

The beginning of the end: a chromosomal assembly of the New World malaria mosquito ends with a novel telomere

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Table S1. Repeat content of the two centromeric scaffolds. The two centromeric scaffolds consist most of repetitive DNA sequences, including tandem repeats of a 120 bp centromeric repeat unit (File S1), AT-repeats (File S1), and other repeats found in the repeat library (File S2).

Name	Total length	120 bp centromeric Repeat Unit ^a	AT-repeat ^b	Repeat Library ^c	%masked ^d
HiC_Centromere_chr2	334502	199427 (59.6%)	NA	112030 (33.5%)	93.1
HiC_Centromere_chr3	957180	335045 (35%)	149542 (15.6%)	338540 (35.4%)	86

a. Number of bases (percentages) masked using 9 representatives of the 120 bp centromeric repeat unit (File S1). *b.* Number of bases (percentages) masked using a representative AT-rich sequence in the centromeric scaffold of chromosome 3 (File S1). *c.* Number of bases (percentages) masked by the AalBS3 repeat library (File S2). *d.* Total percentage of masked sequences in the scaffolds. Repeat masking was performed in a step-wise manner using libraries specified in 1-3, using default parameters.

Table S2. Repeat content of the AalbS2 and AalbS3 assemblies.

	AalbS2	AalbS3
Total Repeat Content	2.20%	5.06%
SINEs	0.01%	0.01%
LINEs	0.64%	0.83%
LTR retrotransposon	0.16%	0.53%
DNA elements	0.55%	1.31%
Unclassified	0.84%	2.38%
Simple repeats	0.26%	0.18%

Repeat masking was performed using the new repeat library (File S2) and default parameters.

Table S3. BUSCO analysis of the AalbS2 and AalbS3 assemblies.

BUSCO Classification	AalbS2		AalbS3	
	Group hits	Percentage	Group hits	Percentage
Complete	1055	98.9	1055	99.0
Complete and single-copy	1046	98.1	1047	98.2
Complete and duplicated	9	0.8	8	0.8
Fragmented	3	0.3	4	0.4
Missing	8	0.8	7	0.6

Table S4. Summary of heterozygosity

	Mother	Father
X	0.000373633	0.000731478
2	0.001091377	0.000908605
3	0.000803706	0.000944578

To estimate heterozygosity, Illumina reads from each of the parents were mapped to the AalbS3 assembly. Heterozygosity rates were calculated for each chromosome as the total number of heterozygous sites in a chromosome divided by the length of the chromosome excluding ambiguous N bases.

Table S5. BLAST analysis using the FISH probes on the Hi-C and Bionano assemblies

	Position	Order of FISH Probes	Probes Aligned to Hi-C Assembly	Probes Aligned to Bionano Assembly
	1A	AALB006952	AALB006952 AALB006952-RA	AALB006952 AALB006952-RA
	3A	AALB006265	AALB006265 AALB006265-RA	AALB006265 AALB006265-RA
	3A	AALB007629	AALB007629 AALB007629-RA	AALB007629 AALB007629-RA
	3C	AALB007743	AALB007743 AALB007743-RA	AALB007743 AALB007743-RA
	3C	AALB015532	AALB015532 AALB015532-RA	AALB015532 AALB015532-RA
X	4A	AALB003331	AALB003331 AALB003331-RA	AALB003331 AALB003331-RA
	4A	AALB007797	AALB007797 AALB007797-RA	AALB007797 AALB007797-RA
	4C	AALB007749	AALB007749 AALB007749-RA	AALB007749 AALB007749-RA
	5A	AALB003698	AALB003698 AALB003698-RA	AALB003698 AALB003698-RA
	5A	AALB003707	AALB003707 AALB003707-RA	AALB003707 AALB003707-RA
	5A	AALB009742	AALB009742 AALB009742-RA	AALB009742 AALB009742-RA
	5B	AALB009739	AALB009739 AALB009739-RA	AALB009739 AALB009739-RA
	6A	AALB009554	AALB009554 AALB009554-RA	AALB009554 AALB009554-RA
	9A	AALB008804	AALB008804 AALB008804-RA	AALB008804 AALB008804-RA
	9A	AALB002730	AALB002730 AALB002730-RA	AALB002730 AALB002730-RA
	10B	AALB002317	AALB002317 AALB002317-RA	AALB002317 AALB002317-RA
	10C	AALB007120	AALB007120 AALB007120-RA	AALB007120 AALB007120-RA

	10C	AALB002315	AALB002315 AALB002315-RA	AALB002315 AALB002315-RA
	11B	AALB002051	AALB002051 AALB002051-RA	AALB002051 AALB002051-RA
	11B	AALB009555	AALB009555 AALB009555-RA	AALB009555 AALB009555-RA
	12A	AALB009737	AALB009737 AALB009737-RA	AALB009737 AALB009737-RA
	12A	AALB003905	AALB003905 AALB003905-RA	AALB003905 AALB003905-RA
	12A	AALB003937	AALB003937 AALB003937-RA	AALB003937 AALB003937-RA
	13A	AALB008464	AALB008464 AALB008464-RA	AALB008464 AALB008464-RA
2	13A	AALB008058	AALB008058 AALB008058-RA	AALB008058 AALB008058-RA
	13A	AALB002941	AALB002941 AALB002941-RA	AALB002941 AALB002941-RA
	13C	AALB003187	AALB003187 AALB003187-RA	AALB003187 AALB003187-RA
	13C	AALB003689	AALB003689 AALB003689-RA	AALB003689 AALB003689-RA
	14B	AALB003339	AALB003339 AALB003339-RA	AALB003339 AALB003339-RA
	14B	AALB002796	AALB002796 AALB002796-RA	AALB002796 AALB002796-RA
	14C	AALB002731	AALB002731 AALB002731-RA	AALB002731 AALB002731-RA
	14C	AALB008768	AALB008768 AALB008768-RA	AALB008768 AALB008768-RA
	15A	AALB008515	AALB008515 AALB008515-RA	AALB008515 AALB008515-RA
	15A	AALB010878	AALB010878 AALB010878-RA	AALB010878 AALB010878-RA
	15A	AALB002801	AALB002801 AALB002801-RA	AALB002801 AALB002801-RA
	15B	AALB002940	AALB002940 AALB002940-RA	AALB002940 AALB002940-RA
	15B	AALB008803	AALB008803 AALB008803-RA	AALB008803 AALB008803-RA

	15C	AALB008770	AALB008770 AALB008770-RA	AALB008770 AALB008770-RA
	15C	AALB001274	AALB001274 AALB001274-RA	AALB001274 AALB001274-RA
	15C	AALB001290	AALB001290 AALB001290-RA	AALB001290 AALB001290-RA
	16A	AALB007837	AALB007837 AALB007837-RA	AALB007837 AALB007837-RA
	17B	AALB008049	AALB008049 AALB008049-RA	AALB008049 AALB008049-RA
	17B	AALB001218	AALB001218 AALB001218-RA	AALB001218 AALB001218-RA
	23B	AALB000001	AALB000001 AALB000001-RA	AALB000001 AALB000001-RA
	23B	AALB002044	AALB002044 AALB002044-RA	AALB002044 AALB002044-RA
	25B	AALB001296	AALB001296 AALB001296-RA	AALB001296 AALB001296-RA
	26A	AALB003709	AALB003709 AALB003709-RA	AALB003709 AALB003709-RA
	26B	AALB003876	AALB003876 AALB003876-RA	AALB003876 AALB003876-RA
	26B	AALB009763	AALB009763 AALB009763-RA	AALB009763 AALB009763-RA
	32A	AALB010862	AALB010862 AALB010862-RA	AALB010862 AALB010862-RA
	32A	AALB004078	Missing	AALB004078 AALB004078-RA
	32C	AALB003945	AALB003945 AALB003945-RA	AALB003945 AALB003945-RA
	33A	AALB008507	AALB008472 AALB008472-RA	AALB008472 AALB008472-RA
	32C	AALB008472	AALB008507 AALB008507-RA	AALB008507 AALB008507-RA
	33B	AALB006953	AALB007117 AALB007117-RA	AALB007117 AALB007117-RA
	33A	AALB007117	AALB006953 AALB006953-RA	AALB006953 AALB006953-RA
	33B	AALB007443	AALB007443 AALB007443-RA	AALB007443 AALB007443-RA

3	33C	AALB007353	AALB007353 AALB007353-RA	AALB007353 AALB007353-RA
	33C	AALB003893	AALB003893 AALB003893-RA	AALB003893 AALB003893-RA
	34A	AALB003904	Missing	AALB003904 AALB003904-RA
	34A	AALB007568	AALB007568 AALB007568-RA	AALB007568 AALB007568-RA
	34B	AALB007622	AALB007622 AALB007622-RA	AALB007622 AALB007622-RA
	35A	AALB001253	AALB001253 AALB001253-RA	AALB001253 AALB001253-RA
	35A	AALB007555	AALB007555 AALB007555-RA	AALB007555 AALB007555-RA
	35B	AALB007448	AALB007448 AALB007448-RA	AALB007448 AALB007448-RA
	35B	AALB014716	AALB014360 AALB014360-RA	AALB014360 AALB014360-RA
	36A	AALB007835	AALB009746 AALB009746-RA	AALB009746 AALB009746-RA
	36B	AALB014360	AALB009753 AALB009753-RA	AALB009753 AALB009753-RA
	35B	AALB009746	AALB007835 AALB007835-RA	AALB007835 AALB007835-RA
	35B	AALB009753	AALB014716 AALB014716-RA	AALB014716 AALB014716-RA
	36B	AALB007293	Missing	AALB007293 AALB007293-RA
	36B	AALB007352	AALB007352 AALB007352-RA	AALB007352 AALB007352-RA
	37A	AALB003188	Missing	AALB003188 AALB003188-RA
	38A	AALB003282	AALB003282 AALB003282-RA	AALB003282 AALB003282-RA
	38A	AALB004081	AALB004081 AALB004081-RA	AALB004081 AALB004081-RA
	45B	AALB006259	AALB006259 AALB006259-RA	AALB006259 AALB006259-RA

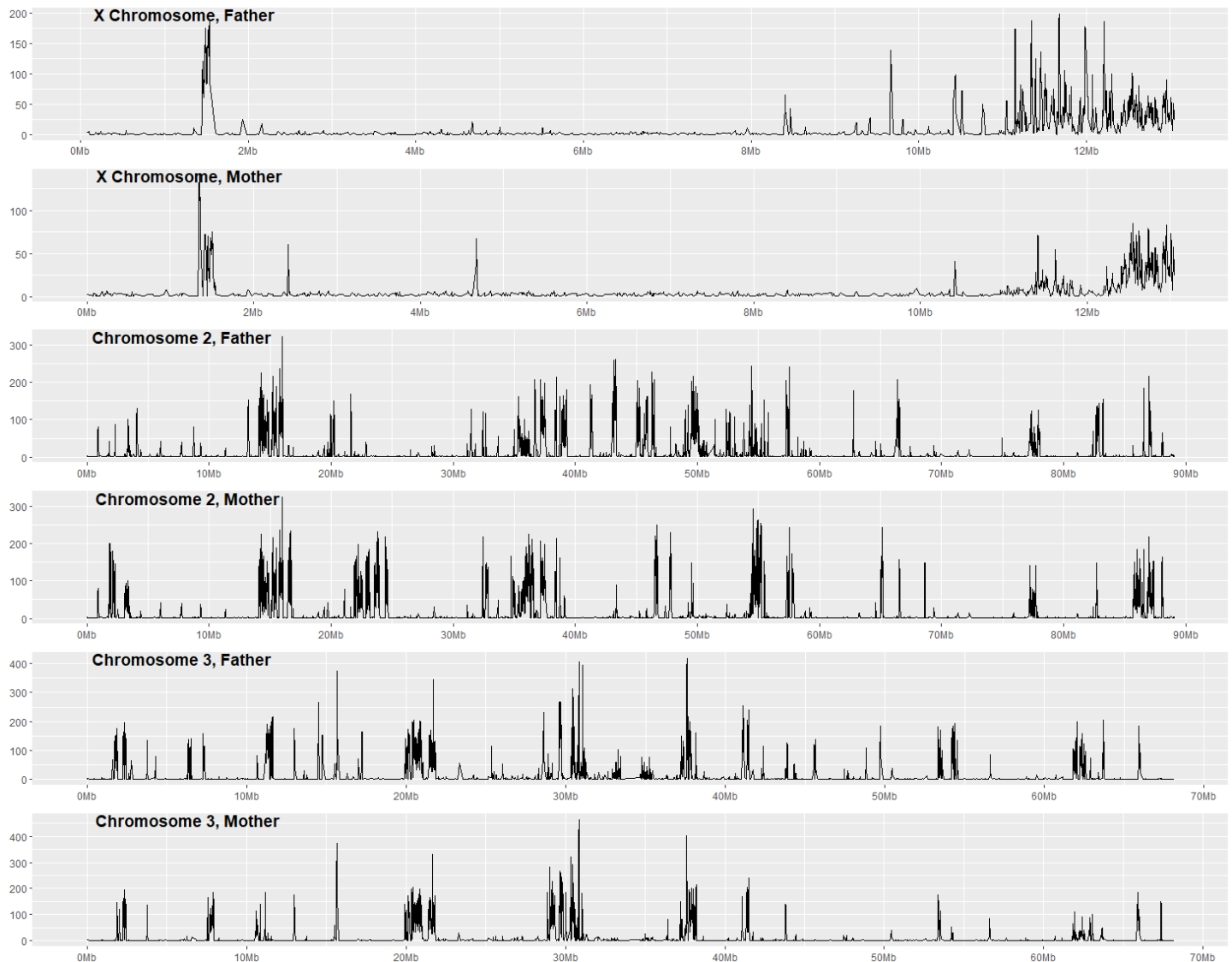


Figure S1. Heterozygosity plot. X-axis is the position along the chromosomes. Y-axis is the number of heterozygous sites per 10 kilobase window. Although the overall heterozygosity is very low, there are clusters of relatively high heterozygosity along each chromosome in both parents.

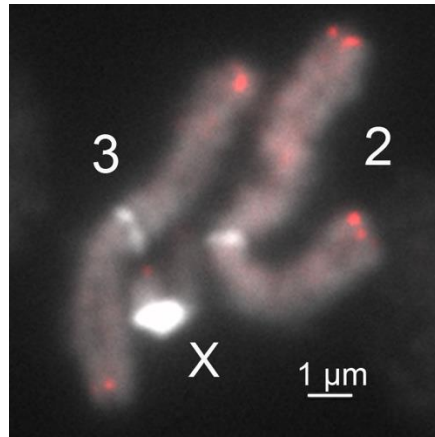


Figure S2. Fluorescence *in situ* hybridization and mapping of the *An. albimanus* telomeric oligonucleotide probe on mitotic chromosomes from the 4th instar larva. The figure shows closely paired homologous chromosomes at the early metaphase stage. Chromosomes hybridized with Cy3-labeled oligonucleotide probe (red) and counterstained with the fluorophore DAPI (white). Chromosome arms are labeled as X, 2, and 3; Scale bar – 1 μm.