Additional file 1

Parameter exploration improves the accuracy of genome assemblies

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Supplementary text

CompareGenomeQualities tool usage

Our tool makes use of multiple programming languages and bioinformatics software [1]. To facilitate usage, we provide a bash script that can install the required dependencies using Bioconda [2]. We also provide our tool and all its dependencies as a Docker image [3]. Below, we first provide an overview of our tool's command-line parameters. We then present example usage of the tool.

Overview of command-line parameters

```
-b, --busco-lineage     One of the 193 BUSCO v5 datasets listed here:
                        https://busco-data.ezlab.org/v5/data/lineages. The
                        dataset is automatically downloaded. Dataset name
                        can be a partial match e.g., insecta instead of
                        insecta_odb10.2020-09-10.tar.gz. Required unless
                        --rank-only is specified.
-g, --genome-size       Expected or estimated genome size in base pairs.
                        Required unless --rank-only is specified.
-1, --illumina-R1       Forward Illumina reads. Required unless --rank-only
                        is specified.
-2, --illumina-R2       Reverse Illumina reads. Required unless --rank-only
                        is specified.
-n, --num-cpus          Used for read mapping and BUSCO steps. Default: 1.
-o, --output-dir        Output directory. Not applicable if --rank-only is
                        specified. Default: /mnt/compare-genome-qualities-
                        yyyy-mm-dd-hhmmss.
--rank-only             Don't compute metrics. Only rank assemblies based
                        on tabular files in the given directory.
-h, --help              View this message docker run
```

Example usage of the tool

There are two ways to run the tool. The default behavior is to run the tool on a series of genome assemblies, providing a set of Illumina reads as additional input. This will compute the assembly quality metrics NG50, BUSCO score, resolved length, and solid Illumina read pairs and subsequently rank the assemblies.

```
compare-genome-qualities.sh -g 450000000 -b insecta_odb9 -1
illumina_R1.fq.gz -2 illumina_R2.fq.gz assembly_1.fa assembly_2.fa
assembly_3.fa
```

Alternatively, our tool can be used to rank genome assemblies based on pre-computed metrics. The pre-computed metrics are presented to the tool in form of tabular files, one file per metric, each file containing one line per assembly indicating the assembly identifier and the value of the metric for that assembly, separated by the tab character.

```
compare-genomes-qualities.sh --rank-only dir_containing_tabular_files
```

Comparison of Canu, flye, and wtdbg2 genome assemblers

Our tool does not require the genome assemblies to be generated using different parameter combinations. For example, we present a comparison of the assemblies generated by three different long-read genome assembly tools: Canu [4], flye [5], and wtdbg2 [6] (Table S5). All assemblies were generated using default parameters of the assembly software. We removed unresolved haplotigs from Canu assembly [7] to get a better sense of resolved assembly lengths, but we did not polish any of the assemblies. In this example, wtdbg2 generated the most contiguous assembly. However, the assembly generated by Canu had the most resolved regions (13 Mb more than the next best) and considerably higher proportion of solidly mapped Illumina reads (57.62% compared to 55.25% of the runner up), followed by Flye. The 0.01% difference in the BUSCO score [8] of Canu and Flye assemblies is minor and likely to be eliminated by subsequent polishing steps. These results validate our choice of using Canu for

assembly parameter optimization and further highlight the benefits of testing different assembly software for a given dataset.

Quality control of Illumina reads

We filtered and trimmed Illumina datasets prior to use. First, we removed optical duplicates using clumpify.sh (version 37) [9]. Second, we removed reads with mean quality threshold lower than 15 using htqc [10]. Third, we compared the mean base quality per cycle, per tile to the mean base quality of that cycle across all tiles to test for air-bubbles becoming trapped in the flow cell [11]. For this, we obtained the difference between per-cycle mean base quality for a tile and the per-cycle mean base quality for all tiles from FastQC's text output (version 0.11.5) [12]. Where this difference was greater than 4, we changed the corresponding base in the reads to 'N'. This was done by creating a BED file of positions from the tile and cycle information and then using seqtk (version 1.2) [13] to convert bases at the positions specified in the file. Next, we considered that reads with multiple occurrences of low-quality bases may be problematic. To eliminate such reads, we turned bases with quality scores lower than 12 to 'N' using seqtk (reads with excessive Ns are removed in the next step). Finally, we used cutadapt (version 1.13) [14] to trim adapter sequences, to trim low quality bases from both the 3' and 5' ends, to trim any leading and trailing 'N's, to eliminate after trimming reads shorter than 50 bp and those with more than 4 'N's. For the Illumina sequences used for assembly comparison, we retained 64,850,542 pairs of 50-150 bp reads (*i.e.*, 79.23% of sequenced bases) after filtering.

Supplementary figures

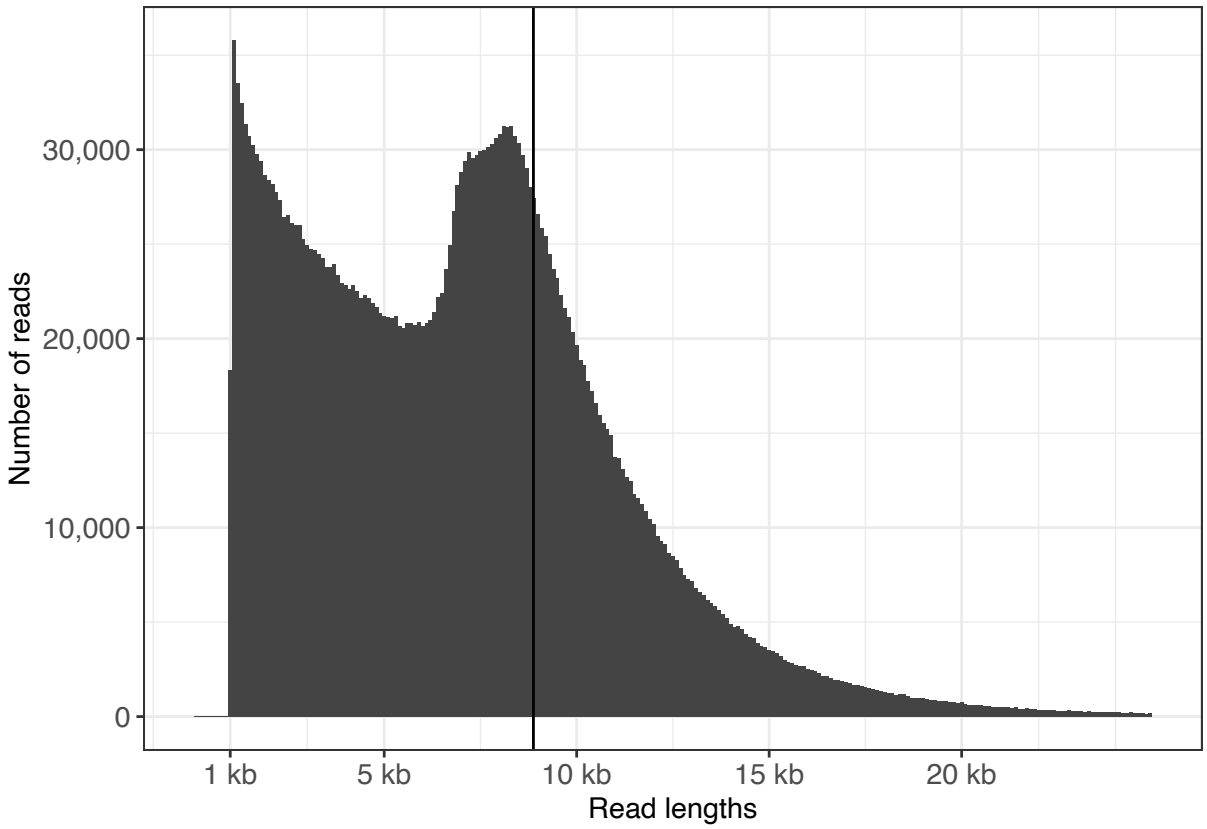


Figure S1: Histogram of lengths of raw Pacbio reads. The black vertical line shows N50 read length (8,876 bp).

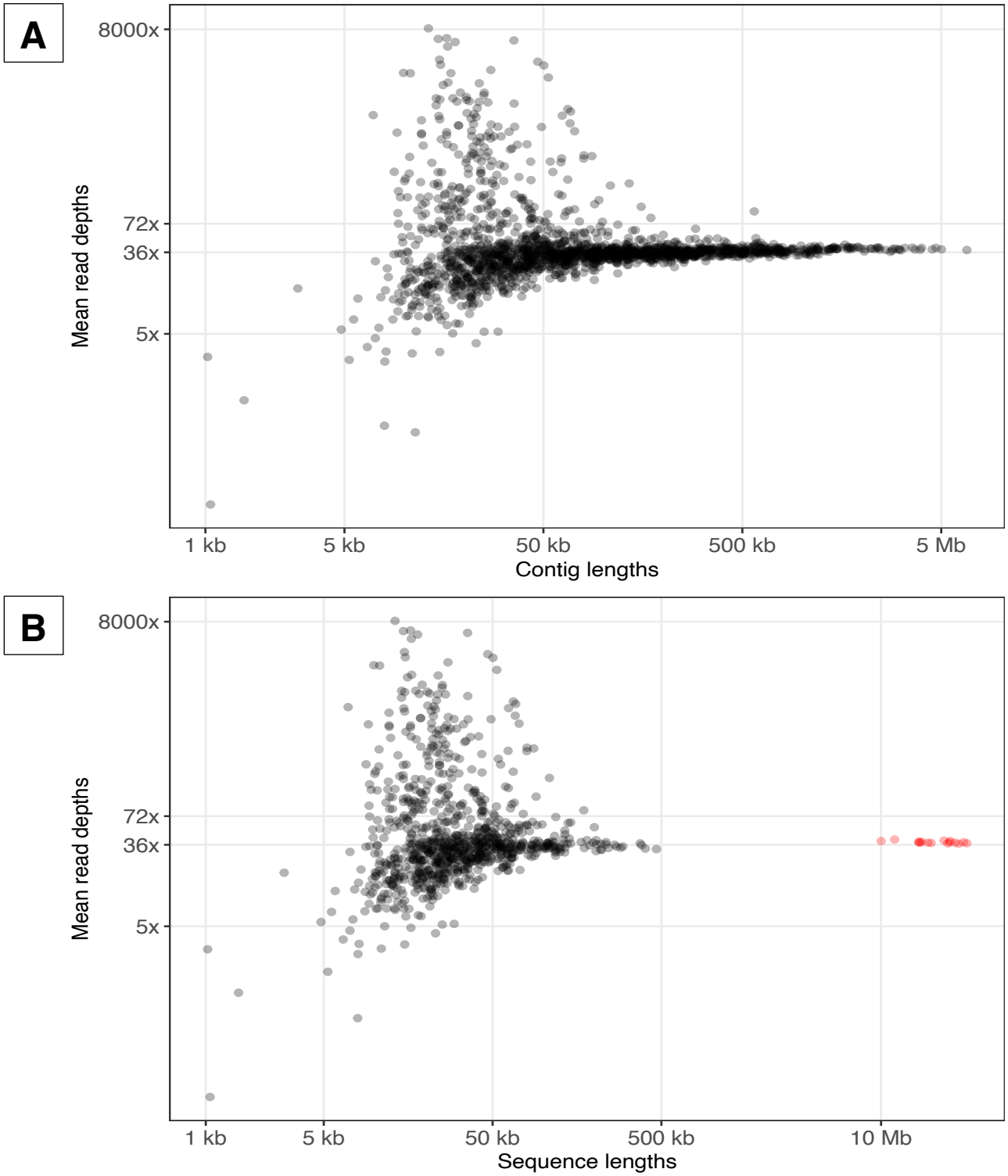


Figure S2: Lengths of the assembled sequences of the best assembly (x axis) and their average read depths (y axis) A) before scaffolding B) and after. In panel B, the sequences longer than 10 Mb (colored red) are the chromosomes, while the cloud of sequences on the left are unplaced contigs. Axes are log scaled. Pacbio reads were mapped to the best assembly using minimap2 (version 2.17; -a -x map-pb) [15]. Read depth of contigs were

calculated using mosdepth (version 0.2.6; -x -n) [16]. Contigs with average depth higher than twice the median coverage (36x) are likely to contain collapsed representation of highly repetitive regions of the genome. Contigs with average depth lower than 5x are likely to contain higher amounts of sequencing error. This is because the SMRTLinks polishing step, which is critical for Canu assemblies, excludes regions with coverage lower than 5x.

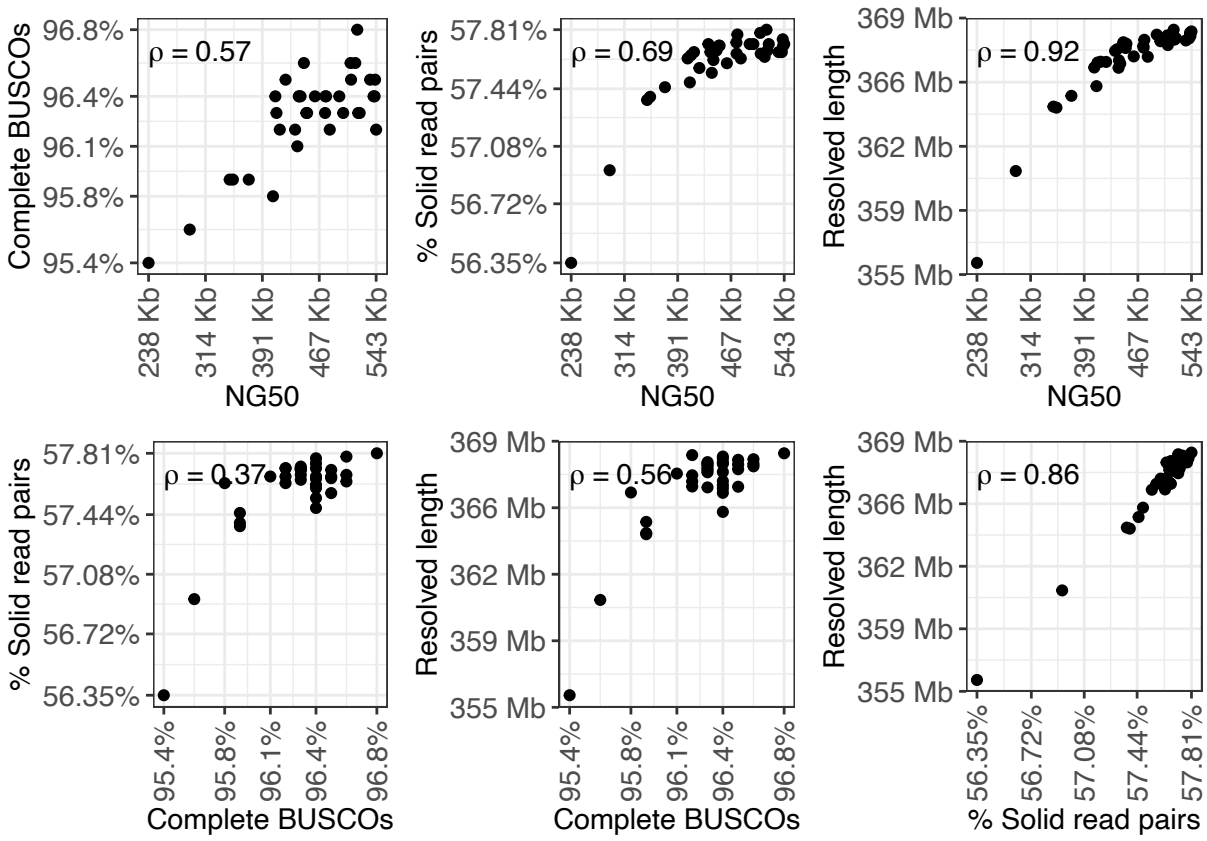


Figure S3: Correlations between the four metrics of genome quality: NG50, BUSCO score, resolved length, and solid Illumina read pairs. Each panel shows the values taken by a pair of metrics on the x and the y axes, and Spearman's rank correlation coefficient (ρ) between the metrics. To account for the general correlation across metrics, the overall ranking of assemblies performed by CompareGenomeQualities is weighted by the complement of the average pairwise correlations (Fig 1).

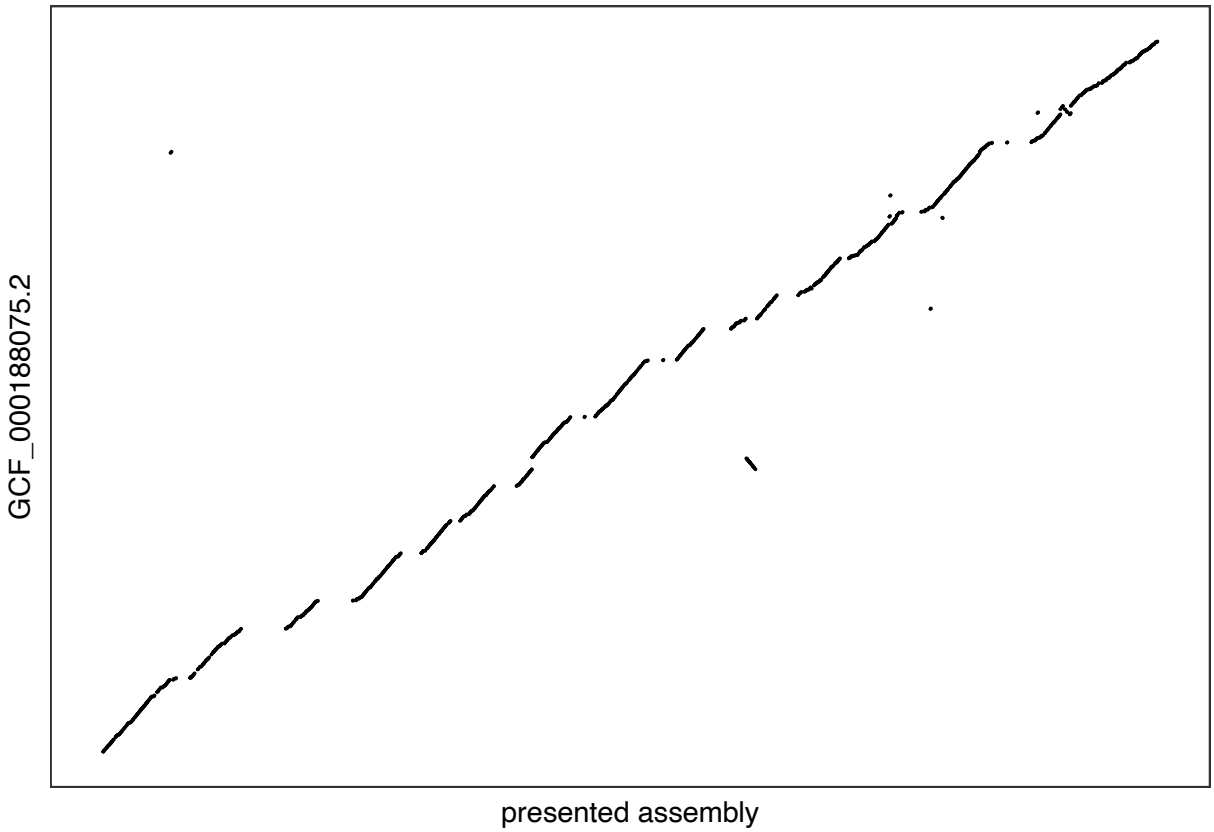
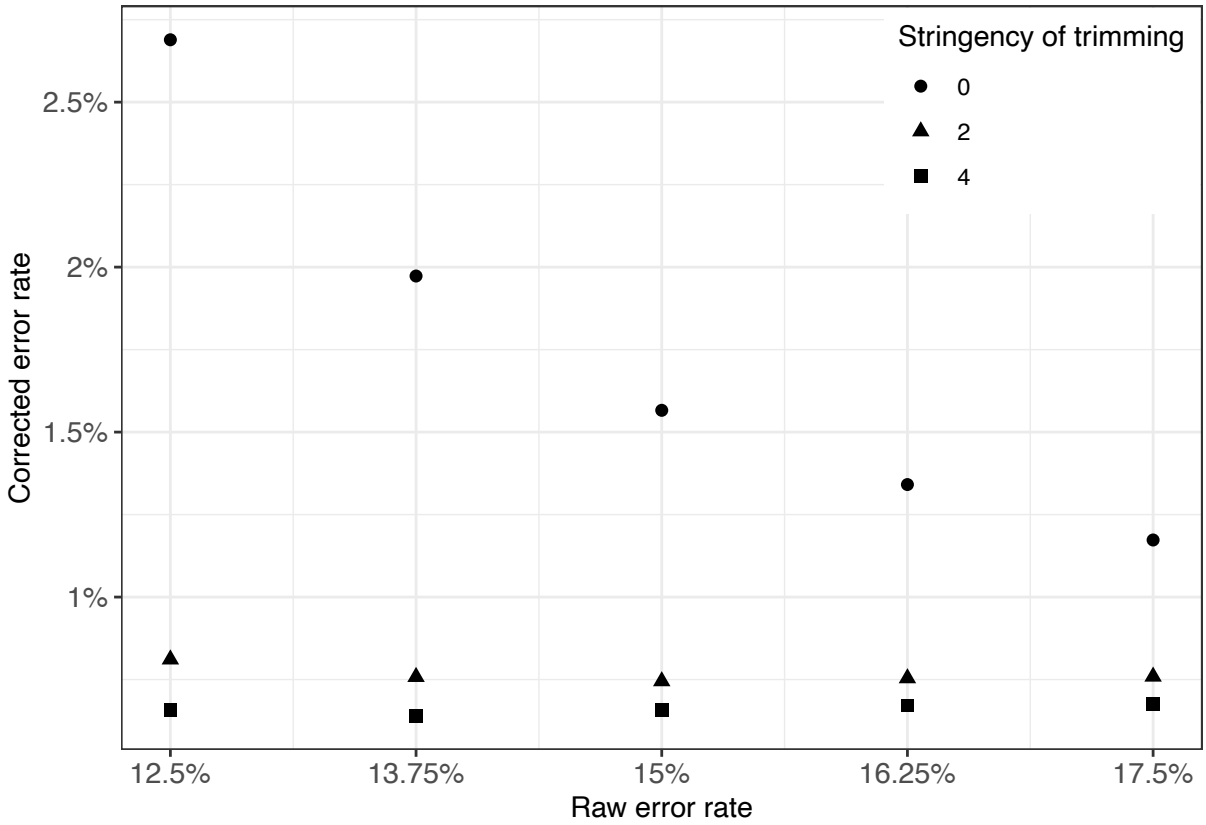


Figure S4: Dotplot comparison of the fire ant genome assembly we present here (x axis) against a previously published draft fire ant genome [17] (y axis). The x axis represents the 16 fire ant chromosomes in the presented assembly and the y axis represents matching sequences in the draft assembly. The assemblies were aligned using minimap2 (version 2.17; -c -P -k19 -w19 -m200) [15] and visualized using dotPlotly (version 11744849; -m 100000) [18]. Most breaks in collinearity are along the x axis. The spacing between diagonals shows how ambiguous regions of the genome that are absent from the previous genome assembly were identified and included in the new assembly.



131 **Figure S5**: Estimated error rates of corrected reads (y axis) against error rate of raw reads (x
132 axis). Shape of the points indicate the stringency of trimming raw reads. Each of the 15 points
133 represents corrected reads obtained by changing the raw error rate threshold and trimming
134 stringency genome assembly parameters used for this study.

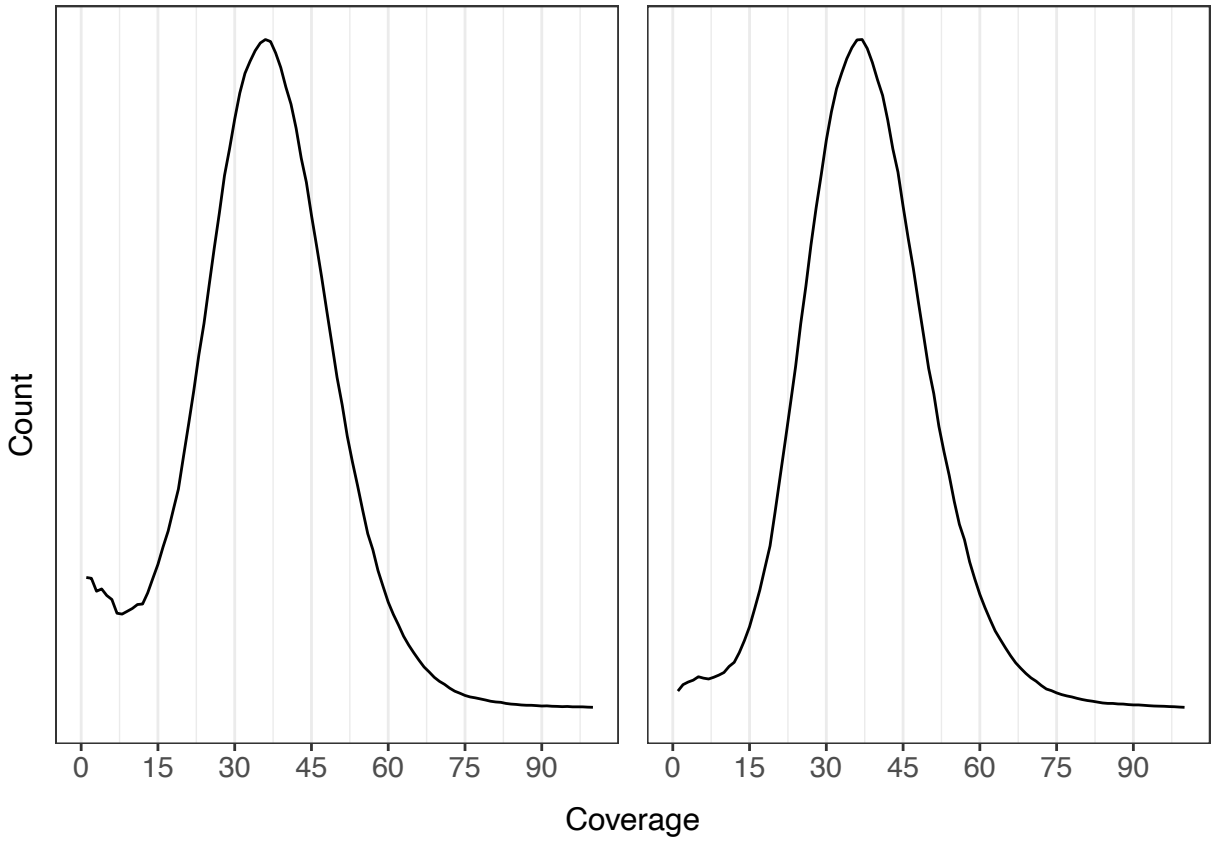


Figure S6: Coverage density plot of the best assembly before (left) and after removing unresolved haplotigs (right). The removal of these unresolved haplotigs clearly reduces the among of contigs with less than half the median coverage (*i.e.*, less than 18x).

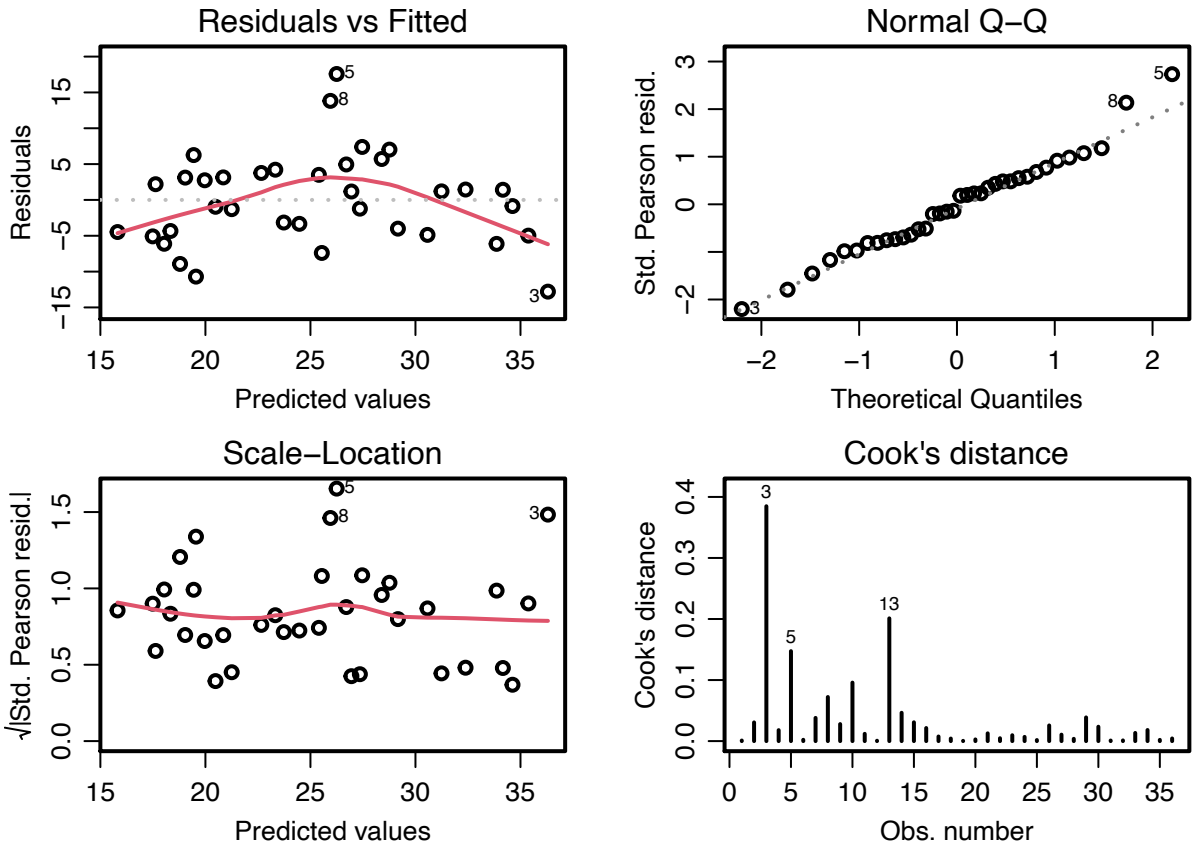
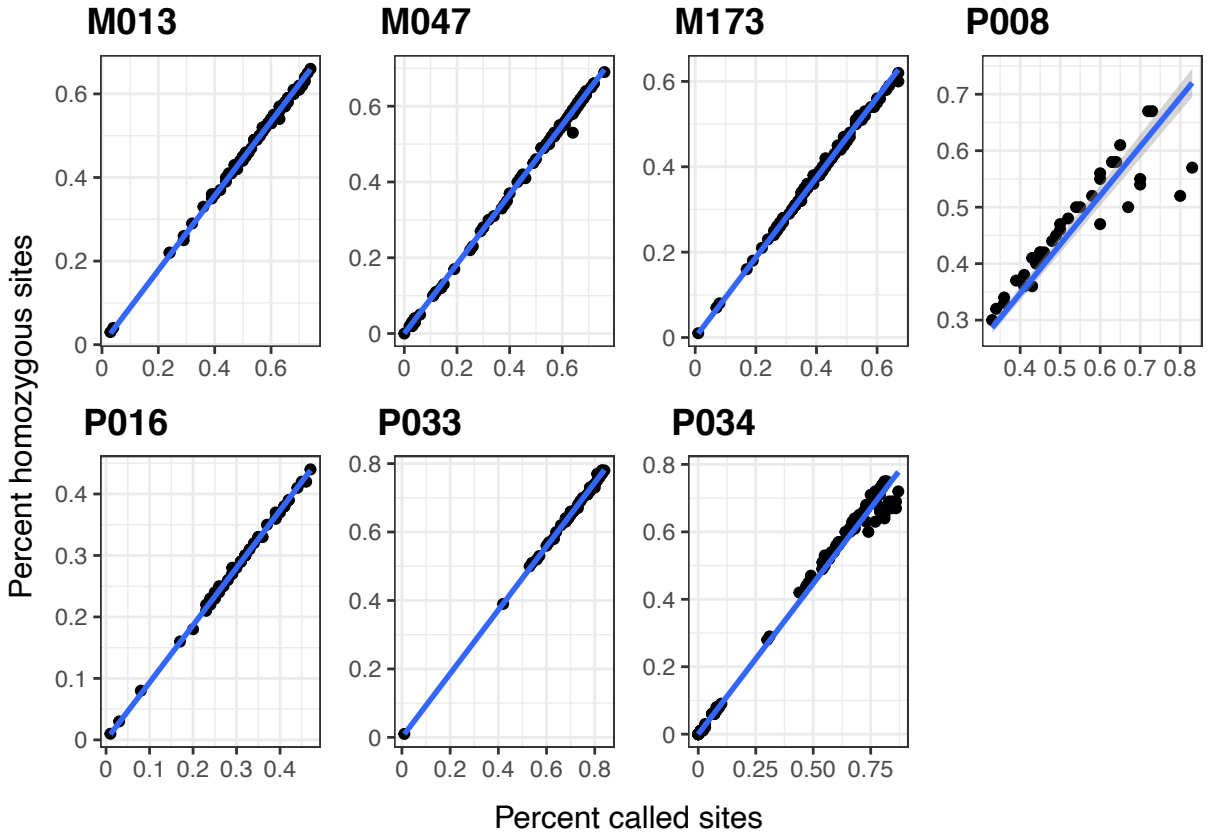
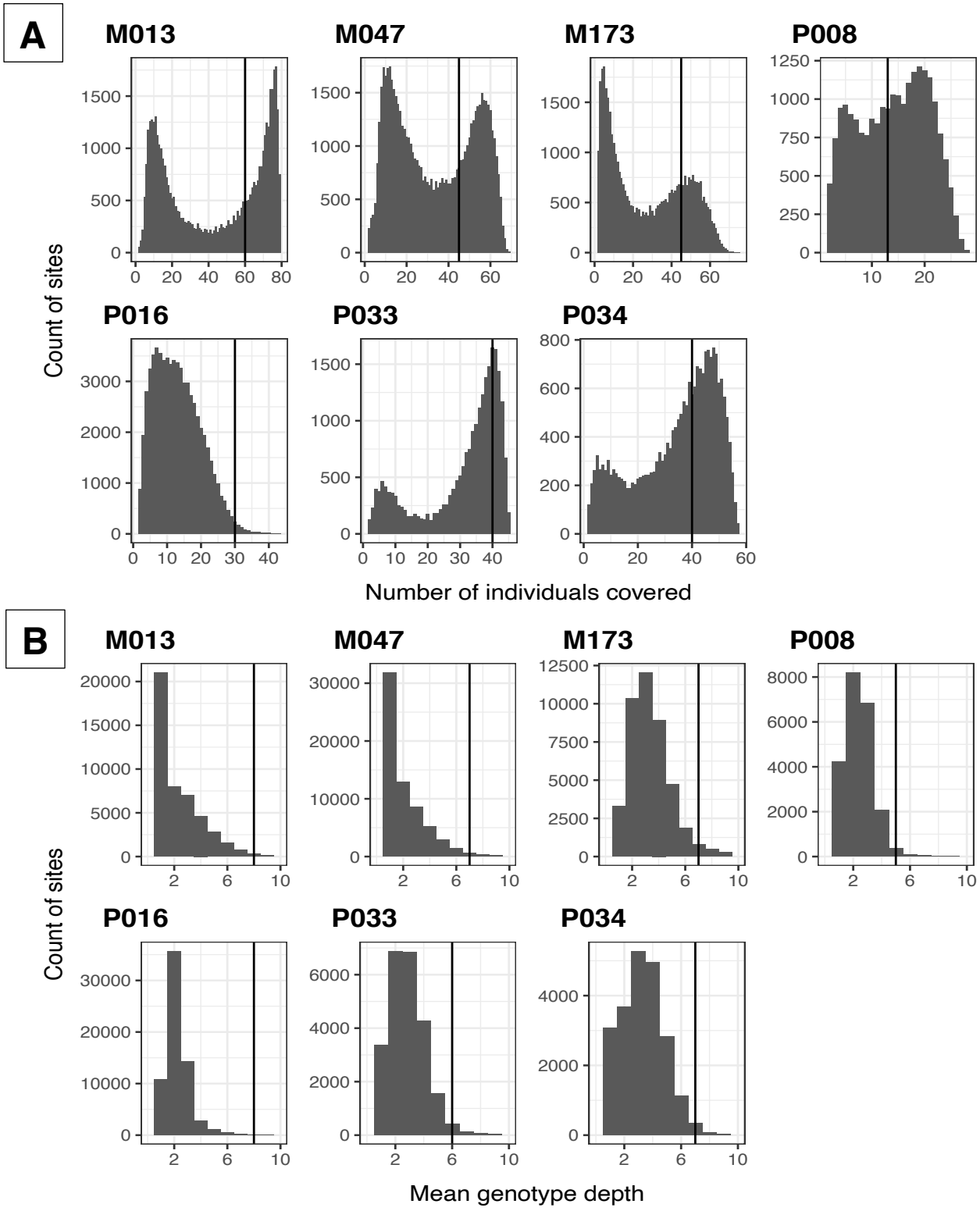


Figure S7: We modelled the overall assembly rank as a function of the three assembly parameters: error rate threshold for raw reads, stringency of trimming raw reads, error rate threshold for corrected reads). The error rate threshold for corrected and for raw reads were significant $p < 10^{-5}$ and $p < 0.05$ respectively.

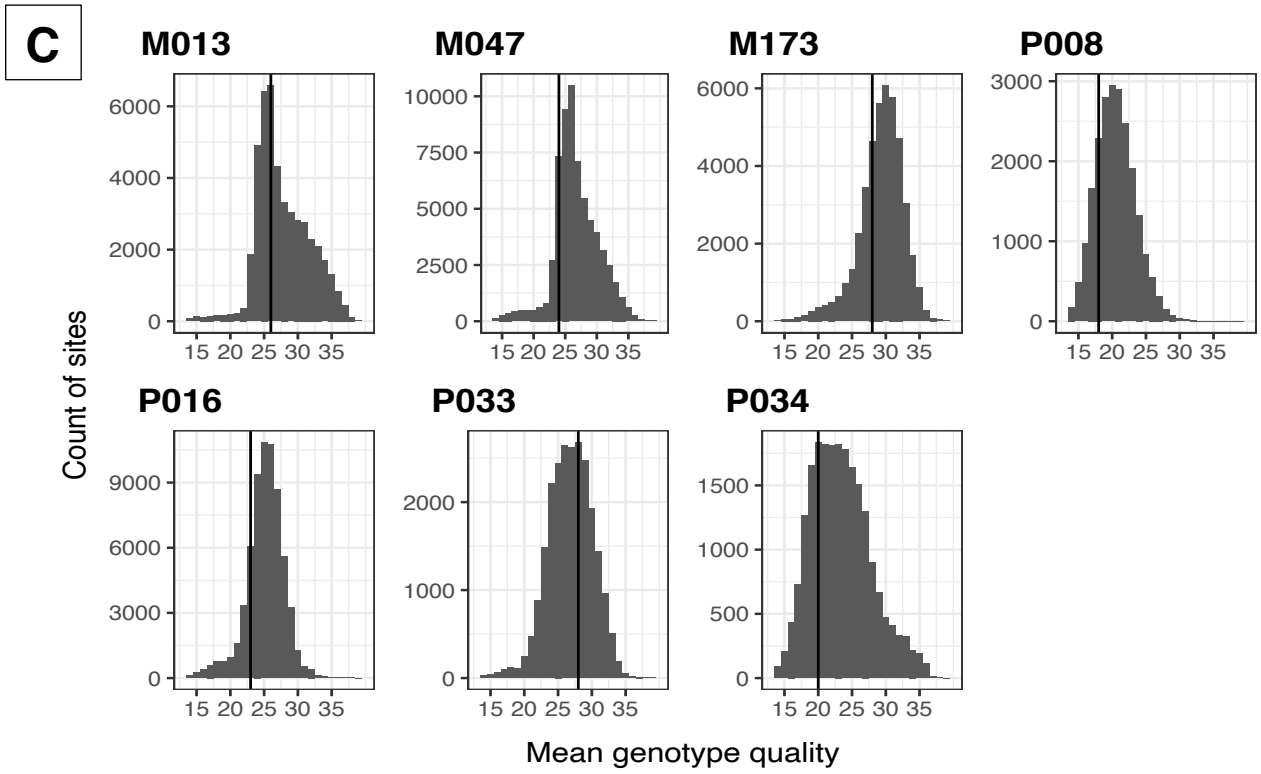


142 **Figure S8**: Proportion of individuals genotyped per site from RAD sequencing of seven fire
143 ant families (M013 - P034; names beginning with M are monogynous colonies and those
144 beginning with P are polygynous colonies) that has single nucleotide polymorphism in the
145 family (x axis) against proportion of homozygous individuals for that site (y axis).



146 **Figure S9**: Site statistics obtained from RAD sequencing of seven fire ant families (M013 -
147 P034; names beginning with M are monogynous colonies and those beginning with P are
148 polygynous colonies). Black vertical line shows the threshold chosen for each family for
149 filtering during linkage map construction. A) Number of individuals genotyped per site (x axis)

against their count (y axis). B) Mean read depth of genotypes per site (x axis) against their count (y axis). C) Mean genotype quality per site (x axis) against their count (y axis). D) Minor allele frequency per site (x axis) against their count (y axis) (continued on next page)



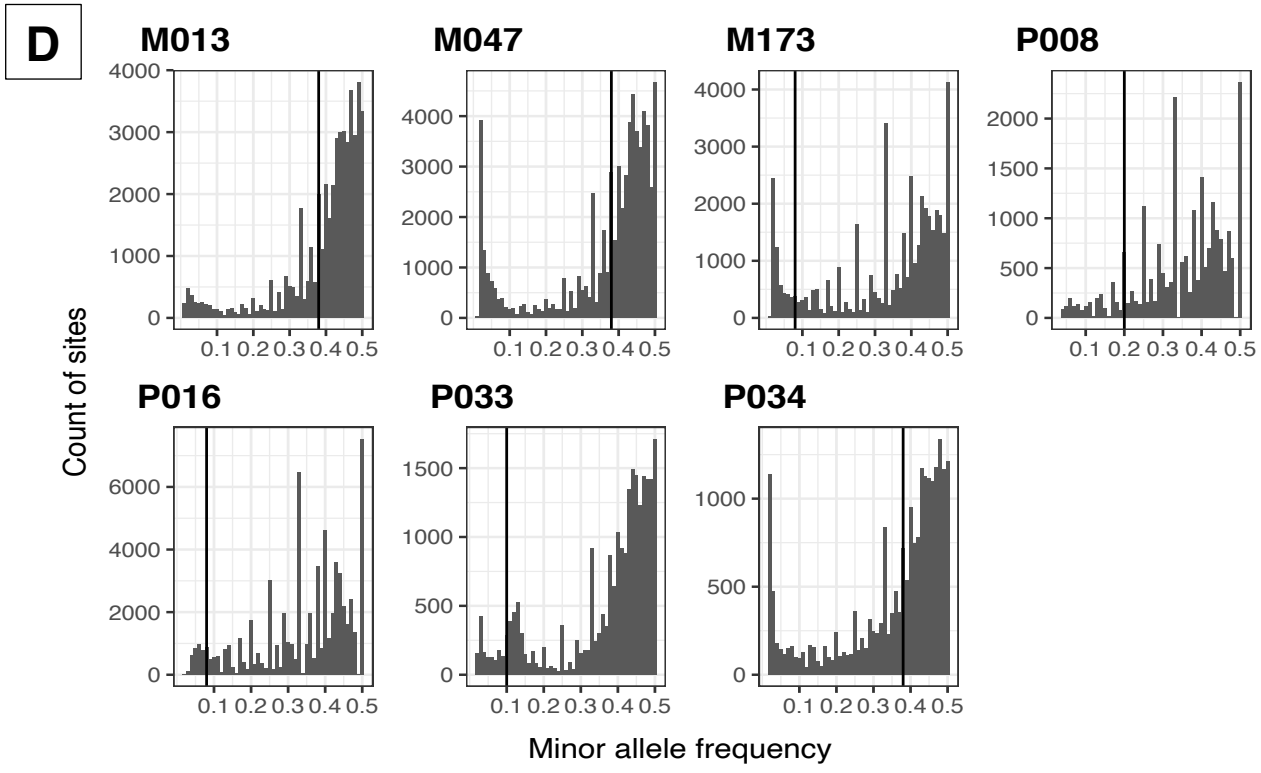


Figure S9 (continued)

Supplementary tables

Table S1: Additional file 2

Table S2: Additional file 3

Table S3: Additional file 4

Table S4: Polishing and haplotype removal improves assembly accuracy

	Raw contigs	After Pacbio polishing	After further Illumina polishing	After further haplotype-filtering
General error rate	1.34	1.30	1.26	-
% Illumina reads with insertion	6.48%	3.91%	1.79%	-
% Illumina reads with deletion	2.75%	2.82%	2.19%	-
Mean mapping quality of Illumina reads	25.73	27.49	28.24	-
% Complete benchmarking universal single-copy orthologs (BUSCO, lineage insecta, n=1664)	98%	98.8%	99%	98.8%

Table S5: Comparison of Canu, Flye, and wtdbg2 genome assemblers

	Resolved length	NG50	BUSCO score	Solid Illumina read pairs
Canu + purge_haplotigs	366,814,754 bp	441,945 bp	96.4%	57.62%
Flye	353,678,069 bp	402,671 bp	96.5%	55.25%
Wtdbg2	320,860,502 bp	502,081 bp	68.6%	48.12%

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