Characterization of the complete mitochondrial genome of the New Zealand parasitic blowfly *Calliphora vicina* (Insecta: Diptera: Calliphoridae)

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ABSTRACT

In this study, the complete mitochondrial (mt) genome of the New Zealand parasitic blowfly *Calliphora vicina* (blue bottle fly) field strain NZ_CalVic_NP was generated using next-generation sequencing technology and annotated. The 16,518 bp mt genome consists of 13 protein-coding genes, two ribosomal RNAs, 22 transfer RNAs, and a 1689 bp non-coding region, similar to the two other available *C. vicina* and most metazoan mt genomes. Phylogenetic analysis showed that *C. vicina* NZ_CalVic_NP forms a monophyletic cluster with the remaining three Calliphorinae species. The complete mt genome sequence of *C. vicina* NZ_CalVic_NP is a resource to facilitate future species- and strain-level identification research and investigations into the evolutionary provenance within the Calliphoridae.

The diminished efficacy demonstrated by current members of the Calliphoridae (blowflies) treatments due to the emergence of resistance in blowflies against many classes of insecticides calls for improved DNA-based diagnostics tools. High-level phylogenetic relationships within the Calliphoridae are still largely unresolved primarily due to their large and highly variable mitochondrial (mt) genomes. *Calliphora vicina* (Robineau-Desvoidy, 1830) NZ_CalVic_NP, was selected for genome sequencing as a representative of an NZ field strain of *C. vicina*.

The *C. vicina* specimen was collected from the Palmerston North area (40°21.3’S, 175°36.7’E), and is stored and available upon request from AgResearch Ltd., Grasslands Research Center (accession number: NPY120886). High molecular weight genomic DNA was isolated from entire *C. vicina* adult males using a modified phenol:chloroform protocol explained in our previous articles (Palevich, Kelly, Ganesh, et al. 2019; Palevich, Kelly, Leahy, et al. 2019; Palevich et al. 2019b). Genomic DNA was prepared for whole-genome sequencing using an Illumina TruSeq Nano library preparation kit (Illumina, Inc., San Diego, CA) according to the manufacturer’s instructions. The Illumina NovaSeq™ 6000 (PE150, Novogene, Beijing, China) platform was used to amplify the entire mt genome sequence. The mt genome was assembled and annotated using the NOVOPlasty pipeline version 3.1 with default parameters (Dierckxsens et al. 2016; Palevich and Maclean 2021), as previously described (Palevich and Maclean 2019a; Palevich, Maclean, Mitreva, et al. 2019; Palevich et al. 2020).

The length of complete mt genome is 16,518 bp, with the overall 77.8% AT content (BioProject ID: PRJNA667961, GenBank accession number: MW266392). The overall nucleotide distribution for the mt genome is 39.5% A, 13.0% C, 9.2% G, and 38.1% T. The structure of the mt genome is typical of insect mt genomes (Cameron 2014) which consists of 13 protein-coding genes, 22 transfer RNAs, and two ribosomal RNAs. Among these 37 genes, 23 genes encoded on the majority strand while remaining 14 genes encoded on the minority strand. There are eight more complete mt genomes recorded belong to the genus Calliphora (*C. vicina*, *C. vomitoria*, *C. nigribarbis*, and *C. chinghaiensis*) (Nelson et al. 2012; Chen et al. 2016; Ren et al. 2016; Karagozlu et al. 2019). In comparison, the reported *C. vicina* NZ_CalVic_NP has the longest complete mt genome and the size difference with the shortest record is 1249 bp (*C. chinghaiensis*). The main reason for the size difference is the control region. The entire ‘control region’ that is non-coding and AT-rich lies between the 12S rRNA and tRNA-Ile in insect mt genomes, and this area in the *C. vicina* NZ_CalVic_NP is 1689 bp in length which is the longest among all Calliphora records.

The phylogenetic position of *C. vicina* NZ_CalVic_NP within the family Calliphorinae was estimated using maximum-likelihood, implemented in RAxML version 8.2.11 (Stamatakis 2014), and the Bayesian inference (BI), implemented in MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001) approaches using default settings. For analysis, the phylogenetic tree was reconstructed using the complete mitogenome sequences of available blowfly species and isolates retrieved from GenBank with the 13 concatenated mt PCGs genes (Figure 1). *Calliphora vomitoria* and *C. nigribarbis* are sister to *C. vicina* and together with *C. chinghaiensis* are monophyletic. Overall, the phylogenetic topology is similar to previous studies (Chen et al. 2016), suggesting that the genus Calliphora is monophyletic. This study provides additional complete and curated mitogenome data for the
nm genome library of the genus Calliphora. Such a resource will be used in future comparative studies investigating the evolutionary provenance of Calliphora by exploring species- and strain-level diversity.

Disclosure statement

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

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Figure 1. A summary of the molecular phylogeny of the Calliphoridae complete mitochondrial genomes. The evolutionary relationship of C. vicina field strain NZ_CalVic_NP (black circle) was compared to the complete mitochondrial genomes of 68 blowfly species or isolates retrieved from GenBank (accession numbers in parentheses) and nucleotide sequences of all protein-coding genes were used for analysis. Phylogenetic analysis was conducted using the Bayesian approach implemented in MrBayes version 3.2.6 (Huelsenbeck and Ronquist 2001) and maximum likelihood (ML) using RAxML version 8.2.11 (Stamatakis 2014). The mtREV with Freq. (+F) model was used for amino acid substitution and four independent runs were performed for 10 million generations and sampled every 1000 generaitions. For reconstruction, the first 25% of the sample was discarded as burnin and visualized using Geneious Prime (Kearse et al. 2012). Nodal support is given: Bayes posterior probabilities/RAxML bootstrap percentage. The phylogram provided is presented to scale (scale bar = 0.05 estimated number of substitutions per site) with the species Haematobia irritans from the family Muscidae used as the outgroup.

Data availability statement

Mitogenome data supporting this study are openly available in GenBank at: https://www.ncbi.nlm.nih.gov/nuccore/MW266392. Associated BioProject, SRA, and BioSample accession numbers are https://www.ncbi.nlm.nih.gov/bioproject/PRJNA667961, SRR13038367, and SAMN16745529, respectively.

References

Cameron SL. 2014. How to sequence and annotate insect mitochondrial genomes for systematic and comparative genomics research. Syst Entomol. 39(3):400–411.

Chen Y, Shi X, Li D, Chen B, Zhang P, Wu N, Xu Z. 2016. The complete nucleotide sequence of the mitochondrial genome of Calliphora ching-haiensis (Diptera: Calliphoridae). Mitochondrial DNA Part B. 1(1):397–398.

Dierckxsens N, Mardulyn P, Smits G. 2016. NOVOPlasty: de novo assembly of organelle genomes from whole genome data. Nucleic Acids Res. 45:e18.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 17(8):754–755.
Karagozlu MZ, Kim JI, Park SH, Shin SE, Kim CB. 2019. The complete mitochondrial genome of a blowfly Calliphora nigribarbis (Vollenhoven, 1863) (Insecta: Diptera: Calliphoridae). Mitochondrial DNA Part B. 4(2):2318–2319.

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.

Nelson LA, Lambkin CL, Batterham P, Wallman JF, Dowton M, Whiting MF, Yeates DK, Cameron SL. 2012. Beyond barcoding: a mitochondrial genomics approach to molecular phylogenetics and diagnostics of blowflies (Diptera: Calliphoridae). Gene. 511(2):131–142.

Palevich N, Kelly WJ, Ganesh S, Rakonjac J, Attwood GT. 2019. Butyrivibrio hungatei MB2003 competes effectively for soluble sugars released by Butyrivibrio proteoclasticus B316 during growth on xylan or pectin. Appl Environ Microbiol. 85(3):e02056–e02018.

Palevich N, Kelly WJ, Leahy SC, Denman S, Altermann E, Rakonjac J, Attwood GT. 2019. Comparative genomics of rumen Butyrivibrio spp. uncovers a continuum of polysaccharide-degrading capabilities. Appl Environ Microbiol. 86(1):e01993–19.

Palevich N, Maclean PH. 2021. Sequencing and reconstructing helminth mitochondrial genomes directly from genomic next-generation sequencing data. In Parasite Genomics Protocols. Humana Press, New York, NY.

Palevich N, Maclean P, Baten A, Scott R, Leathwick DM. 2019a. The complete mitochondrial genome of the New Zealand parasitic roundworm Haemonchus contortus (Trichostrongyloidea: Haemonchidae) field strain NZ_Hco_NP. Mitochondrial DNA Part B. 4(2):2208–2210.

Palevich N, Maclean PH, Baten A, Scott RW, Leathwick DM. 2019b. The genome sequence of the anthelmintic-susceptible New Zealand Haemonchus contortus. Genome Biol Evol. 11(7):1965–1970.

Palevich N, Maclean PH, Choi Y-J, Mitreva M. 2020. Characterization of the complete mitochondrial genomes of two sibling species of parasitic roundworms, Haemonchus contortus and Teladorsagia circumcincta. Front Genet. 11(1066):573395.

Palevich N, Maclean PH, Mitreva M, Scott R, Leathwick D. 2019. The complete mitochondrial genome of the New Zealand parasitic roundworm Teladorsagia circumcincta (Trichostrongyloidea: Haemonchidae) field strain NZ_Teci_NP. Mitochondrial DNA Part B. 4(2):2869–2871.

Ren L, Guo Q, Yan W, Guo Y, Ding Y. 2016. The complete mitochondrial genome of Calliphora vomitoria (Diptera: Calliphoridae). Mitochondrial DNA Part B. 1(1):378–379.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 30(9):1312–1313.