Bacterial Diversity Profiling of the New Zealand Parasitic Blowfly 
*Lucilia sericata* Based on 16S rRNA Gene Amplicon Sequencing

**ABSTRACT** Here, we present a 16S rRNA gene amplicon sequence data set and profiles demonstrating the bacterial diversity of larval and adult *Lucilia sericata*, collected from Ashhurst, New Zealand (May 2020). The two dominant genera among adult male and female *L. sericata* were *Serratia* and *Morganella* (phylum Proteobacteria), while the larvae were also dominated by the genera *Lactobacillus*, *Carnobacterium*, and *Lactococcus* (phylum Firmicutes).

Members of Calliphoridae (blowflies) are economically important for medical and veterinary management worldwide (1). Larvae of this fly invade their animal host, feed on tissues and excretions, and progressively cause severe skin disease, commonly referred to as flystrike (myiasis) (2, 3). Currently, control relies heavily on the prophylactic application of long-acting chemicals to all sheep, but this approach is increasingly under threat due to the development of resistance to current treatments. *Lucilia sericata* NZ_LucSer_NP (4) was selected for microbiome assessment as a representative of a New Zealand field strain of *L. sericata*. In this study, we investigated the bacterial microbiomes of *L. sericata* larvae, adult males, and adult females to gain a better understanding of the microbial communities and especially symbionts to blowflies that could lead to entirely novel treatments against flystrike and blowfly control.

The *L. sericata* specimen larvae were collected from a farm site in the Ashhurst area in New Zealand (40°18′S, 175°45′E). Species identification and rearing of the blowflies on beef liver as a protein source and a 10% sugar solution were done according to Dear (5). Lab-reared separate pools of larval, adult male, and adult female *L. sericata* blowflies were washed twice in sterile phosphate-buffered saline (PBS; pH 7.4) to remove surface-adherent bacteria, snap-frozen in liquid nitrogen, and transferred to −80°C storage prior to DNA extraction. High-molecular-weight genomic DNA was isolated from *L. sericata* pooled samples of 100 larvae as well as 10 entire adult males and females per replicate (*n* = 5 for each). Genomic DNA was prepared for metagenomic 16S rRNA gene amplicon sequencing of the V3-V4 hypervariable region using a modified phenol-chloroform protocol recently described for complex samples, such as parasitic roundworms (6, 7), fastidious anaerobic rumen bacteria (8–10), and spore-forming psychrotolerant *Clostridium* sp. isolated from spoiled meat (11, 12). A DNA library was prepared using the 16S V3-V4 rRNA library preparation method (Illumina, Inc., San Diego, CA) according to the manufacturer’s instructions (13) and sequenced on the Illumina MiSeq platform with the 2 × 250-bp paired-end (PE) reagent kit v2, producing a total of 5,208,027 PE raw reads.

The processing of the amplicon reads followed a modified version of the pipeline described in reference 14. The reads produced by the sequencing instrument were paired using the program FLASH2 v2.2.00 (15). Paired reads were then quality trimmed using Trimmomatic v0.38 (16). The trimmed reads were reformatteed as fasta, and the read headers were modified to include the sample name. All reads were compiled into a single file, and Mothur v1.45.2 (17) was used to remove reads with homopolymers longer than 10 nucleotides (nt) and to collapse the reads into unique representatives. The collapsed reads...
were clustered using Swarm v2 (18). The clustered reads were filtered based on their abundance, keeping representatives that were (i) present in one sample with a relative abundance of >0.1%, (ii) present in >2% of the samples with a relative abundance of >0.01%, or (iii) present in 5% of the samples at any abundance level. The selected representatives were annotated using QIIME 2 v2017.4 (19) with the SILVA database v138 (20). The annotated

TABLE 1 Details of all Lucilia sericata samples used in this study and information for sequencing reads

| Samplea | Life cycle stage | No. of raw reads | No. of quality-filtered reads | SRA accession no. |
|---------|------------------|------------------|-------------------------------|-------------------|
| Adult_Male_1 | Adult male | 437,598 | 437,584 | SRR13779722 |
| Adult_Male_2 | Adult male | 391,709 | 391,686 | SRR13779721 |
| Adult_Male_3 | Adult male | 372,187 | 372,165 | SRR13779720 |
| Adult_Male_4 | Adult male | 342,606 | 342,588 | SRR13779719 |
| Adult_Male_5 | Adult male | 442,342 | 442,324 | SRR13779718 |
| Adult_Female_1 | Adult female | 389,860 | 389,841 | SRR13779716 |
| Adult_Female_2 | Adult female | 376,696 | 376,677 | SRR13779715 |
| Adult_Female_3 | Adult female | 422,959 | 422,938 | SRR13779714 |
| Adult_Female_4 | Adult female | 359,511 | 359,497 | SRR13779713 |
| Adult_Female_5 | Adult female | 298,290 | 298,284 | SRR13779712 |
| L3_Larvae_1 | Larvae L3 | 326,910 | 326,904 | SRR13779711 |
| L3_Larvae_2 | Larvae L3 | 270,953 | 270,946 | SRR13779710 |
| L3_Larvae_3 | Larvae L3 | 290,495 | 290,492 | SRR13779709 |
| L3_Larvae_4 | Larvae L3 | 276,269 | 276,265 | SRR13779708 |
| L3_Larvae_5 | Larvae L3 | 209,642 | 209,638 | SRR13779707 |

a All samples were collected in March 2020 from the Ashhurst area in New Zealand (40°18′S, 175°45′E).
tables were then used for downstream statistical analysis. Sample and sequence data are summarized in Table 1.

In all samples, the predominant phylum was Proteobacteria (Fig. 1) and the predominant genera were Serratia and Morganella, while the larvae were also dominated by Lactobacillus, Carnobacterium, and Lactococcus (phylum Firmicutes). The metagenomic 16S rRNA gene amplicon sequencing of L. sericata field strain NZ_LucSer_NP reported here is a valuable resource for future studies investigating the role of bacteria in flystrike. In order to improve the phylogenetic resolution of the microbial community structures and improve our knowledge of flystrike caused by L. sericata, future efforts should focus on the generation of amplicon sequencing data from numerous locations around New Zealand and across a wider range of blowfly species (21).

Data availability. The 16S rRNA gene amplicon sequence data have been deposited in the GenBank Sequence Read Archive (SRA) under the BioProject accession number PRJNA667961.

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REFERENCES

1. Githeko AK, Lindsay SW, Confalonieri UE, Patz JA. 2000. Climate change and vector-borne diseases: a regional analysis. Bull World Health Organ 78:1136–1147.

2. Hall M, Wall R. 1995. Myiasis of humans and domestic animals. Adv Parasitol 35:257–334. https://doi.org/10.1016/s0065-308x(08)60073-1.

3. Fischer O, Matlova L, Dvorska L, Svastova P, Bartl J, Weston R, Pavlik I. 2004. Blowflies Calliphora vicina and Lucilia sericata as passive vectors of Mycobacterium avium subs. avium, M. a. paratuberculosis and M. a. hominisuis. Med Vet Entomol 18:116–122. https://doi.org/10.1111/j.0269-283X.2004.00477.x.

4. Palevich N, Carvalho L, Maclean P. 2021. The complete mitochondrial genome of the New Zealand parasitic blowfly Lucilia sericata (Insecta: Diptera: Calliphoridae). Mitochondrial DNA Part B 6:2127–2129. https://doi.org/10.1080/23802359.2021.1906774.

5. Dear JP. 1986. Calliphoridae (Insecta: Diptera): Fauna of New Zealand 888.

6. Palevich N, Maclean PH, Baten A, Scott RW, Leathwick DM. 2019. The genome sequence of the anathelminc-susceptible New Zealand Haemonchus contortus. Genome Biol Evol 11:1970–1979. https://doi.org/10.1093/gbe/evz141.

7. 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 11:573395. https://doi.org/10.3389/fgene.2020.573395.

8. Palevich N, Kelly WJ, Ganesh S, Rakonjac J, Attwood GT. 2018. Butyrivibrio hungatei MB2003 competes effectively for soluble sugars released by Butyrivibrio proteoclasticus B316 during growth on xylan or pectin. Appl Environ Microbiol 85:e02056-18. https://doi.org/10.1128/AEM.02056-18.

9. 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:e01993. https://doi.org/10.1128/AEM.01993-19.

10. Palevich N, Maclean PH, Kelly WJ, Leahy SC, Rakonjac J, Attwood GT. 2020. Complete genome sequence of the polysaccharide-degrading rumen bacterium Pseudobutyrivibrio xylanivorans MA3014 reveals an incomplete glycolytic pathway. Genome Biol Evol 12:1566–1572. https://doi.org/10.1093/gbe/eva165.

11. Palevich N, Palevich FP, Maclean PH, Altermann E, Gardner A, Burgess S, Mills J, Brightwell G. 2019. Comparative genomics of Clostridium species associated with vacuum-packed meat spoilage. Food Microbiol 95:103687. https://doi.org/10.1016/j.fm.2020.103687.

12. Palevich N, Palevich FP, Maclean PH, Jauregui R, Altermann E, Mills J, Brightwell G. 2019. Draft genome sequence of Clostridium estertheticum subsp. laramiensis DSM 14864T, isolated from spoiled uncooked beef. Microbiol Resour Announc 8:e01275-19. https://doi.org/10.1128/MRA.01275-19.

13. Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl Environ Microbiol 79:5112–5120. https://doi.org/10.1128/AEM.01493-13.

14. Camarinha-Silva A, Jáuregui R, Pieper DH, Wos-Oxley ML. 2012. The temporal dynamics of bacterial communities across human anterior nares. Environ Microbiol Rep 4:126–132. https://doi.org/10.1111/j.1758-2229.2011.00313.x.

15. Magoc T, Salzberg SL. 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27:2957–2963. https://doi.org/10.1093/bioinformatics/btq507.

16. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

17. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7573–7541. https://doi.org/10.1128/AEM.01541-09.

18. Mahé F, Rognes T, Quince C, de Vargas C, Dunthorn M. 2014. Swarm: robust and fast clustering method for amplicon-based studies. PeerJ 2:e593. https://doi.org/10.7717/peerj.593.

19. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, CostaLO, Fierer N, Pena AG, Goodrich JK, Gordon JJ, Huttenlocher GA, Kelley ST, Knights D, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. https://doi.org/10.1038/nmeth.f.303.

20. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2012. The SILVA ribosomal RNA gene database project: improved data processing and Web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219.

21. Palevich N, Carvalho L, Maclean P. 2021. Characterization of the complete mitochondrial genome of the New Zealand parasitic blowfly Calliphora vicina (Insecta: Diptera: Calliphoridae). Mitochondrial DNA Part B 6:1270–1272. https://doi.org/10.1080/23802359.2021.1906775.