

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

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6 **ABSTRACT**

7 In the present study, the complete mitochondrial genome of the New Zealand parasitic blowfly  
8 *Calliphora vicina* (blue bottle blowfly) field strain NZ\_CalVic\_NP was generated using next-  
9 generation sequencing technology and annotated. The 16,518 bp mitochondrial genome  
10 consists of 13 protein-coding genes, two ribosomal RNAs, 22 transfer RNAs, and a 1,689 bp  
11 non-coding region, similar to most metazoan mitochondrial genomes. Phylogenetic analysis  
12 showed that *C. vicina* NZ\_CalVic\_NP does not form a monophyletic cluster with the remaining  
13 three Calliphorinae species. The complete mitochondrial genome sequence of *C. vicina*  
14 NZ\_CalVic\_NP is a resource to facilitate future species identification research within the  
15 Calliphoridae.

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17 **KEYWORDS:** Diptera, Calliphoridae, Calliphorinae, complete mitochondrial genome,  
18 *Calliphora vicina*.

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

25 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 Centre  
26 (accession number: NPY120886). High molecular weight genomic DNA was isolated from  
27 entire *C. vicina* adult males using a modified phenol:chloroform protocol explained in our  
28 previous articles (Palevich et al. 2019a; Palevich et al. 2019b; Palevich et al. 2019d). The  
29 Illumina NovaSeq™ 6000 (PE150, Novogene, China) platform was used to amplify the entire  
30 mitochondrial genome sequence. The mitochondrial genome was assembled and annotated as  
31 previously described (Palevich et al. 2019c; Palevich et al. 2019e; Palevich et al. 2020).

33 The length of complete mitochondrial genome is 16,518 bp, with the overall 77.8% AT content  
34 (BioProject ID: PRJNA667961, GenBank accession number: MW123003). The overall  
35 nucleotide distribution for the mitochondrial genome is 39.5 % A, 13.0 % C, 9.2 % G, and 38.1  
36 % T. The structure of the mitochondrial genome is typical of insect mitochondrial genomes  
37 (Cameron 2014) which consists of 13 protein-coding genes, 22 transfer RNAs, and 2 ribosomal  
38 RNAs. Among these 37 genes, 23 genes encoded on the majority strand while remaining 14  
39 genes encoded on the minority strand. There are eight more complete mitochondrial genomes  
40 recorded belong to the genus Calliphora (*C. vicina*, *C. vomitoria*, *C. nigribarbis* and *C.*  
41 *chinghaiensis*) (Nelson et al. 2012; Chen et al. 2016; Ren et al. 2016; Karagozlu et al. 2019).  
42 In comparison, the reported *C. vicina* NZ\_CalVic\_NP has the longest complete mitochondrial  
43 genome and the size difference with the shortest record is 1,249 bp (*C. chinghaiensis*). The  
44 main reason for the size difference is the control region. The entire ‘control region’ that is non-  
45 coding and AT-rich lies between the 12S rRNA and tRNA-Ile in insect mitochondrial genomes,  
46 and this area in the *C. vicina* NZ\_CalVic\_NP is 1,689 bp in length which is the longest among  
47 all Calliphora records.

48 The phylogenetic position of *C. vicina* NZ\_CalVic\_NP within the family Calliphorinae was  
49 estimated using maximum-likelihood, implemented in RAxML version 8.2.11 (Stamatakis

50 2014), and the Bayesian inference (BI), implemented in MrBayes version 3.2.6 (Huelsenbeck  
51 et al. 2001) approaches using default settings.

52 For analysis, the phylogenetic tree was reconstructed using the complete mitogenome  
53 sequences of available blowfly species and isolates retrieved from GenBank with the 13  
54 concatenated mitochondrial PCGs and rRNA genes (Figure 1). *Calliphora vomitoria* was the  
55 most related species with *C. nigribarbis* and *C. chinghaiensis*. Overall, the phylogenetic  
56 topology is similar to previous studies (Chen et al. 2016), suggesting that the genus Calliphora  
57 is not monophyletic. This study provides additional complete mitogenome data for the  
58 improvement and future investigation of the Calliphoridae phylogeny.

## 59 **Disclosure statement**

60 No potential conflict of interest was reported by the authors.

## 61 **Data availability statement**

62 The data that support the findings of this study are openly available in GenBank of NCBI at  
63 <https://www.ncbi.nlm.nih.gov>, reference number MW123003.

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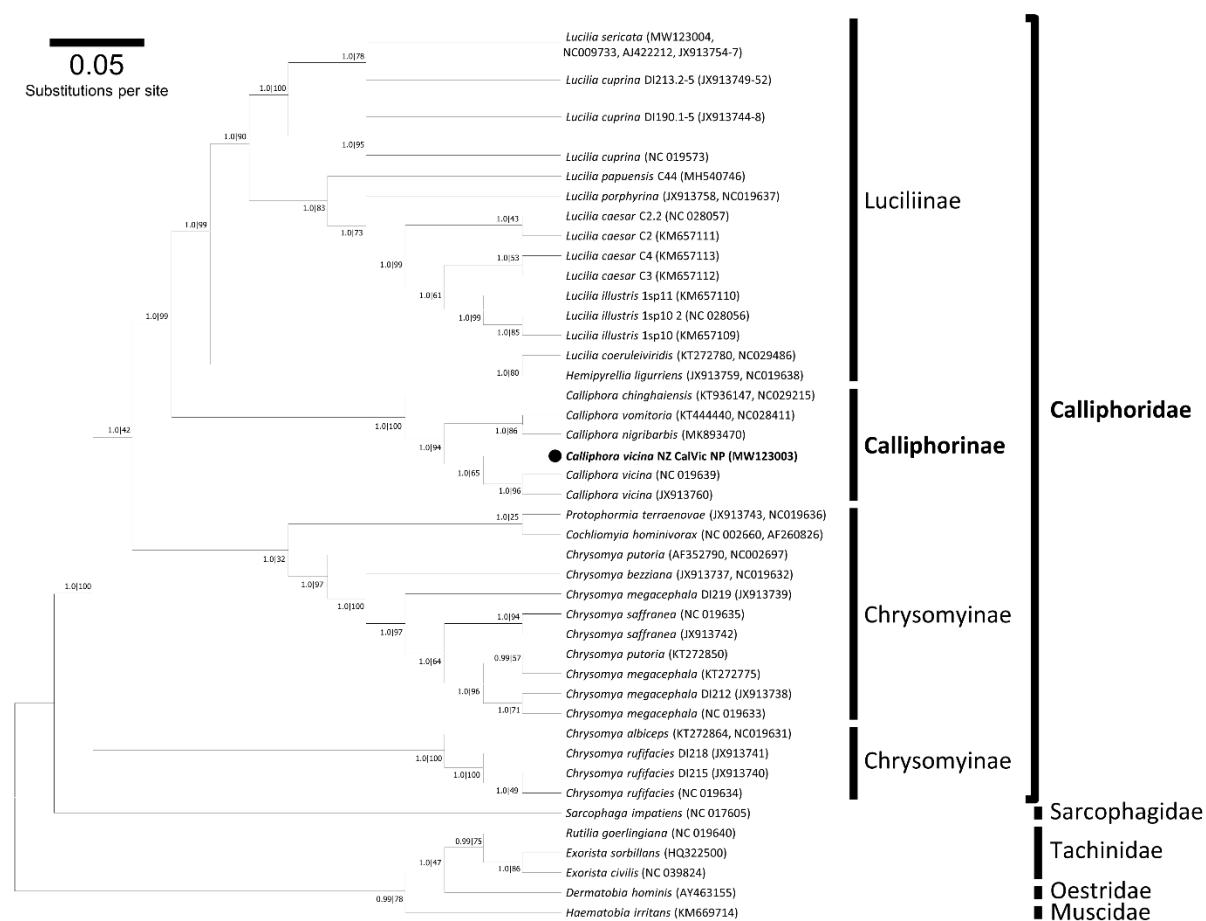
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**Figure 1.** A summary of the molecular phylogeny of the Calliphoridae complete mitochondrial genomes. The evolutionary relationship of *C. vicina* field strain NZ\_CalVic\_NP (black circle) was compared to the complete mitochondrial genomes of 68 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 et al. 2001) and maximum likelihood (ML) using RAxML version 8.2.11 (Stamatakis 2014). The mtREV with Freqs. (+F) model was used for amino acid substitution and four independent runs were performed for 10 million generations and sampled every 1,000 generations. 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.