







Draft Whole-Genome Sequences of *Campylobacter* Strains Isolated from Brushtail Possums in New Zealand

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ABSTRACT Draft genomes of five *Campylobacter* isolates recovered from New Zealand brushtail possums are described. Genome sizes ranged from 1.591 Mbp to 1.594 Mbp, with G+C contents of 29.9% to 29.95%. Comparison to Australian *Campylobacter* 16S rRNA gene sequences suggests that the species may be common to possums.

In the 1850s (1), the Australian brushtail possum (*Trichosurus vulpecula*; Maori, *paihamu*) was first introduced to New Zealand, where it rapidly became a significant invasive pest (1). Although *Campylobacter* spp. have been described in the Australian brushtail possum population (2, 3), they have not previously been isolated from the New Zealand population (4, 5). We hypothesized that brushtail possums play a role in the epidemiology of *Campylobacter* spp. in New Zealand, contributing to the contamination of waterways and other environments (6).

Swabs from the cecum, intestine, or feces were taken from road-killed possums or possums killed as routine pest control in an urban or perirural setting (Palmerston North, New Zealand). Swabs were cultured on cefoperazone amphotericin teicoplanin (CAT) agar (Fort Richard Laboratories, Auckland, New Zealand) in an H₂-enriched microaerobic atmosphere at 37°C, and colonies typical of *Campylobacter* spp. were seen after 3 days. Single colonies were subcultured on Columbia horse blood agar (Fort Richard Laboratories) and grown under the same conditions for genomic DNA preparation. Genomic DNA was extracted using a QIAamp DNA minikit (Qiagen, Hilden, Germany). DNA was checked for quality using Qubit assay kits (Life Technologies, Oregon, USA) and for fragmentation using gel electrophoresis. Genomic DNA was sequenced at New Zealand Genomics, Ltd. (Massey University, Palmerston North, New Zealand), using either an Illumina MiSeq or Illumina HiSeq 2500 instrument (Scoresby, Victoria, Australia) according to the manufacturer's instructions with paired-read lengths of 250 and 150 bp, respectively. Sequence data were trimmed using Trimmomatic v.0.3.8 (7) (trim parameters, 1:30:11 LEADING:10 TRAILING:10 MINLEN:30), assembled using SKESA v.2.2.1 (8) using the default settings, and further processed and annotated online by the NCBI Prokaryotic Genome Annotation Pipeline (9). Relevant sequencing, assembly, and genome statistics are described in Table 1.

Genomes ranged in size from 1,591,228 bp to 1,594,282 bp with between 1,659 and 1,671 predicted coding sequences. The G+C contents were between 29.9% and 29.95%. All genomes had single copies of 5S, 16S, and 23S rRNA gene sequences and 33 identifiable tRNAs. The 16S rRNA sequences from all isolates were identical and showed the closest BLAST similarity to *Campylobacter* isolate BTP1Tcr (GenBank accession number [AY554142](https://www.ncbi.nlm.nih.gov/nucl/AY554142)), with a pairwise sequence identity of 99.6% over 1,427 bp. This sequence was obtained from a study of Australian brushtail possums (2), which identified both *Helicobacter* and *Campylobacter* carriage. This species of *Campy-*

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TABLE 1 *Campylobacter* sp. genome statistics

Strain	Length (bp)	No. of contigs	N ₅₀ (bp)	Coverage (×)	G+C		NCBI assembly accession no.	GenBank accession no.	SRA accession no.	Sequence type	Raw sequences (Mbp)
					No. of CDSs ^a	content (%)					
LR185c	1,591,228	55	64,039	101	1,659	29.95	GCA_008633905	VJNR000000000	SRR9678926	MiSeq 2 × 250 bp	160.4
LR196d	1,592,443	41	117,332	463	1,665	29.91	GCA_008633865	VJNS000000000	SRR9678927	HiSeq 2 × 150 bp	736.9
LR264d	1,593,663	40	129,728	381	1,671	29.9	GCA_008633895	VJNT000000000	SRR9678928	HiSeq 2 × 150 bp	606.6
LR286c	1,592,793	47	78,517	414	1,671	29.92	GCA_008633875	VJNU000000000	SRR9678929	HiSeq 2 × 150 bp	659.2
LR291e	1,594,282	39	129,696	455	1,668	29.92	GCA_008633915	VJNV000000000	SRR9678925	HiSeq 2 × 150 bp	725.7

^a CDSs, coding DNA sequences.

lobacter possibly forms an association with the gastrointestinal tract of possums. Comparison with representative 16S rRNA sequences from all other *Campylobacter* species showed that the closest similarity was to *Campylobacter helveticus* (98.7% identity, NCBI assembly accession numbers [GCF_002080395](#) and [GCF_900176295](#)), with similar observed levels of identity with *Campylobacter upsaliensis* (98% to 98.4% identity, [GCA_000167395](#), [GCA_000185345](#), and [GCA_000620965](#)) and *Campylobacter avium* (98.2% identity, [GCA_002238335](#) and [GCA_002245935](#)).

Data availability. *Campylobacter* genomes from this article are submitted under BioProject accession number [PRJNA552733](#) and BioSample accession numbers [SAMN12216776](#) through [SAMN12216780](#), with GenBank accession numbers [VJNR000000000](#), [VJNS000000000](#), [VJNT000000000](#), [VJNU000000000](#), and [VJNV000000000](#), corresponding to SRA accession numbers [SRR9678926](#), [SRR9678927](#), [SRR9678928](#), [SRR9678929](#), and [SRR9678925](#), respectively.

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REFERENCES

- Lawton C, Cown P, Bertolino S, Lurz PWW, Peters AR. 2010. The consequences of introducing non-indigenous species: two case studies, the grey squirrel in Europe and the brushtail possum in New Zealand. *Rev Sci Tech* 29:287–298. <https://doi.org/10.20506/rst.29.2.1983>.
- Coldham T, Rose K, O'Rourke J, Neilan BA, Dalton H, Lee A, Mitchell H. 2011. Detection, isolation, and characterization of *Helicobacter* species from the gastrointestinal tract of the brushtail possum. *Appl Environ Microbiol* 77:1581–1587. <https://doi.org/10.1128/AEM.01960-10>.
- Ahmed W, Hodggers L, Sidhu JPS, Toze S. 2012. Fecal indicators and zoonotic pathogens in household drinking water taps fed from rainwater tanks in Southeast Queensland, Australia. *Appl Environ Microbiol* 78:219–226. <https://doi.org/10.1128/AEM.06554-11>.
- Devane ML, Nicol C, Ball A, Klena JD, Scholes P, Hudson JA, Baker MG, Gilpin BJ, Garrett N, Savill MG. 2005. The occurrence of *Campylobacter* subtypes in environmental reservoirs and potential transmission routes. *J Appl Microbiol* 98:980–990. <https://doi.org/10.1111/j.1365-2672.2005.02541.x>.
- Phiri BJ. 2015. Estimating the public health risk associated with drinking water in New Zealand. PhD thesis. Massey University, Palmerston North, New Zealand.
- Shrestha RD, Midwinter AC, Marshall JC, Collins-Emerson JM, Pleydell EJ, French NP. 27 September 2019. *Campylobacter jejuni* associated with wild birds and those causing human disease in six high-use recreational waterways in New Zealand. *Appl Environ Microbiol*. <https://doi.org/10.1128/AEM.01228-19>.
- 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>.
- Souvorov A, Agarwala R, Lipman DJ. 2018. SKESA: strategic k-mer extension for scrupulous assemblies. *Genome Biol* 19:153. <https://doi.org/10.1186/s13059-018-1540-z>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.