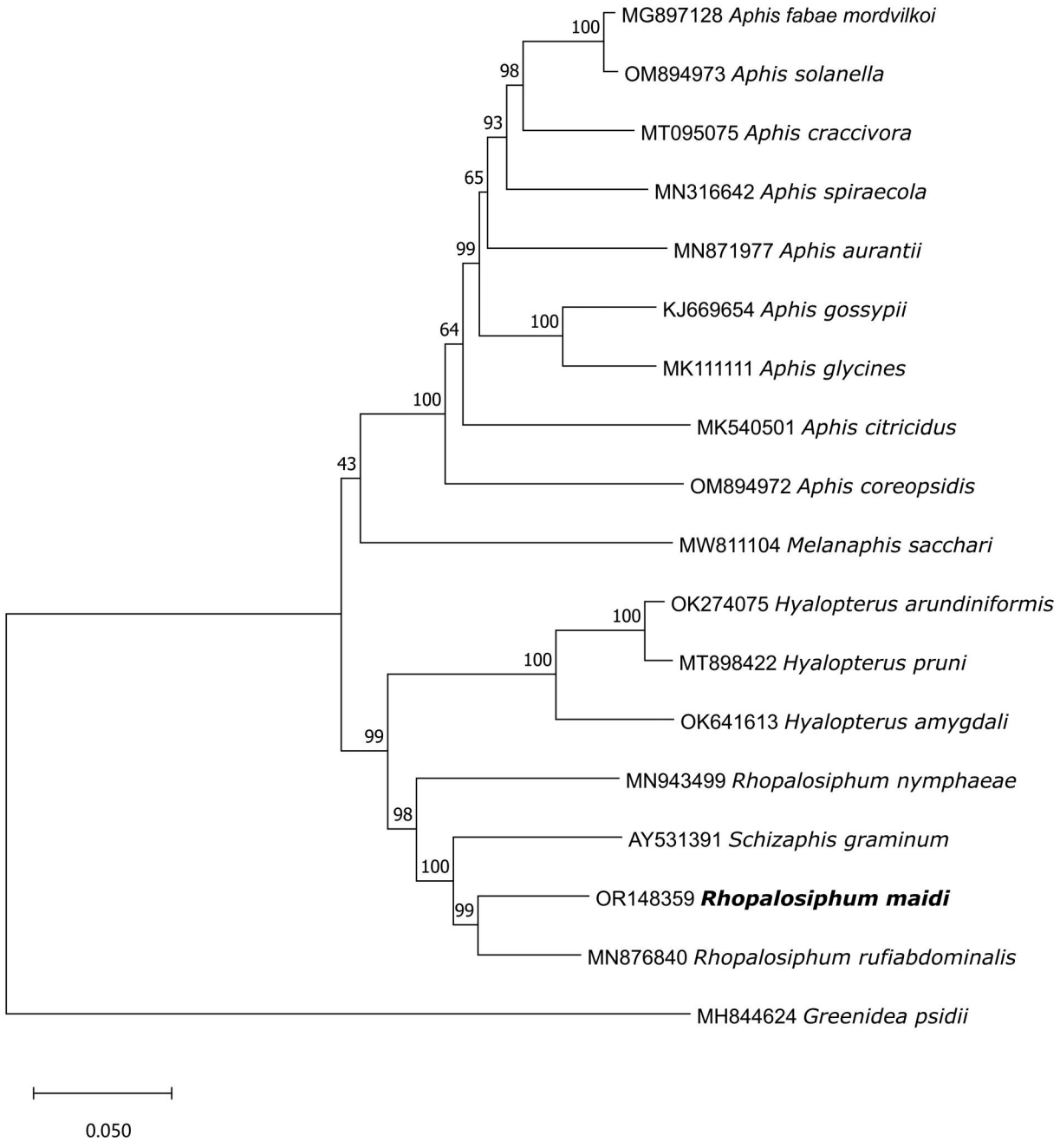

Figure 3. Phylogenetic relationships of 17 aphidini, including *Rhopalosiphum maidis*, based on the nucleotide sequences of complete mitochondrial genomes using ML methods. The sequences used for tree reconstruction are listed in Table 1. The scale bar is the distance scale. The numbers beside the nodes are bootstrap values. The bootstrap value based on 1000 replicated is represented on each node. *Greenidea psidii* is used as outgroup to root the tree.

2007) in MEGA-11 (Tamura et al. 2021) with default parameters. The maximum likelihood model with the lowest Bayesian Information Criterion (BIC) score is regarded as the best model. According to BIC of 104464.25, GTR (General Reversible Mitochondrial) + a discrete Gamma distribution (G) + evolutionarily invariable (I) with 1000 replicates was selected to construct a phylogenetic tree.

Results

The complete mitogenome of *R. maidis* (OR148359.3) is a circular DNA molecule 17,021 bp in length. The mitogenome contains 13 protein-coding genes (PCGs), 22 transfer RNA genes, large and small ribosomal RNA unit genes (*rrnL* and *rrnS*, respectively), and two large noncoding regions (putative control regions; Figure 2).

The nucleotide composition of *R. maidis* was significantly AT-biased, with A, G, C, and T accounting for 44.89, 5.69, 10.10, and 39.32%, respectively. In this genome, the GC and AT skews were −0.279 and 0.066, respectively. The overall length of all overlaps was 75 bp and overlaps were present at 14 gene junctions. The largest overlap (29 bp) was observed between *trnY* and *cox1*. Intergenic spacers, totaling 1,746 bp, appeared at 12 positions and ranged from 1 to 1,655 bp. The control region had an A + T content of 88.82%, was 912 bp long, and was located between *rrnS* and *trnI*.

The *rrnL* gene was located between $trnL^{tag}$ and *trnV* and had an A + T content of 85.15% and was 1259 bp long. The *rrnS* gene was 759 bp long with an A + T content of 83.79%. The first two bases of start codons were "AT," which was the same across all the 13 PCGs, and the third base of start codons was different. The PCGs *cox3*, *nad4l*, and *cob* started with ATG; *cox1*, *atp6*, *atp8*, *nad6*, and *nad1* started with ATT; and *nad2*, *cox2*, *nad3*, *nad5*, and *nad4* started with ATA. Twelve, one, and two PCGs were terminated with TAA, TAG (*cob*), and an incomplete stop codon T (*cox1* and *nad4*), respectively.

Seventeen complete mitochondrial sequences in Aphidini and outgroups from *Greenidea psidii* were identified for the phylogenetic analysis (Table 1).

The phylogenetic tree indicated that the genome of *R. maidis* was similar to that of *R. rufiabdominalis* and there was strong support for the clustering of *R. maidis* with *R. rufiabdominalis*, *Schizaphis graminum*, and *R. nymphaeae* (Figure 3).

Discussion and conclusion

Herein, the complete mitogenome of *R. maidis* was assembled and analyzed using Illumina Novaseq and PacBio Sequel techniques. The mitochondrial genome of *R. maidis* is 17,021 bp long, has standard, metazoan set of genes in the typical insect order (Cameron 2014). Base composition is heavily AT-biased. This is consistent with previous results for other species of Aphididae. For instance, the AT contents of *Aphis aurantii* is 83.5% (Pu et al. 2020), *A. citricidus* is 84.0% (Wei et al. 2019), *A. gossypii* is 83.7% (Zhang et al. 2016). Voronova et al. (2020) sequenced the complete mitochondrial genomes of *A. fabae mordvilkoi*, *A. craccivora*, and *M. persicae* from Aphidinae, as well as *Therioaphis tenera* and *Appendiseta robiniae* from Calaphidinae, and determined that the A + T content of all five mitogenomes is >80%.

Phylogenetic analysis suggests that *Rhopalosiphum* is more closely related to *R. rufiabdominalis*, with a bootstrap rate of 96, indicating that the two species share more recent common ancestor gene. We also found that there was strong support for the clustering of *Schizaphis graminum* with *R. maidis*. This is not consistent with traditional taxonomy. Phylogenetic trees based on genome-wide sequence data may not always represent the true evolutionary history for a variety of reasons. One process that can lead to incorrect reconstruction of species phylogenies is gene flow, especially if interspecific gene flow has affected large parts of the genome (Zhang et al. 2021). We expect that these results will provide new insights and act as a reference for future studies on the phylogenetics and genetics of *R. maidis*.

Author contributions

CK and FZ: conceptualization, methodology, formal analysis, resources, investigation, writing-original draft, writing-review and editing, and visualization. KX: investigation, writing-review and editing. KX and FZ: resources, formal analysis, investigation, writing-review and editing, visualization, supervision, project administration, and funding acquisition.

Ethical approval

The collection of the reported sample was carried out in accordance with guidelines provided by the national regulations. The sampling site is not located in any protected area. The research was conducted with the permission of Shanxi Agricultural University.

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/genbank under the accession no. OR148359.3. Illumina reads were deposited under SRR25470088, and PacBio reads under SRR29709340. The associated BioProject and Bio-Sample numbers are PRJNA1000643 and SAMN36765787.

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