Complete *de novo* assembly of *Wolbachia* endosymbiont of contemporary *Drosophila simulans* using long-read genome sequencing.

Running Title: *De novo* assembly of *Wolbachia* in *Drosophila simulans*

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Authorship order was determined based on contributions to data generation and analysis. For authors with equal contributions, ties were resolved alphabetically by first name.

**Abstract**
We present a contemporary high-quality, complete *de novo* assembly of *Wolbachia pipientis* (*w*Ri Merrill 23, CP199298), an alphaproteobacterial endosymbiont of *Drosophila simulans*. This assembly was generated using long read sequencing of *w*Ri-infected *D. simulans* embryos collected from the Merrill College at the University of California, Santa Cruz in October 2023.

*Wolbachia pipientis* infects diverse arthropods and nematodes, manipulating host phenotypes through cytoplasmic incompatibility (CI), male killing, and fertility rescue [1,2]. The Riverside strain (*w*Ri) was first identified in California *Drosophila simulans* in the 1980s [3] and rapidly spread statewide due to exceptionally strong CI [4]. Despite its significance in shaping *D. simulans* populations [5], a modern *w*Ri genome has not been assembled and the existing reference genome reflects the *w*Ri present in 1984 [3,6]. Here, we present a complete *de novo* *w*Ri genome assembly from contemporary *D. simulans* collected at the UC Santa Cruz Alan Chadwick Garden, located in Merrill College, in October 2023 (CP199298), providing an updated reference for future studies.

To generate a contemporary *w*Ri genome assembly, we collected wild *D. simulans* flies, established isofemale lines, and performed long-read DNA sequencing of *w*Ri-infected embryos. We established isofemale lines by deploying banana-baited bottles for ~5 days and collecting gravid females onto white food medium. After offspring eclosed, species identity was confirmed by phenotyping males and by PCR using silf-F/R primers to distinguish *D. simulans* from *D. melanogaster* [7] and wsp_1F/592R primers to confirm *w*Ri identity [8]. We extracted DNA from *w*Ri-infected embryos using the Wizard HMW DNA Extraction Kit (Promega #A2920, Lot: 0000575812) and prepared libraries with the Native Barcoding Kit V14 (SQK-NBD114-24, Lot: NDP1424.10.0010). We sequenced these libraries on the Nanopore MinION Mk1B with a R10 version flow cell (FLO-MIN-114, Lot: 11004365) and MinKNOW v23.07.8 with adaptive sampling (fast model) to deplete *D. simulans* reads (GCF_016746395.2), yielding 5.4M reads after 20 hours that were subsequently basecalled with Dorado (v0.7.3, hac model). After filtering for host-free reads >3kb, we assembled the *w*Ri genome using Flye [9] following Jacobs and Nakamoto *et al.* (2024) [10], yielding a 1.26 Mb circular assembly with 30x coverage.

To polish the assembly, we generated Illumina short-read whole-genome sequencing data from whole *w*Ri-infected *D. simulans* flies (Merrill 23 stocks). Illumina libraries were prepared using the Tn5 protocol [11] and sequenced on a NovaSeqX Plus. We polished the assembly with Pilon [12] v1.24 using short reads following Jacobs and Nakamoto *et al.* (2024). We assessed the quality of the polished assembly with BUSCO [13] (v5.7.0, rickettsiales_odb10), which achieved a completeness score of 99.2%, annotated the assembly with Prokka [14] (v1.1.1, kingdom:bacteria) to identify coding sequences (CDS), tRNAs, rRNAs, and ncRNA (Table 1) and calculated and visualized GC content and GC skew with Proksee [15] v1.1.2 (Figure 1). Default parameters were used unless otherwise specified.

Raw sequencing reads and the assembled genome are available under BioProject accession number PRJNA1312834. Analysis scripts are available at https://github.com/jodiejacobs/Jacobs_et_al_2026_de_novo_wRi_merrill_23_assembly.

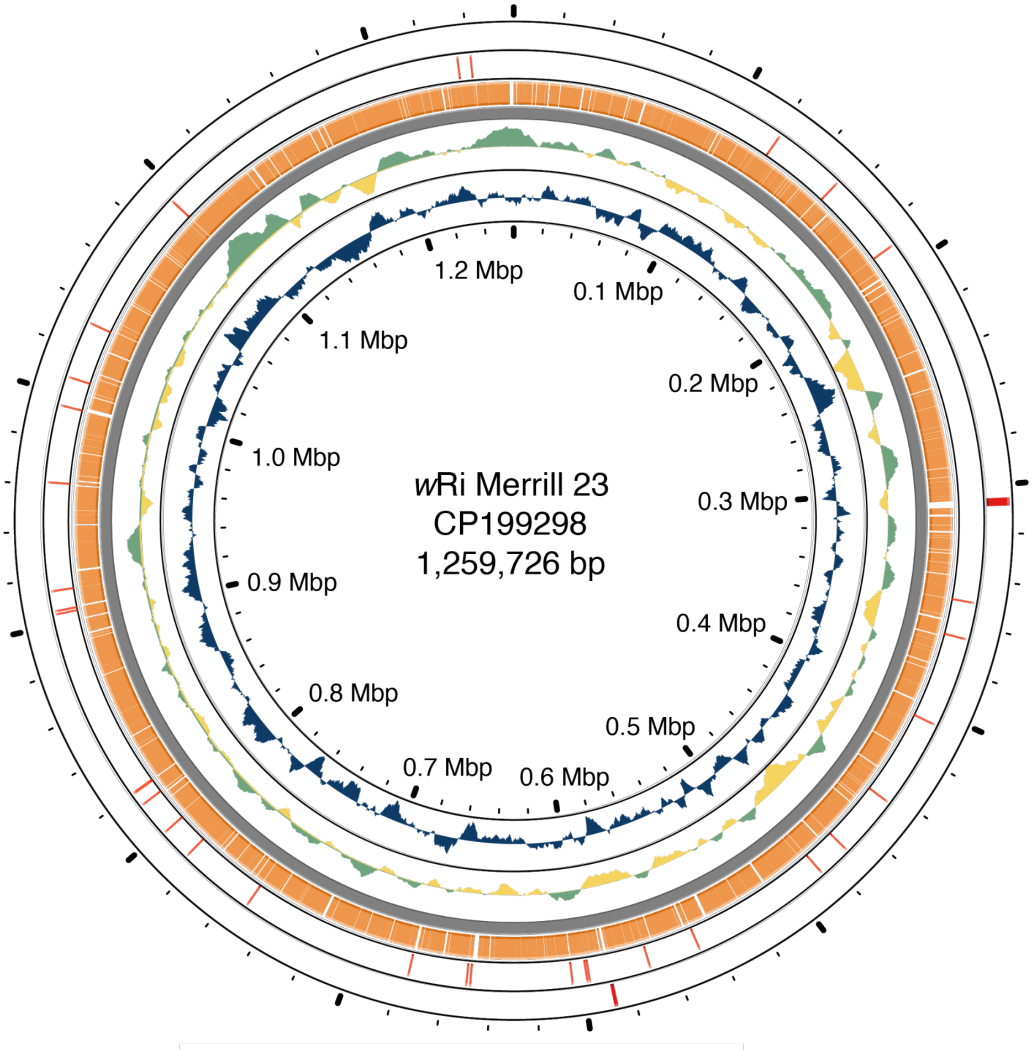


**Figure 1. *Wolbachia* *w*Ri genome map.** Concentric circles show (outer to inner): rRNA genes (red), tRNA genes (red), coding sequences (orange), GC skew (green/yellow for high/low), and GC content (blue), with GC metrics plotted as deviations from genome-wide average.

| ***w*Ri Merrill Annotation summary** | |
|---|---|
| Annotation pipeline | Prokka v1.1.1 |
| Annotation method | kingdom:bacteria |
| Length (bp) | 1,259,726 |
| GC Content | 35.22% |

| | |
|---|---|
| Genes (total) | 1,283 |
| CDSs (total) | 1,246 |
| Genes (RNA) | 37 |
| rRNAs | 1, 1, 1 (5S, 16S, 23S) |
| tRNAs | 34 |
| ncRNAs | 0 |
| Pseudogenes (total) | 3 |

Table 1. Annotation summary statistics.

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