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Complete mitochondrial genome of the corn leafhopper *Dalbulus maidis* (Hemiptera: Cicadellidae)

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ABSTRACT. The corn leafhopper, *Dalbulus maidis*, is the most important pest of maize (*Zea mays*) in the Neotropics, due to its efficiency in pathogen transmission and close evolutionary association with the crop. This host specialization has enabled *D. maidis* to spread with maize, from their center of origin in Mesoamerica to all tropical and subtropical America, including Brazil. The population dynamics and survival strategies of *D. maidis* could be better understood by studying the species' genetics, which has been little explored thus far. To fill in this knowledge gap, we provide here the complete mitochondrial DNA genome (mitogenome) of *D. maidis* (GenBank accession number: ON756137). Six adult specimens collected in Southern Brazil (Novo Machado/RS and Salvador das Missões/RS) were used for DNA extraction and sequencing on an Illumina MiSeq, followed by

mitogenome assembling using NOVOPlasty v4.3.1, and annotation using MITOS v2.0.8. We confirmed the species identity by molecular diagnostics that showed >98% similarity between our reported partial cytochrome oxidase subunit I (mtCOI) gene and other *D. maidis* mtCOI sequences reported to date. The mitogenome of *D. maidis* is 16,488 base pairs long and contains 37 genes: 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs), and two ribosomal RNA (rRNA) genes. Similar to other arthropods, the mitogenome of *D. maidis* is A–T biased (A: 32.3 – 32.9 %, T: 31.9 – 33.0 %). Primers for the mitochondrial COI gene were also designed to assist in future studies. This report of the *D. maidis* mitogenome will facilitate the development of effective molecular markers, rapid detection of field infestations and identification of migration pathways available for this insect pest in Brazil and neighboring countries.

Key words: Corn stunt disease; Deltocephalinae; Mitogenome; *Zea mays*

INTRODUCTION

The corn leafhopper, *Dalbulus maidis* (Hemiptera: Cicadellidae), is the most important pest of maize (*Zea mays*) in the Neotropics, primarily due to its efficiency as a vector of three pathogens: corn stunt spiroplasma, maize bushy stunt phytoplasma and maize rayado fino virus (Nault, 1990; Jones and Medina, 2020). The combined effect of pathogen transmission and sap-sucking by *D. maidis* can reduce maize yield up to 100%, depending on the severity of symptoms (Hogenhout et al., 2008; Oliveira and Frizzas, 2021). The close evolutionary association of *D. maidis* with maize has enabled this strategist species to spread with the crop (Bernal et al., 2019), from their center of origin in Mesoamerica to all tropical and subtropical America, including Brazil (Oliveira and Frizzas, 2021). The pest is currently found from southern USA to northern Argentina (Oman, 1948; Triplehorn and Nault, 1985; Carloni et al., 2013), although still absent in other continents (Pozebon et al., 2022).

Population outbreaks of *D. maidis* and the associated corn stunt disease have increased in frequency across South America since 2015 (Oliveira and Frizzas, 2021), probably due to modifications in the cultivation system, close succession of maize crops and long-distance migration of leafhoppers (Pozebon et al., 2022). The mechanisms underpinning population movement and survival strategies of *D. maidis* could be better understood by studying the species' genetic diversity and identifying if gene flow is taking place across different regions (Beche et al., 2022). Although partial cytochrome oxidase subunit I (mtCOI) gene sequences for *D. maidis* are available (Medina et al., 2012; Palomera et al., 2012), a complete mitochondrial DNA genome for this species was still lacking, preventing further research in the area.

In addition to providing a basis for future genetic studies, having the complete mitogenome available will help understand the differences between populations of *D. maidis* and their impact on maize production (Dietrich et al., 1998; Zucchi et al., 2019). Detection of field infestations will also become more rapid and precise, as novel molecular markers and primers can be designed based on any desired region of the species'

mitogenome (Arnemann et al., 2016a). Other authors have highlighted the need for mitogenome characterization in the study of major and emerging agricultural pests, such as *Helicoverpa armigera* (Tay et al., 2013; Arnemann et al., 2019), *Spodoptera frugiperda* (Tay et al., 2022), and *Melanagromyza sojae* (Arnemann et al., 2016b; Pozebon et al., 2021).

To fill in this knowledge gap, we report in this study the complete mitochondrial genome of *D. maidis* for the first time, providing a better understanding of the phylogenetic relationships within the Hemiptera order and paving the way for further genetic studies involving this important maize pest.

MATERIAL AND METHODS

Sample collection and DNA extraction

Adult leafhopper specimens found damaging maize plants in Rio Grande do Sul State (Southern Brazil) were collected on August 3, 2021. Four specimens (hereafter A1, A2, A3 and A4) came from the municipality of Novo Machado (27°32'33''S, 54°29'00"W), and two specimens (hereafter B1 and B2) from Salvador das Missões (28°08'09"S, 54°49'14"W). An additional seventh specimen collected in Novo Machado was selected as a voucher and preserved in the Pest Management and Genetics Laboratory of the Federal University of Santa Maria, RS, Brazil (contact person: Gustavo Ugalde, gandradeugalde@yahoo.com.br). This is a pest leafhopper species and no ethical approval or permission to collect was required.

Upon confirmation of unique *D. maidis* morphological characters by one of the authors (G.R.S.), total gDNA from the six leafhopper adults was extracted using the Invisorb Spin Tissue Mini kit (Stratec Biomedical, Birkenfeld, Germany) following the modified protocol as detailed in Piot et al. (2019). Quality control of the genomic DNA samples was done on E-Gel EX 1% (Invitrogen) and quantified using a Quant-iT PicoGreen assay (Invitrogen). Between 19 and 162 ng of input gDNA were fragmented with a Covaris S2 (Covaris) aiming for an average length of 600 bp.

Mitochondrial genome sequencing and annotation

For each sample, a sequencing library was constructed using the NEBNext Ultra II DNA Library Kit for Illumina (New England Biolabs) and the NEBNext Multiplex Oligos for Illumina (New England Biolabs) for dual indexing. Library size selection of 600-1000 bp fragments was done on E-Gel EX 2% (Invitrogen) and purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research). The purified fragments were enriched using six to eight PCR cycles depending on the input concentration. Amplified libraries were purified using 0.9x AMPure XP beads (Beckman Coulter). Final quality control was done with a High Sensitivity DNA Kit (Agilent Technologies) and concentration was determined with qPCR according to the Sequencing Library qPCR Quantification Guide from Illumina.

Sequencing was done as a paired-end 250 nano-run on an Illumina MiSeq and yielded between 0.11 and 0.14 million read pairs per sample. Raw read quality was checked with FastQC (v0.11.9), revealing good quality reads. We screened for common lab contaminants using FastQ_Screen (v0.15.0) and included a small selection of *D. maidis*

mitochondrial sequences obtained from NCBI. No contaminants were detected. Quality trimming was done using Cutadapt (v3.4), including removal of reads with ambiguities and resulting in a final length shorter than 100 nt. The trimmed reads were used to assemble the mitochondrial genome of all samples with NOVOPlasty (v4.3.1), using *D. maidis* mitochondrial genes NAD1 and COI retrieved from NCBI as seed sequences. The NAD1 seed resulted in the best assemblies.

Sample A1 yielded the longest (16,488 bp) and probably complete assembly. Sample A2 produced a slightly shorter assembly (16,357 bp), whereas samples A3 (10,052 bp) and B1 (6,105 bp) yielded only fragmented mitogenomes. Samples A4 and B2 failed because not enough mitogenome-specific reads were available in the sample. The four draft assemblies (A1, A2, A3 and B1) were aligned using T-Coffee's M-Coffee extension (v13.41), and the mitogenome from sample A1 (GenBank accession number: ON756137) was annotated using MITOS v2.0.8 (Bernt et al., 2013) and Tandem Repeats Finder v4.0.9 (Benson, 1999) as described (Tay et al., 2016). The mitochondrial genome cycle graph was mapped using CIRCOS (v0.69-6). Primers for the mitochondrial COI gene were designed using Primer3 (Untergasser et al., 2012). PCR conditions were estimated based on melting temperature (T_m) and on similar estimates made by Palomera et al. (2012).

Sequence analysis

Phylogenetic analysis based on partial mtCOI gene included our reported *D. maidis* and related Cicadellidae leafhopper sequences from the Deltocephalinae tribe, with *Aphrodes aestuarinus* and *Aphrodes makarovi* as outgroups (GenBank database, accessed 12, 2022). Sequences from the genus *Dalbulus* (other than *D. maidis*) were not used due to lack of available mtCOI data. The sequences were downloaded and aligned with the mtCOI gene from our sample A1 (GenBank accession number: ON678134). Aligned partial mtCOI gene sequences were trimmed to 568 bp. We used ClustalW (gap opening penalty: 15.00; gap extension penalty: 6.66) within MEGA-X (Kumar et al., 2018) for sequence alignment, followed by phylogenetic inference using PhyML 3.0 (Guindon et al., 2010) with 1,000 bootstrap replications for estimating node confidence. The ''automatic model selection'' option was selected to optimize the substitution model.

RESULTS

Mitogenomic organization and nucleotide composition

The two longest assemblies (A1 and A2) shared 99.0% nucleotide identity (*ca.* 16,317 bp compared), but only assembly A1 was annotated and reported (Figure 1). As with other arthropod mitogenomes, we observed high A + T content (65.7 %) compared to G + C (34.3 %). We identified 13 protein-coding genes (PCGs), 22 transfer RNAs (tRNAs) and two ribosomal RNA (rRNA) genes, based on the annotated mitogenome of sample A1 (ON756137). We also observed three non-coding regions, two of them

with tandem repeats (hereafter RR1 and RR2). The RR1 repeat region consists of 16.7 copies of a 52 bp sequence, while the RR2 region consists of 2.3 copies of a 209 bp sequence. The MITOS software could not identify a trnF gene and OL regions.

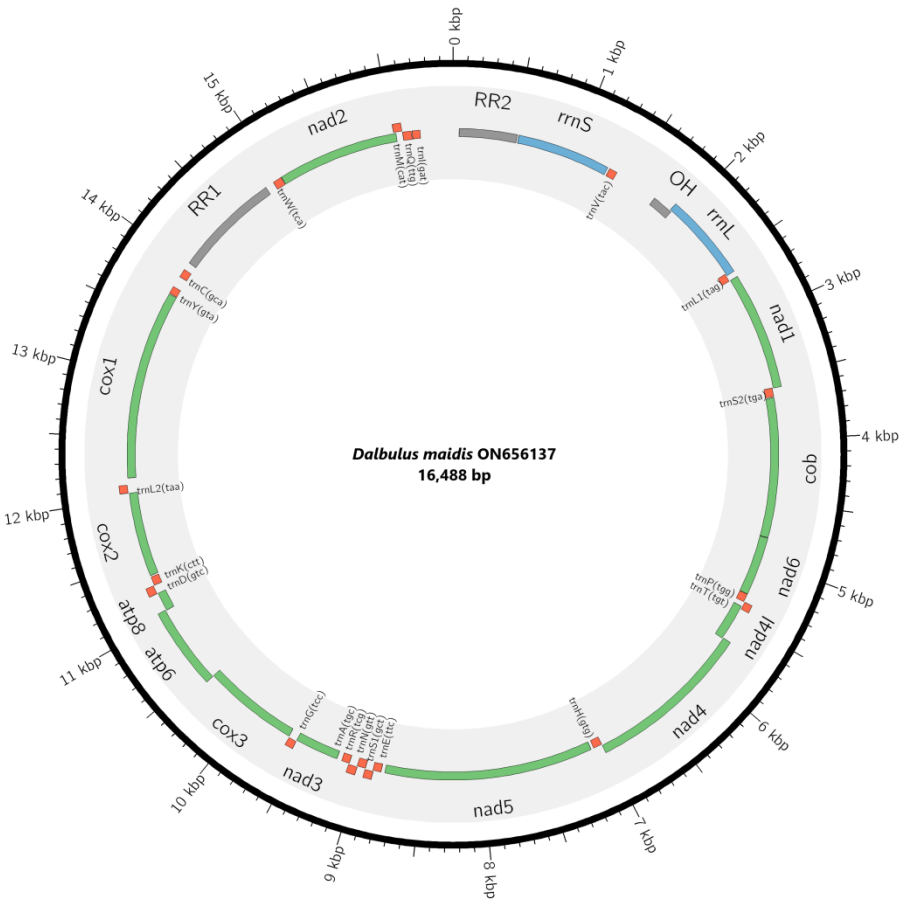

Figure 1. Mitochondrial genome cycle graph of *Dalbulus maidis.* Protein-coding genes (complex I, complex IV and ATP synthase) are green, transfer RNAs are red, ribosomal RNAs are blue. Figure created using CIRCOS (v0.69-6).

Protein-coding genes and mtCOI primers

Among the 13 PCGs, six were initiated with ATT start codon (NAD1, NAD3, NAD4L, NAD5, NAD6, COII), six by ATG (CytB, NAD2, NAD4, COI, COIII, ATP6), and one by ATC (ATP8). All PCGs were terminated with TAA stop codon, except for NAD2 and NAD5, which ended at TAG and a single T, respectively. Primers were designed to allow replication of the mtCOI gene region in future studies (Table 1). PCR conditions for mtCOI amplification are as follows: 94 ºC for 5 min; followed by 35 cycles each of 30 s at 94 ºC, 60 s at 55 ºC, and 60 s at 72 ºC; ending with final cycles of 30 s at 94 ºC, 60 s at 52 ºC and 10 s at 72 ºC.

Table 1. Primers designed for replication of mtCOI (cytochrome oxidase subunit I) gene from *Dalbulus maidis*.

ID	Product size (bp)	Position	T_m (°C) [1]	GC content (%)	Sequence
dmaiCOI1	824	Left	58.66	55.00	TGAGACAGGGACAGGAACAG
		Right	59.81	50.00	ACCCCGCCATGATAGCAAAT
dmaiCOI2	766	Left	57.77	55.00	CCAGACATAGCTTTCCCTCG
		Right	58.20	50.00	CGAATCCCGCTGCTCATATT
dmaiCOI3	761	Left	59.52	55.00	GCAGGCCCCAGAGTTGATAT
		Right	58.22	47.62	CCCGCCATGATAGCAAATACT
dmaiCOI4	777	Left	58.95	50.00	AGCTCAACCTGGGTCGTTTA
		Right	58.55	45.45	TCAACATCTATCCCCACTGTGA

[1] T_m = Melting temperature.

Phylogenetic analysis and potential impacts

Molecular diagnostics based on partial mtCOI gene (568 bp) confirmed our specimens were *D. maidis*, with high nucleotide similarity (98-100%) to the partial mtCOI gene sequences of other reported *D. maidis* (GenBank accession numbers: MH591425.1, MH591423.1, MH591422.1, MH591421.1, MH591424.1, MN345272.1, JN411692.1, KF152948.1, JN411697.1, KF152945.1, KF152944.1, KF152943.1, KF152946.1). Phylogenetic analysis with *Aphrodes* species as outgroups placed *D. maidis* within the same clade as *Sonronius dahlbomi* and near the *Macrosteles* clade (Figure 2). A basal position is suggested for *Davisonia* as compared to the other analyzed genera (100% bootstrap support). Low bootstrap confidence (≤55%) for certain clades suggests that further research is needed to understand the phylogenetic relationships within this agriculturally important hemipteran group.

Previous phylogeny assessments based on mitochondrial DNA sequences have suggested that the ancestral hosts of *Dalbulus* were gamagrasses (*Tripsacum* spp.) (Dietrich et al., 1998), with subsequent maize domestication favoring corn leafhopper genotypes preadapted for exploiting maize (Bernal et al., 2019). Among the 13 species identified so far within the genus *Dalbulus*, only *D. maidis* achieved pest status and wide distribution because of its close association with maize and efficiency in pathogen transmission (Pozebon et al., 2022). Other leafhopper species, however, are also capable of transmitting corn stunting pathogens, such as the African species *Leptodelphax maculigera* (Hemiptera: Delphacidae), which was recently found in Brazil (Ferreira et al., 2023) and whose pest potential remains unknown. Although the species are morphologically distinct, they share the same habitat and may be misidentified by common methods, raising the need for genetic identification.

Epidemic outbreaks of *D. maidis* are expected to increase in frequency and intensity in Brazil and other maize-producing countries that present suitable conditions for this species (Santana et al., 2019; Pozebon et al., 2022). The annotated *D. maidis* mitogenome and mtCOI primers provided in this study will facilitate detection of field infestations and identification of migration pathways available for this insect pest, as recently done for *Chrysodeixis includens* and *Rachiplusia nu* (Perini et al., 2020). Population dynamics of *D. maidis* should also be explored using the genetic tools provided here, which would help plan management programs for this insect pest that are both economically efficient and environmentally sustainable.

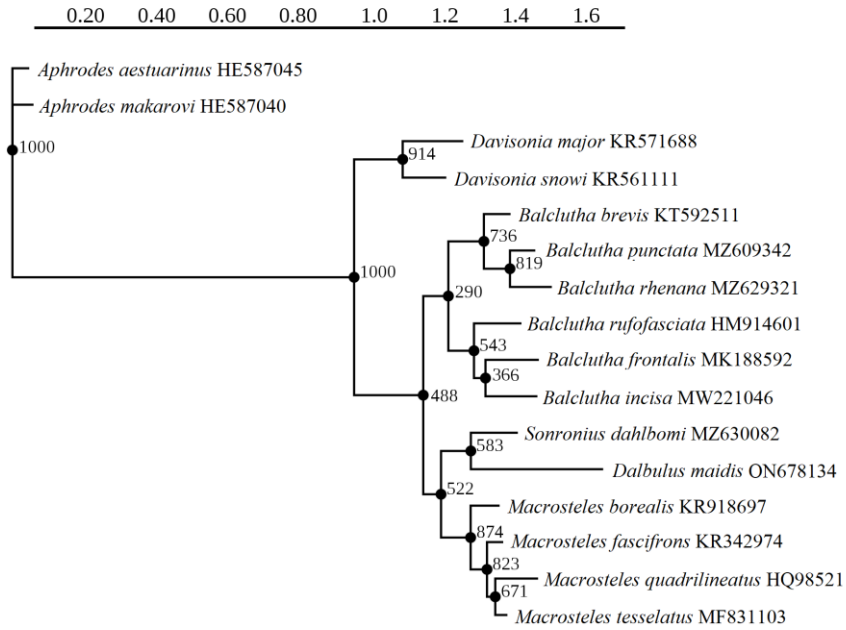

Figure 2. Partial mtCOI gene (568 bp) maximum likelihood (ML) phylogeny of Cicadellidae species inferred using PhyML (Guindon et al. 2010) (substitution model: GTR + G + I; 8 311,88 (AIC); Gamma shape parameter: 1.001, proportion of invariable sites: 0.486). The outgroups are *Aphrodes aestuarinus* (HE587045) and *Aphrodes makarovi* (HE587040) (Zahniser and Dietrich, 2013). The GenBank accession numbers for all samples used are provided. All sequences used are aligned to nucleotide positions 12,240-12,808 of the rotated (100 nt) *D. maidis* mitogenome from sample A1 (ON756137).

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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 numbers ON678134 and ON756137. The associated BioProject accession number is PRJNA852223. The associated SRA accession numbers are SRR19801437, SRR19801438, SRR19801439, SRR19801440, SRR19801441 and SRR19801442. The associated BioSample numbers are SAMN29267070, SAMN29267071, SAMN29267074, SAMN29267075, SAMN29267076 and SAMN29267077.

AUTHOR CONTRIBUTIONS STATEMENT

H.P., J.A.A. and G.S. designed the study. G.R.S. acquired relevant specimens for this study. G.U. carried out laboratory work to generate DNA data. Y.G. and F.V.N. produced the mitogenome assemblies and annotations. H.P., J.A.A. and W.T.T. interpreted the results and wrote the manuscript. All authors contributed to improve the final submitted version for intellectual content and integrity.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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