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MITOGENOME REPORT

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De novo assembly and annotation of the *Empoasca fabae* mitochondrial genome

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ABSTRACT

This study presents the assembly and annotation of the full-length mitochondrial genome for the leafhopper species *Empoasca fabae* Harris, 1841. The mitogenome was obtained from a contig-level assembly with the identified mitochondrial genome being 14,873 bp in length. The base composition was A (38.8%), T (39.1%), C (11.7%), and G (10.4%). The mitogenome comprised 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), two ribosomal RNA genes (rRNAs), and showed a unique, non-AT-rich D-loop region. Phylogenetic analysis confirmed the placement of *E. fabae* within the subfamily Typhlocybinae, clustering with other species in the *Empoasca* genus.

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Introduction

Empoasca fabae Harris, 1841, is a significant insect pest in North American agriculture, belonging to the family Cicadellidae in the order Hemiptera. Its economic impact stems from its polyphagous feeding habits, annual migratory patterns, and suspected ability to transmit viral and bacterial plant pathogens across a wide range of economically important crops (Santos et al. 2024a, 2024b). Our group has also been using this migratory leafhopper as a model to study the effects of climate change on insect migration, population dynamics, disease transmission, and insecticide resistance (Plante et al. 2024). To advance this research, population genomic studies are essential. However, we are currently facing a bottleneck due to the lack of available mitochondrial genomes for *E. fabae*, which has prompted us to produce and annotate the first complete mitochondrial genome of this species using high-throughput Illumina sequencing.

Materials and methods

Sample collection and DNA extraction

Leafhoppers used in this study were captured using yellow sticky traps between July and August 2023 from a strawberry farm in Southern Québec, Canada (45°34′24.0″N 73°03′46.0″W) (Figure 1). Samples were visually identified using taxonomic morphology to the species level as previously described (Chasen et al. 2014), preserved in 70% ethanol and stored at 4 °C until DNA extraction. Specimens were deposited at the Canadian National Collection of Insects, Arachnids, and Nematodes, under the voucher numbers CNC2098398-2098407, with Dr. Joel Kits as responsible of Hemiptera division (joel.kits@agr.gc.ca).

E. fabae specimens were pooled into 3 subsamples of 10 insects each and washed with sterile ddH_2O. DNA was then extracted by homogenizing the insects with a mini-pestle in 700 µL of lysis buffer containing 20 mM EDTA (pH 8.0), 100 mM Tris-HCl (pH 7.5), 1.4 M NaCl, 2% w/v CTAB, and 4% w/v PVP-40. After homogenization, an additional 700 µL of lysis buffer was added, and samples were incubated at 65 °C for 1 h, inverting the tubes every 10 min. The supernatant was then washed twice with chloroform-isoamyl alcohol (24:1), and DNA was precipitated using 70% v/v ice-cold isopropanol. The DNA pellet was washed with 70% ethanol and air-dried for 10 min before eluting in a buffer containing 10 mM Tris-HCl and 0.1 mM EDTA at a pH of 8.0.

Sequencing and preprocessing

Preparation of Illumina short-read libraries and subsequent sequencing were performed by Genome Québec (Montréal, Canada), resulting in three paired-end (PE) files of 43 M, 49 M, and 50 M reads, respectively. Raw data files were preprocessed with BBTools (v.36.92) to trim adapter sequences ($k = 23$, mink = 11, hdist = 1), with flags for paired-end trimming and overlap detection (Bushnell 2024). Additional quality filtering was performed (trimq = 10), and low complexity sequences were removed (entropy = 0.7, entropywindow =

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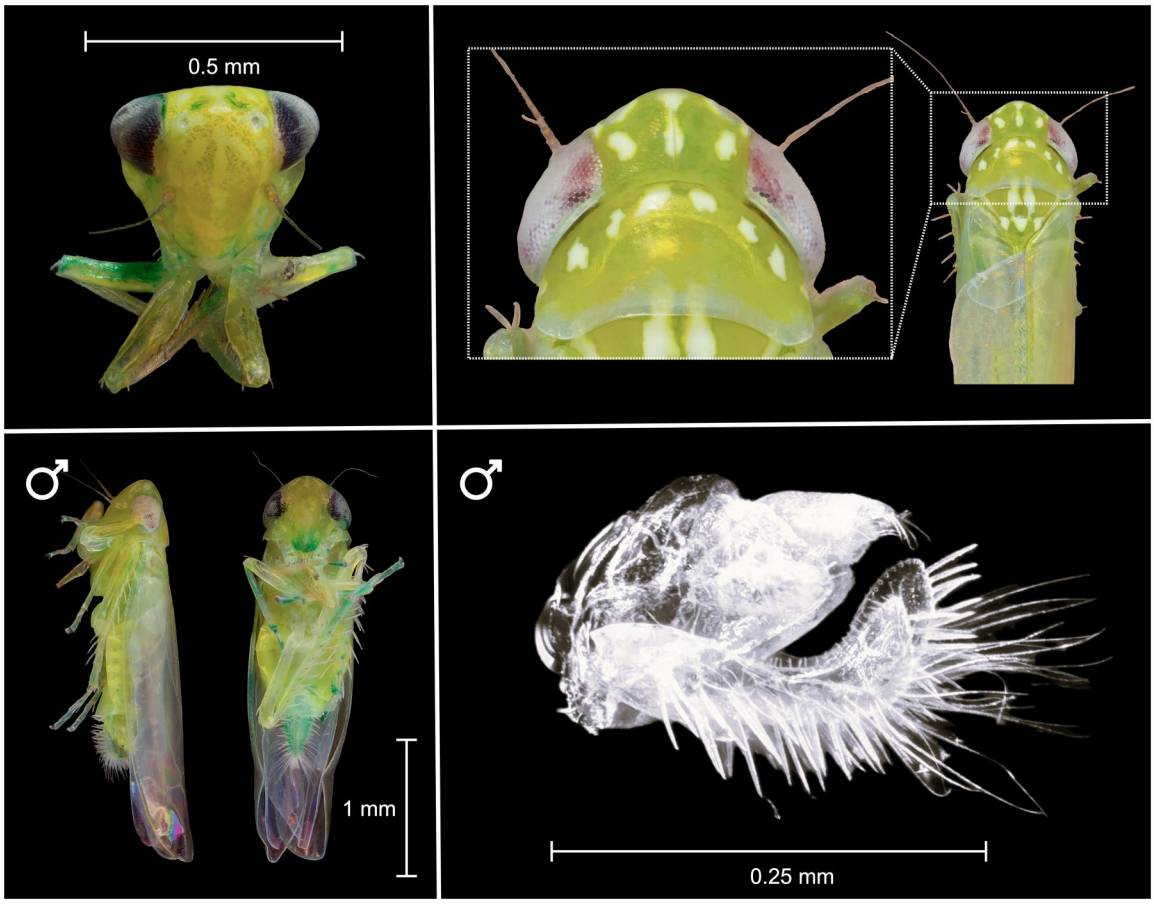

Figure 1. Dorsal and ventral view of *Empoasca fabae* males, with dissected male genitalia from a lateral view. Original pictures taken by Joshua Molligan of the specimens submitted to the Canadian national collection of insects, arachnids, and nematodes, under voucher numbers CNC2098398-2098407.

50, and entropyk = 5). Finally, reads shorter than 20 bp were then filtered and discarded.

Assembly and mitogenome identification

After preprocessing, the forward and reverse PE files were merged, totaling 142 M reads. A *de novo* assembly was performed using SPAdes (v.4.0.0), with an adjusted kmer size (21, 33, 55, 77, 99, and 127) (Prjibelski et al. 2020). The assembly used repeat resolution, mismatch careful mode, and a mismatch corrector to polish contigs. This produced a total of 2.5 M contigs, which were filtered for contigs $\geq$ 5 kb and $\geq$ 5x coverage, reducing the total to 1583 contigs.

The filtered contigs were blasted using NCBI BLAST (v.2.13.0) to identify top 10 matches with $>$ 80% identity (Altschul et al. 1990). Contigs were further filtered for $>$ 40% coverage to the queried nodes. The top matches were retrieved, and their accession numbers were then queried with edirect (v.14.6) to retrieve taxonomy data from the nucleotide database (NCBI 2024). Out of 41 candidate contigs, a contig of 14.8 kb showed 81.3% similarity to *Empoasca flavescens* strain As_1 mitochondrion, spanning 14.2 kb (Accession No. MK211224.1), with a coverage of 95.9% (E-value = 0.0). All concatenated raw reads were then remapped to the contig using BBMap (v.36.92).

Annotation

A hybrid annotation approach was used to find and validate the presence of all protein-coding genes (PCGs), ORFs, tRNAs, and rRNAs. Firstly, MITOS2 (Galaxy Version 2.1.9 + galaxy0) identified 13 PCGs, 22 tRNAs, two rRNA genes (suspected 16S and 12S) (Bernt et al. 2013). Seqtk (v.1.2) was then used to rearrange the contig based on the cluster of tRNAs before NAD2, specifically trnI, which possesses a GAT anticodon for isoleucine (Shen et al. 2016). Geneious (Dotmatics, USA, v.2024.0) was then used for visualization of the mitogenome (Figure 2), to plot open reading frames (ORFs) $>$ 300 bp, and elucidate base composition (Kearse et al. 2012).

Finally, Mfannot was then used to re-annotate the sequence, confirming 12 of the 13 CDS, with NAD6 being annotated as an ORF covering precisely the same region (Lang et al. 2019). To confirm tRNA predictions, tRNAscan-SE (v.2.0) was used with "Infernal" search mode and without the HMM filter, using invertebrate mitochondrial codes (Lowe and Eddy 1997). A score cutoff of 10 increased predicted tRNAs from 12 to 14 and lowering the cutoff to 1 increased total tRNA predictions to 19. Each predicted tRNA matched precisely those identified by MITOS2.

Phylogenetic analysis

For phylogenetic placement, a multisequence alignment of all 13 PCGs was performed. GenBank was queried using a refined

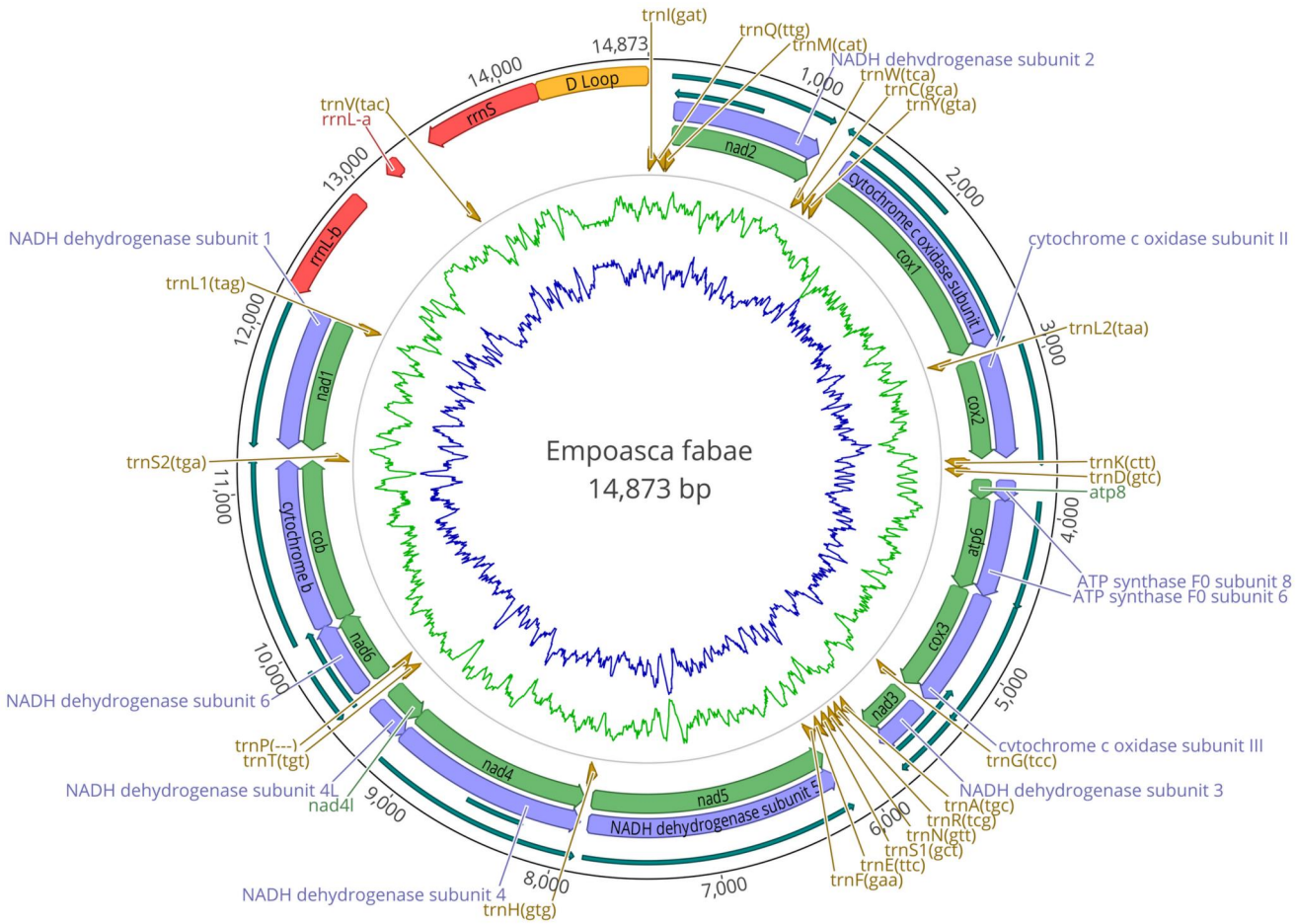

Figure 2. *Empoasca fabae* mitogenome annotation showing placement of all 13 PCGs in green and respective CDSs in purple, rRNAs in red, D Loop in orange, and ORFs in outermost blue lines.

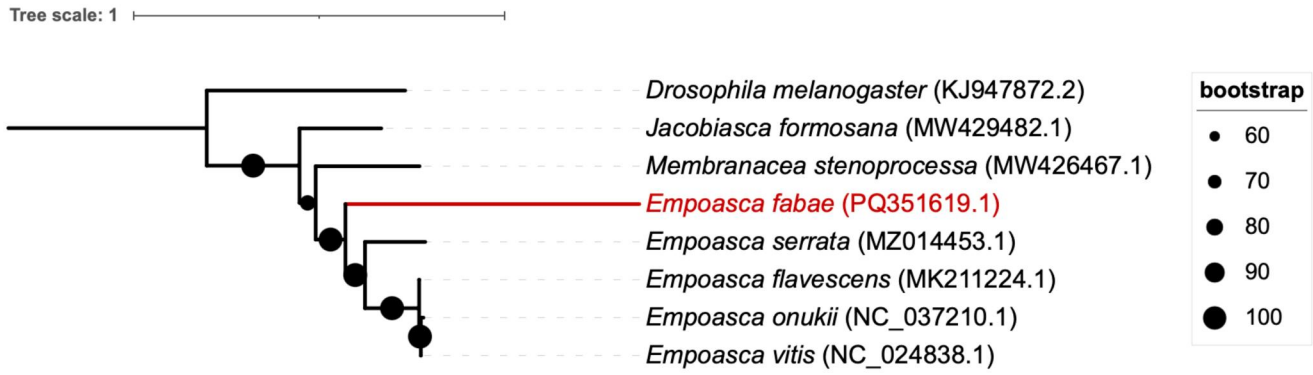

Figure 3. Phylogenetic placement amongst the *Empoasca* genues. The phylogenetic tree was constructed using the maximum likelihood method with individual best-fit models for each of the 13 mitochondrial PCGs. Bootstrap values emphasize the respective placement of leafhopper species. The following sequences were used: KJ947872.2 (unpublished), MW429482.1 (unpublished), and MW426467.1 (unpublished), MZ014453.1, (Lin et al. 2021), MK211224.1 (Lou et al. 2019), NC_037210.1 (Liu et al. 2017), and NC_024838.1, (Zhou et al. 2016). Further placement amongst all subfamilies is included in Supplementary figure S2 with the respective species name and literature references provided in Supplementary table S1.

Boolean search to include complete mitochondrial genomes of Cicadellidae subfamilies Aphrodinae, Cicadellinae, Coelidiinae, Deltocephalinae, Eurymelinae, Evacanthinae, Iassinae, Ledrinae, Megophthalminae, Neocoelidiinae, Nioniinae, and Typhlocybinae within the size range of 10,000–15,000 bp and published between 2010 and 2024 resulting in 153 species (Benson et al. 2013). All PCGs identified for *E. fabae* were then merged with the queried GenBank file and sorted for each mitochondrial PCG using "grep" and "awk" commands declaring all possible gene ID pattern combinations. Isolated genes were then translated using EMBOSS transeq (v.6.5.7) for their respective amino acid sequences, then aligned using MAFFT (v.7.453) and re-concatenated (Rice et al. 2000, Katoh and Standley 2013). Genes were then pooled based on their unique accession numbers, with files being omitted from further analysis if they did not contain all 13 PCGs or if sequences were identified as identical duplicates. This resulted in a total of 73 unique species from nine families, including Ledrinae,

Mileewini, Cicadellinae, Deltocephalinae, Eurymelinae, Evacanthinae, Iassinae, Nioniinae, and Typhlocybinae, as well as *Drosophila melanogaster* (Accession No. KJ947872.2) as outgroup (Supplementary Table S1). The aligned sequences were then analyzed using IQ-TREE (v.2.1.3) using the ModelFinder Plus parameter with limits set restricting model substations to mitochondrial codes (-msub mitochondrial). The consensus tree was then visualized with iTOL (Tree of Life Web Project, USA, v.2024.0) (Figure 3) (Letunic and Bork 2021; Nguyen et al. 2015). Branch support was assessed using maximum likelihood method, 1,000 bootstrap replicates and 1,000 approximate likelihood ratio test replicates to ensure robustness.

Results

The final mitochondrial genome of *E. fabae* was 14,873 bp in length (Figure 2), and a mean coverage of 1,689x coverage (Supplementary Figure S1). The genome (Genbank accession no. PQ351619) comprised 13 PCGs, 22 tRNAs, and two rRNAs, with a unique D-loop region (Table 1). Total base composition was A (38.8%), T (39.1%), C (11.7%), and G (10.4%). Nearly all PCGs begin with the start codon ATN, consisting of four ATTs, six ATGs, and one ATA. The only two PCGs without ATN codons were NAD5 and COX2, which began with the start codons TTG and GTG, respectively. All predicted PCGs from MITOS2 were confirmed with MFannot, and tRNA predictions were validated using MITOS2 and tRNAscan-SE.

After applying the best-fit partitioning scheme to the dataset, the alignment was divided into 13 genes with individual substitution models for each partition, such as the mtInv + F+R6 model for COX1 (Table 2). The consensus phylogenetic tree using invertebrate-specific, mitochondrial substation models confirmed the placement of *E. fabae* among leafhoppers within the Typhlocybinae subfamily. Additionally, the phylogenetic analysis showed that all members of genus *Empoasca* were clustered together, although a discrete phylogeny of *E. fabae* was shown (Figure 2, Table 2, Supplementary Figure S2).

Discussion and conclusions

The leafhopper genus *Empoasca* Walsh, 1862 has been previously reported to include eleven subgenera (Oman et al. 1990), with the subgenus *Empoasca* (*Empoasca*) grouping over 600 known species worldwide of which 177 have been reported in North America, 27 in Canada and only *E. bifurcata* DeLong, 1931, and *E. fabae* being present in Quebec (Dmitriev 2003). Surprisingly, only four complete mitogenomes of *Empoasca* species are currently available in GenBank: *E. serrata* Vilbaste, 1965; *E. flavescens* Fabricius, 1794; *E. vitis* Göthe, 1875; and *E. onukii* Matsuda, 1952, all

Table 1. Combined feature table comparing three annotators for the presence of all protein-coding genes and tRNA annotations, including their respective positions and start codons.

Feature	Position	Strand	Start codon/anticodon	MFannot	tRNAscan	Mitos
trnI (gat)	1–66	+	GAT (Ile)			✓
trnQ (ttg)	63–132	–	TTG (Gln)		✓	✓
trnM (cat)	133–201	+	CAT (Met)		✓	✓
nad2	171–1176	+	ATT	✓		✓
trnW (tca)	1174–1237	+	TCA (Trp)		✓	✓
trnC (gca)	1229–1290	–	GCA (Cys)		✓	✓
trnY (gta)	1290–1354	–	GTA (Tyr)		✓	✓
cox1	1355–2894	+	ATG	✓		✓
trnL2 (taa)	2889–2954	+	TAA (Leu)		✓	✓
cox2	2954–3636	+	GTG	✓		✓
trnK (ctt)	3633–3705	+	CTT (Lys)		✓	✓
trnD (gtc)	3706–3769	+	GTC (Asp)			✓
atp8	3787–3922	+	ATT			✓
atp6	3915–4566	+	ATG	✓		✓
cox3	4566–5346	+	ATG	✓		✓
trnG (tcc)	5346–5408	+	TCC (Gly)		✓	✓
nad3	5408–5762	+	ATT	✓		✓
trnA (tgc)	5760–5824	+	TGC (Ala)		✓	✓
trnR (tcg)	5825–5887	+	TCG (Arg)			✓
trnN (gtt)	5885–5950	+	GTT (Asn)		✓	✓
trnS1 (gct)	5949–6016	+	GCT (Ser)			✓
trnE (ttc)	6017–6077	+	TTC (Glu)			✓
trnF (gaa)	6076–6142	–	GAA (Phe)		✓	✓
nad5	6125–7817	–	TTG	✓		✓
trnH (gtg)	7814–7876	–	GTG (His)		✓	✓
nad4	7875–9189	–	ATG	✓		✓
nad4l	9182–9458	–	ATG	✓		✓
trnT (tgt)	9460–9523	+	TGT (Thr)		✓	✓
trnP (tgg)	9523–9585	–	TGG (Pro)			✓
nad6	9587–10076	+	ATT	✓		✓
cob	10068–11205	+	ATG	✓		✓
trnS2 (tga)	11203–11266	+	TGA (Ser)		✓	✓
nad1	11283–12201	–	ATA	✓		✓
trnL1 (tag)	12198–12262	–	TAG (Leu)		✓	✓
rrnL	12241–13260	–	–	✓		✓
trnV (tac)	13406–13470	–	TAC (Val)		✓	✓
rrnS	13470–14196	–	–	✓		✓

Table 2. Individual models determined for each protein-coding gene's amino acid sequence using the IQ-TREE ModelFinder.

Gene	Substitution model	Position range in partition
ATP6	mtInv + F+R5	1–684
ATP8	mtInv + F + I + G4	685–849
COX1	mtInv + F+R6	850–2409
COX2	mtInv + F + I + G4	2410–3096
COX3	mtInv + F+R5	3097–3891
CYTB	mtInv + F+R6	3892–5034
NAD1	mtInv + F+R5	5035–6126
NAD2	mtInv + F+R6	6127–7229
NAD3	mtInv + F+G4	7230–7592
NAD4	mtInv + F+R6	7593–9156
NAD4L	mtInv + F + I + G4	9157–9486
NAD5	mtInv + F+R6	9487–11455
NAD6	mtInv + F+R5	11456–12034

Note: Models were subsequently used for phylogenetic placement and candidate tree generation.

derived from specimens collected in China (Supplementary Table S1). Recent studies, based on morphology and phylogenetic placement, have proposed reclassifying Empoasca to include only New World species, excluding these Old-World species (Xu et al. 2021). Our analysis reveals that the mitogenomes of *E. serrata*, *E. flavescens*, *E. onukii*, and *E. vitis* exhibit distinct phylogenetic relationships branching from *E. fabae* supporting Xu et al. (2021) proposition. Furthermore, the branch length, PCG similarity, and placement of *E. onukii* and *E. vitis* in the phylogenetic tree corroborate previous reports suggesting they represent a single species (Figure 2; Qin et al. 2015; Fu et al. 2014).

In this study, we successfully sequenced and annotated the complete mitochondrial genome of *E. fabae* Harris, 1841. This is the first mitogenome for the species, but also the first mitochondrial genome available for a species in the *Empoasca* genus from Canada, North America and the Nearctic region. This study paves the way for more detailed genomic analyses that can improve our understanding of the evolutionary relationships within the *Empoasca* genus and contribute to pest management strategies in agriculture.

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J.M. Conceptualization, Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. J.J. Sample collection, Writing - review & editing. S.M. Conceptualization, Writing - review & editing., and E.P.L. Conceptualization, Resources, Supervision, Writing - original draft, Writing - review & editing.

Author contribution

CRediT: **Joshua Molligan**: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing; **Jordanne Jacques**: Investigation, Resources, Writing – review & editing; **Soham Mukhopadhyay**: Software, Visualization, Writing – review & editing.

Ethical approval

No specific permits were required for the insect specimens collected for this study. The field studies did not involve endangered or protected species. The insect species sequenced is a common leafhopper species in Canada and is not included in the 'List of Protected Animals in Canada'.

Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The genome sequence data that supports the findings of this study are openly available in GenBank of NCBI at https://www.ncbi.nlm.nih.gov/ under the accession no. PQ351619.1. The associated BioProject, SRA, and BioSample numbers are PRJNA1160200, SRR30817348, and SAMN43663934, respectively.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol. 215(3):403–410. doi:10.1016/S0022-2836(05)80360-2.

Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW. 2013. GenBank. Nucleic Acids Res. 41(Database issue):D36–D42. doi:10.1093/nar/gks1195.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved de novo metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69(2):313–319. doi:10.1016/j.ympev.2012.08.023.

Bushnell B. 2024. BBTools: A Suite of Fast, Multithreaded Bioinformatics Tools Designed for Analyzing and Manipulating Sequence Data. DOE Joint Genome Institute. [Accessed 2024 Aug 15]. https://jgi.doe.gov/data-and-tools/bbtools/.

Chasen EM, Dietrich C, Backus EA, Cullen EM. 2014. Potato leafhopper (Hemiptera: cicadellidae) ecology and integrated pest management focused on alfalfa. J Integ Pest Manage. 5(1):1–8. doi:10.1603/IPM13014.

Dmitriev DA. 2003. *Subgenus Empoasca (Empoasca) Walsh, 1862*. 3I Interactive Keys and Taxonomic Databases. Retrieved September 14, 2024, from http://dmitriev.speciesfile.org/3i_keys.asp.

Fu JY, Han BY, Xiao Q. 2014. Mitochondrial COI and 16sRNA Evidence for a Single Species Hypothesis of E. vitis, J. formosana and E. onukii in East Asia. PLoS One. 9(12):e115259. doi:10.1371/journal.pone.0115259.

Katoh K, Standley DM. 2013. MAFFT multiple sequence alignment software Version 7: improvements in performance and usability. Mol Biol Evol. 30(4):772–780. doi:10.1093/molbev/mst010.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28(12):1647–1649. doi:10.1093/bioinformatics/bts199.

Lang BF, Laforest M-J, Burger G. 2019. MFannot: Automated annotation of mitochondrial and plastid genomes. http://megasun.bch.umontreal.ca/RNAweasel/.

Letunic I, Bork P. 2021. Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. Nucleic Acids Res. 49(W1):W293–W296. doi:10.1093/nar/gkab301.

Lin S, Huang M, Zhang Y. 2021. Structural features and phylogenetic implications of 11 new mitogenomes of Typhlocybinae (Hemiptera: Cicadellidae). Insects. 12(8):678. doi:10.3390/insects12080678.

Liu J-H, Sun C-Y, Long Ju, Guo J-J. 2017. Complete mitogenome of tea green leafhopper, Empoasca onukii (Hemiptera: Cicadellidae) from Anshun, Guizhou Province in China. Mitochondrial DNA B Resour. 2(2):808–809. doi:10.1080/23802359.2017.1398616.

Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25(5):955–964. doi:10.1093/nar/25.5.955.

Luo X, Chen Y, Chen C, Pu D, Tang X, Zhang H, Lu D, Mao J. 2019. Characterization of the complete mitochondrial genome of *Empoasca*

sp. (Cicadellidae: Hemiptera). Mitochondrial DNA Part B. 4(1):1477–1478. doi:10.1080/23802359.2019.1579066.

National Center for Biotechnology Information (NCBI). 2024. EDirect: E-utilities on the UNIX command line (v.14.6). https://www.ncbi.nlm.nih.gov/books/NBK179288/.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 32(1):268–274. doi:10.1093/molbev/msu300.

Oman PW, Knight WJ, Nielson MW. 1990. Leafhoppers (Cicadellidae): a bibliography, generic checklist and index to the world literature 1956–1985. London, UK: CAB International Institute of Entomology. p. 368.

Plante N, Durivage J, Brochu A-S, Dumonceaux T, Almeida Santos A, Torres D, Bahder B, Kits J, Dionne A, Légaré J-P, et al. 2024. Leafhoppers as markers of the impact of climate change on agriculture. Cell Reports Sustain. 1(2):100029. doi:10.1016/j.crsus.2024.100029.

Prjibelski A, Antipov D, Meleshko D, Lapidus A, Korobeynikov A. 2020. Using SPAdes de novo assembler. Curr Protoc Bioinformatics. 70(1):e102. doi:10.1002/cpbi.102.

Qin D, Zhang L, Xiao Q, Dietrich C, Matsumura M. 2015. Clarification of the identity of the tea green leafhopper based on morphological comparison between Chinese and Japanese specimens. PLoS One. 10(9):e0139202. doi:10.1371/journal.pone.0139202.

Rice P, Longden I, Bleasby A. 2000. EMBOSS: the European molecular biology open software suite. Trends Genet. 16(6):276–277. doi:10.1016/S0168-9525(00)02024-2.

Santos AA, Jacques J, Plante N, Fournier V, Pérez-López E. 2024a. Leafhoppers as vectors of phytoplasma diseases in Canadian berry crops: a review in the face of climate change. Ann Entomol Soci Am. 117(1):14–20. doi:10.1093/aesa/saad038.

Santos AA, Jacques J, Pérez-López E. 2024b. Potential impact of climate change on Nearctic leafhopper distribution and richness in North America. Npj Sustain Agric. 2(1):12. doi:10.1038/s44264-024-00020-6.

Shen W, Le S, Li Y, Hu F. 2016. SeqKit: a cross-platform and ultrafast toolkit for FASTA/Q file manipulation. PLoS One. 11(10):e0163962. doi:10.1371/journal.pone.0163962.

Xu Y, Dietrich CH, Zhang Y, Dmitriev DA, Zhang L, Wang Y, Lu S, Qin D. 2021. Phylogeny of the tribe Empoascini (Hemiptera: Cicadellidae: Typhlocybinae) based on morphological characteristics, with reclassification of the Empoasca generic group. Syst Entomol. 46(1):266–286. doi:10.1111/syen.12461.

Zhou N, Wang M, Cui L, Chen X, Han B. 2016. Complete mitochondrial genome of *Empoasca vitis* (Hemiptera: Cicadellidae). Mitochondrial DNA A DNA Mapp Seq Anal. 27(2):1052–1053. doi:10.3109/19401736.2014.928863.