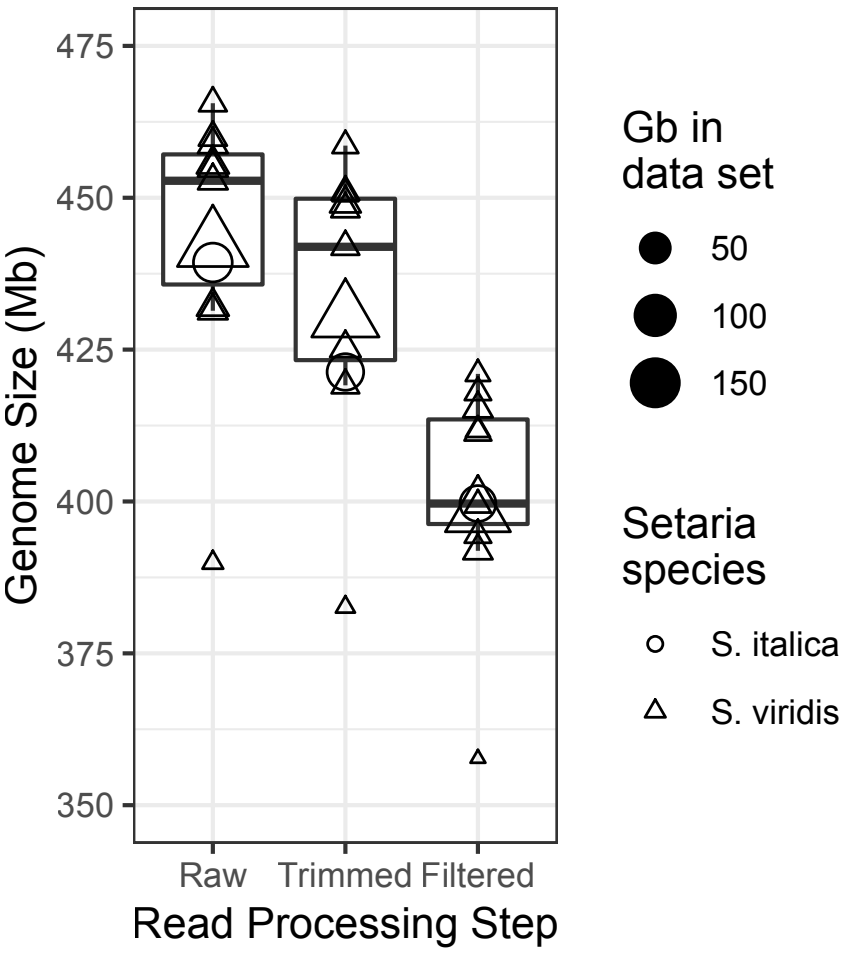
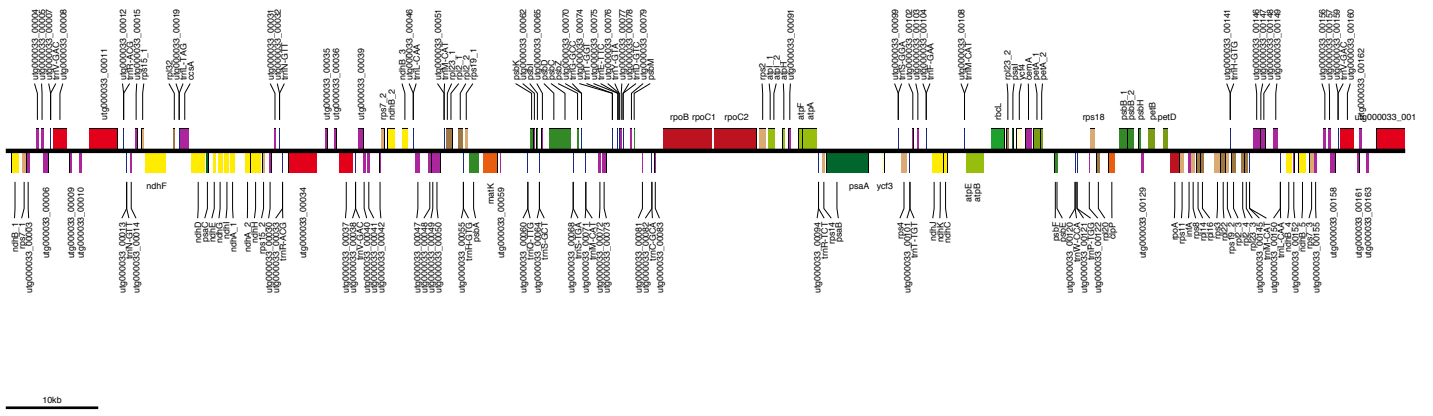
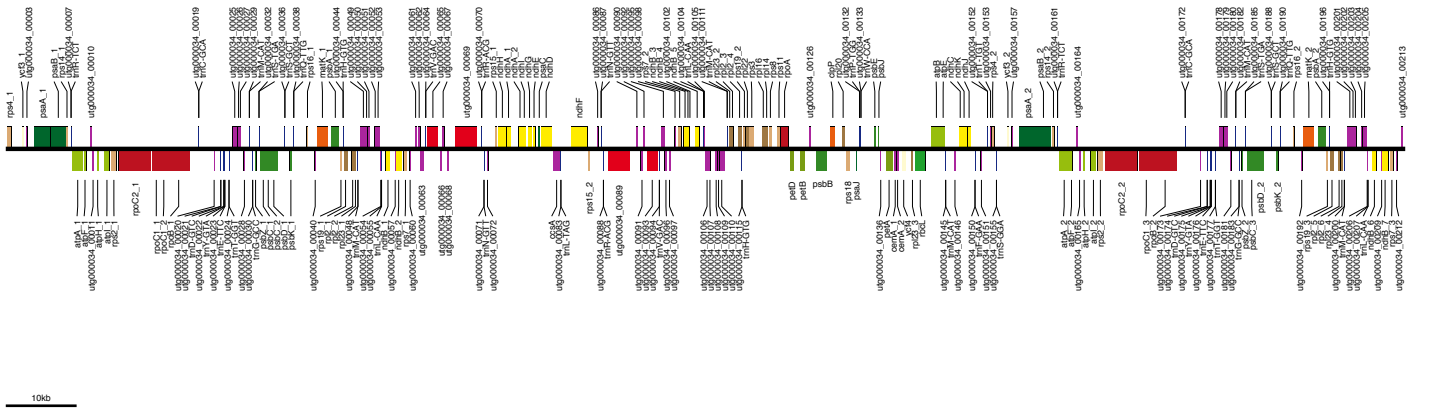



**Figure S1. Estimated haploid genome length for *S. viridis* and *S. italica.*** Data were evaluated without processing (Raw), after read quality trimming (Trimmed), and after quality trimming plus the removal of any reads that align to the chloroplast or mitochondria (Filtered). The amount of reads that remained following any processing is indicated by the relative size of the circles and triangles for cultivars of *S. italica* and *S. viridis,* respectively.

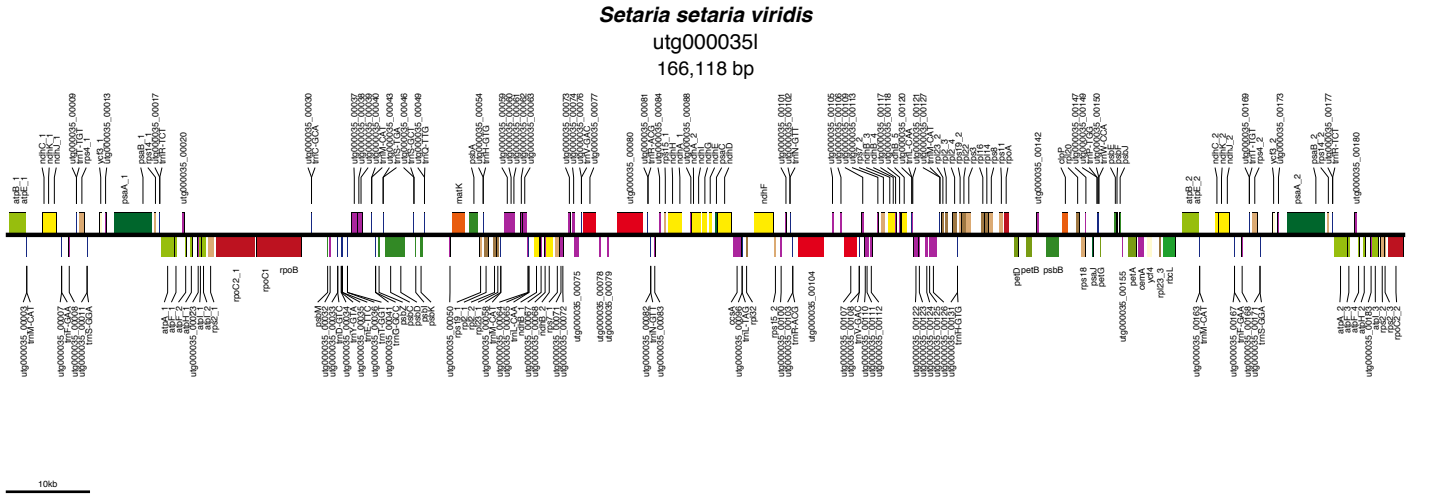
**a**




**b**




**c**




**d**

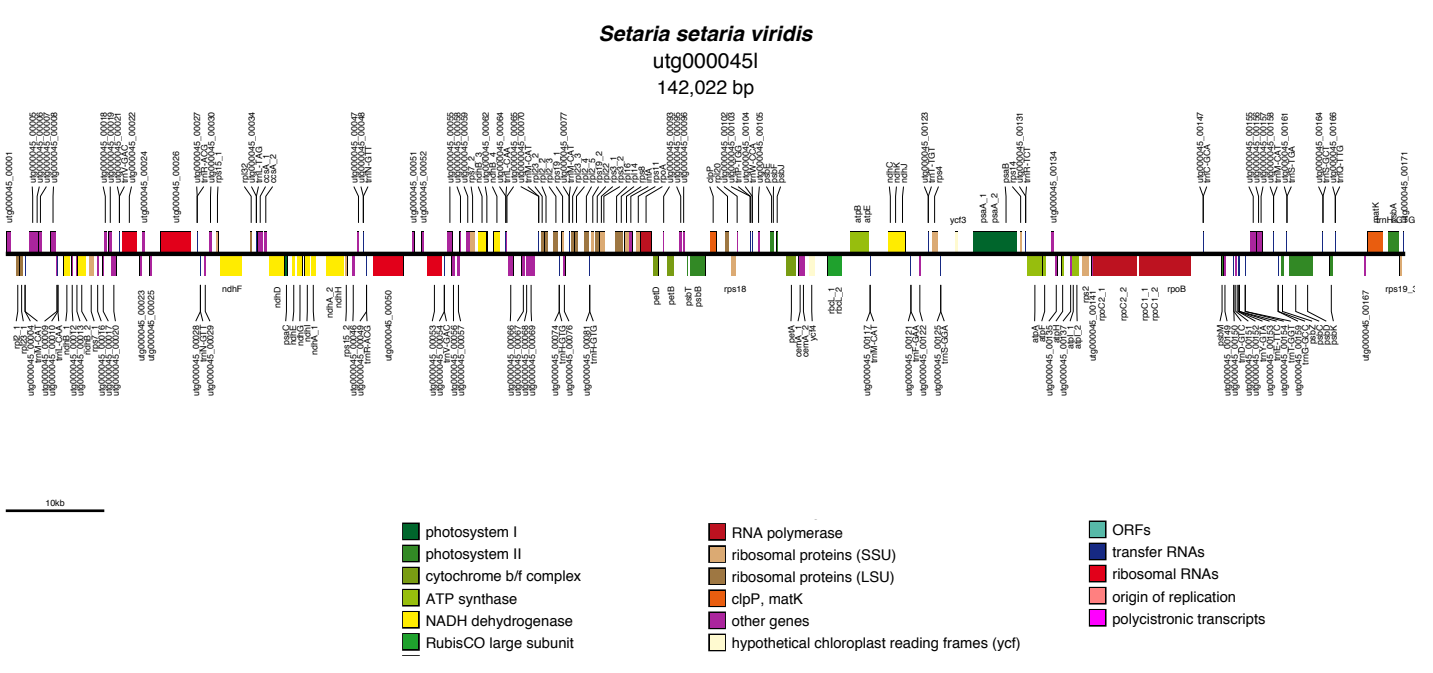



**Figure S2. Physical maps of the four chloroplast-derived contigs** a) utg000033l, b) utg000034l, c) utg000035l, d) utg000045l.

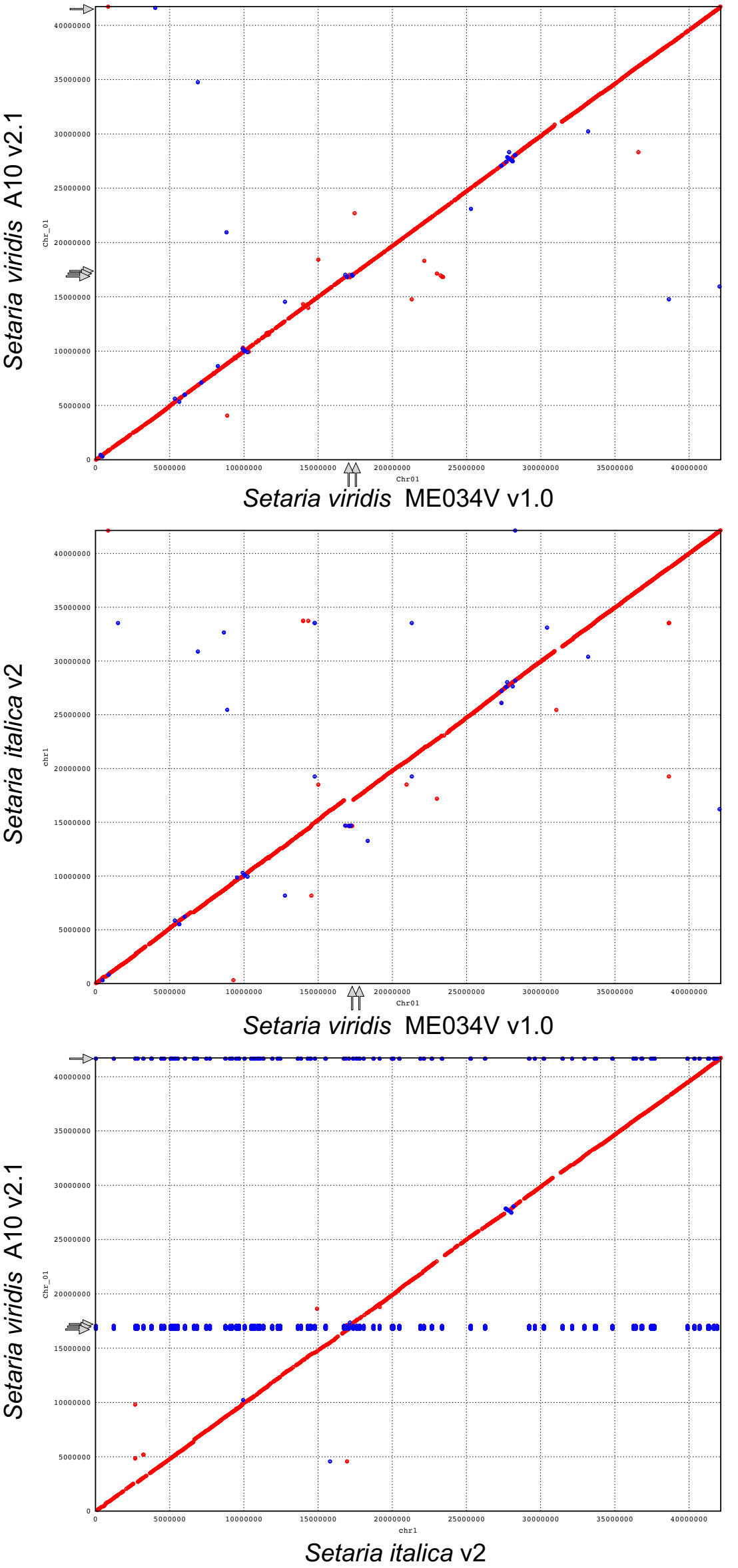



**Figure S3. Chromosome alignments of (top) ME034V to A10.1, (middle) ME034V to *S. italica,* and (bottom) *S. italica* to A10.1.** a) Chromosome 1.

*Note: Alignments were generated by MUMmer4 (nucmer, mincluster=65, minmatch=500; Marçais *et al.* 2018). In the *S. italica* to A10.1 comparisons, the blue horizontal lines indicate positions of gaps in both assemblies; *S. italica* having many more gaps. Because assembly gap lengths in ME034V were shorter (represented by 100 “N’s”) than the minimum length of a single match (minmatch = 500 bp), they were not large enough to be detected by the nucmer analysis. Therefore, positions of assembly gaps (“N’s”) larger than 50 bp in A10.1 and ME034V are indicated along the x or y axes with gray arrows.

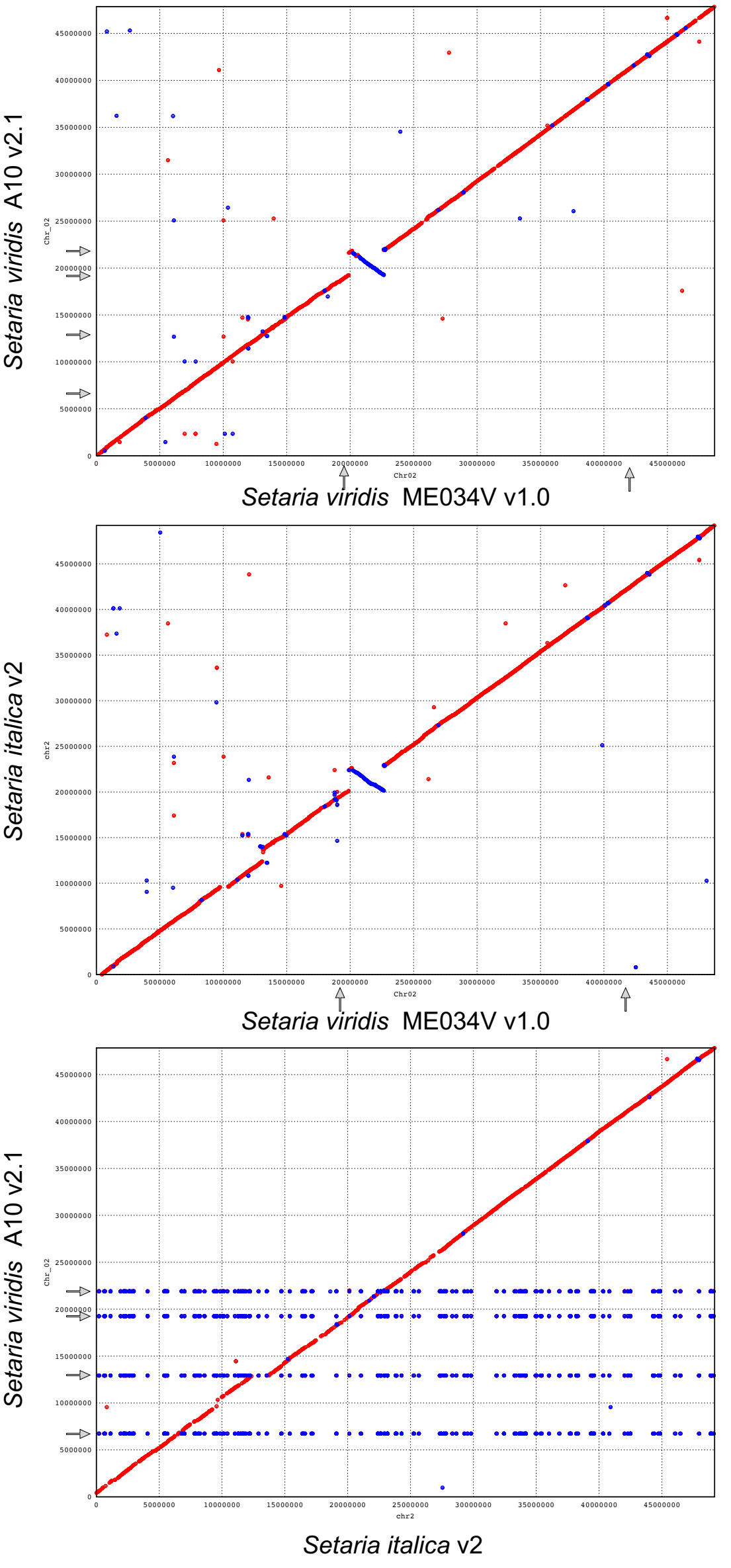



**Figure S3. Chromosome alignments of (top) ME034V to A10.1, (middle) ME034V to *S. italica,* and (bottom) *S. italica* to A10.1 cont.** b) Chromosome 2. Full legend on part a.

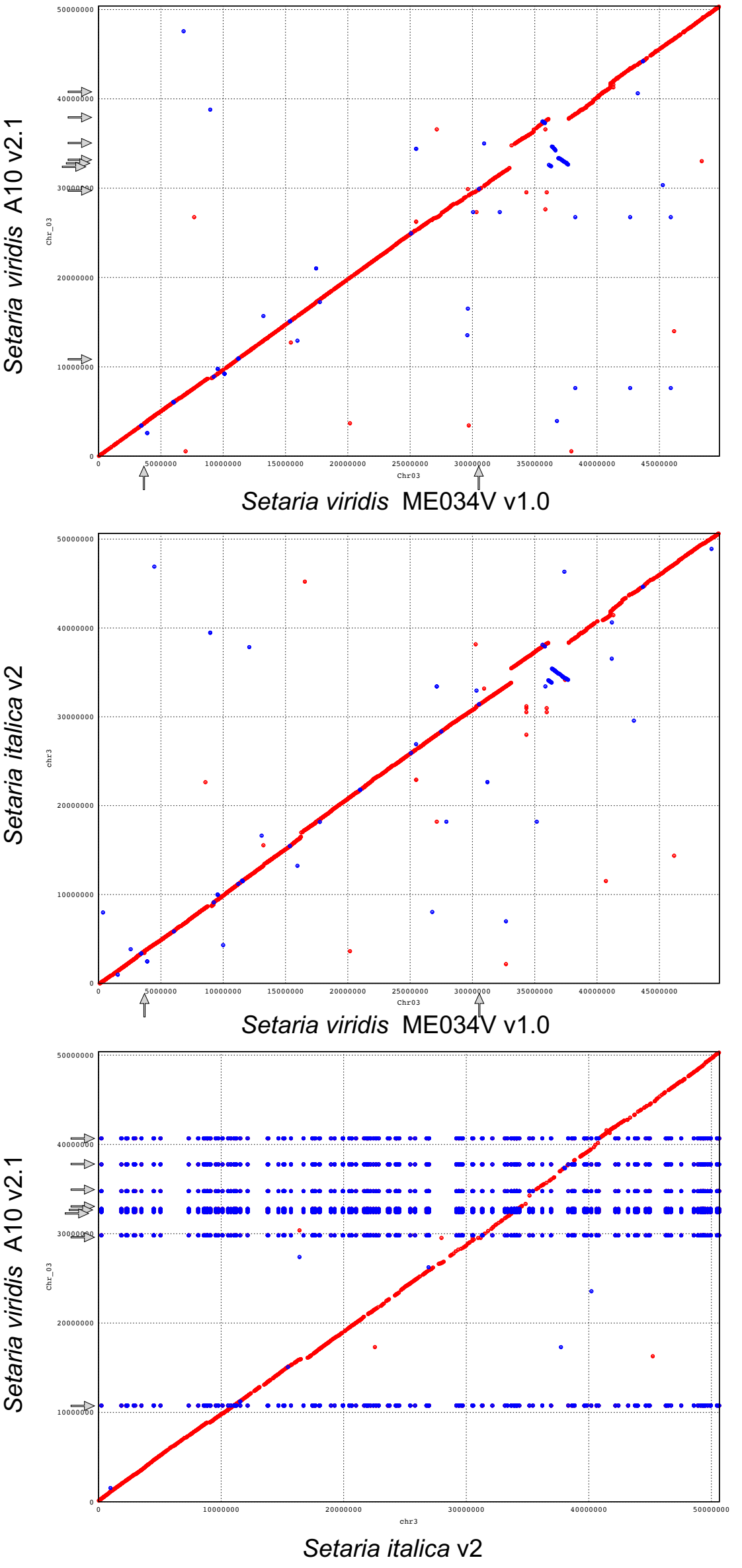



**Figure S3. Chromosome alignments of (top) ME034V to A10.1, (middle) ME034V to *S. italica,* and (bottom) *S. italica* to A10.1 cont.** c) Chromosome 3. Full legend on part a.

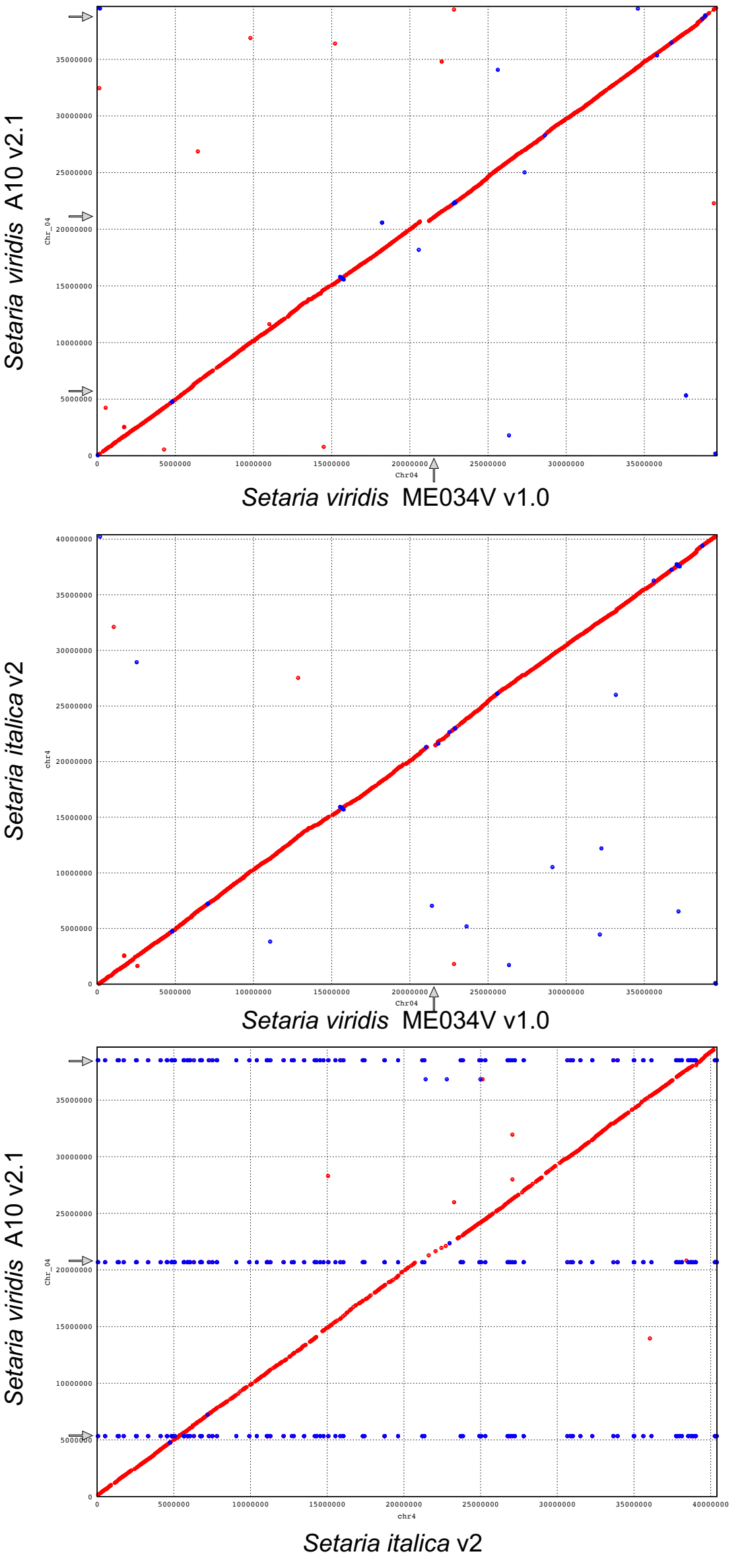



**Figure S3. Chromosome alignments of (top) ME034V to A10.1, (middle) ME034V to *S. italica,* and (bottom) *S. italica* to A10.1 cont.** d) Chromosome 4. Full legend on part a.

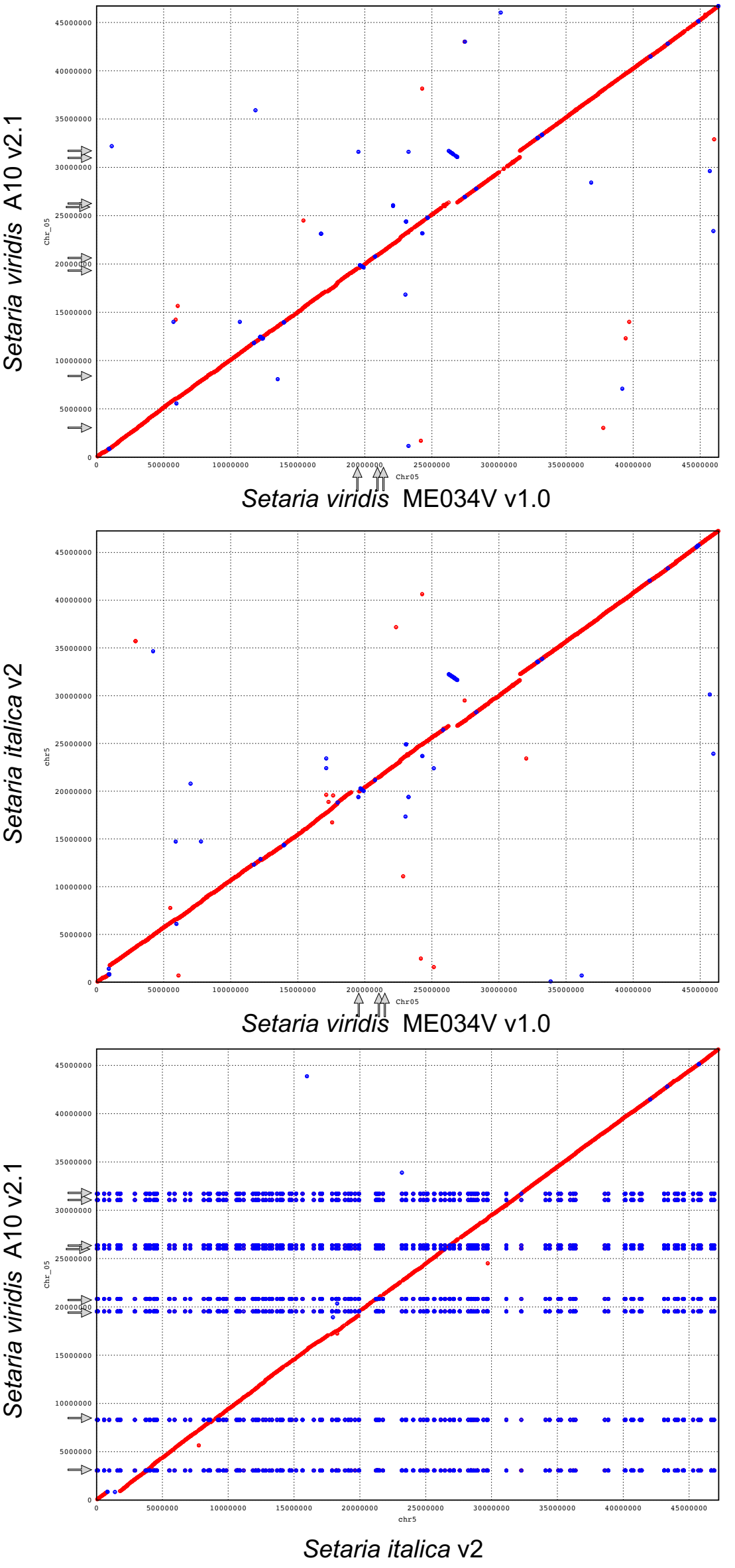



**Figure S3. Chromosome alignments of (top) ME034V to A10.1, (middle) ME034V to *S. italica,* and (bottom) *S. italica* to A10.1 cont.** e) Chromosome 5. Full legend on part a.

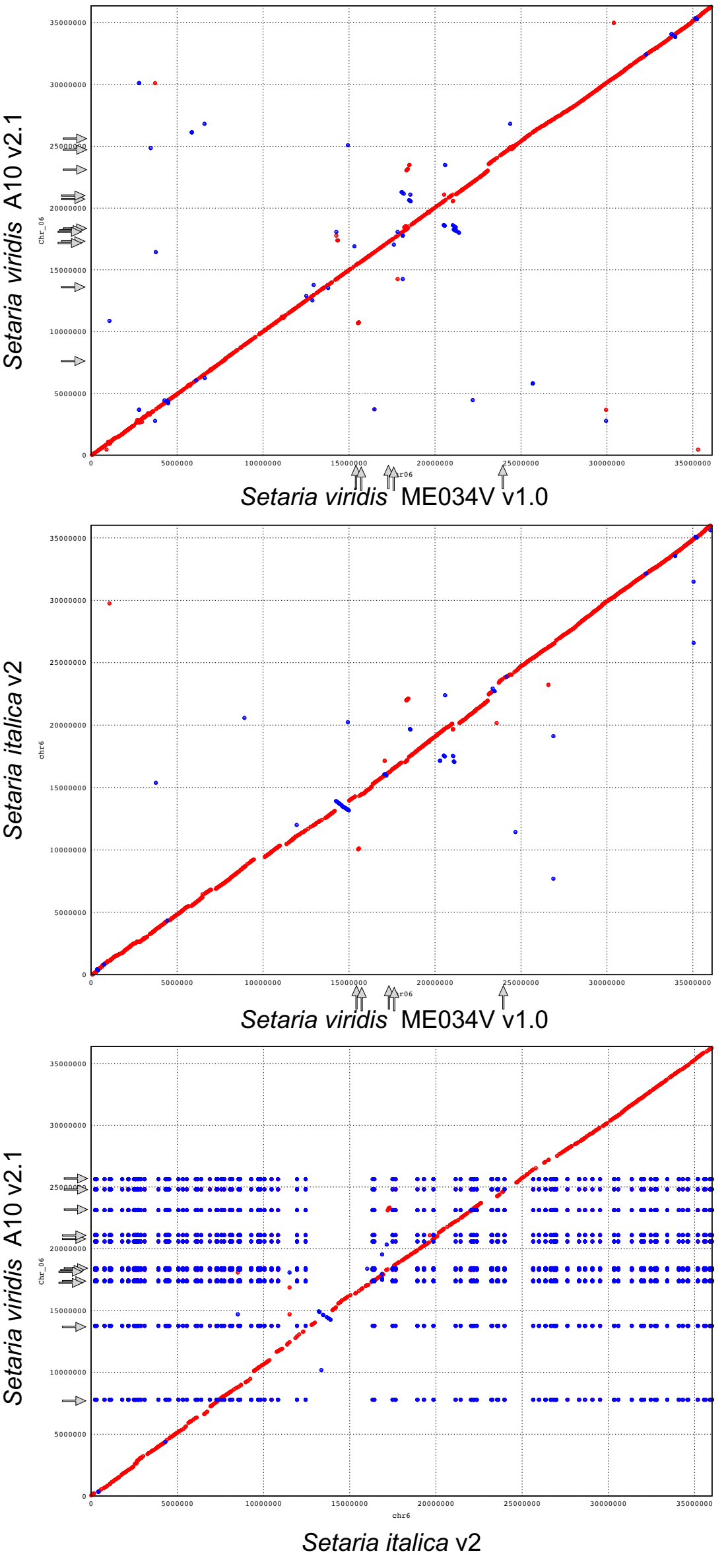



**Figure S3. Chromosome alignments of (top) ME034V to A10.1, (middle) ME034V to *S. italica,* and (bottom) *S. italica* to A10.1 cont.** f) Chromosome 6. Full legend on part a.

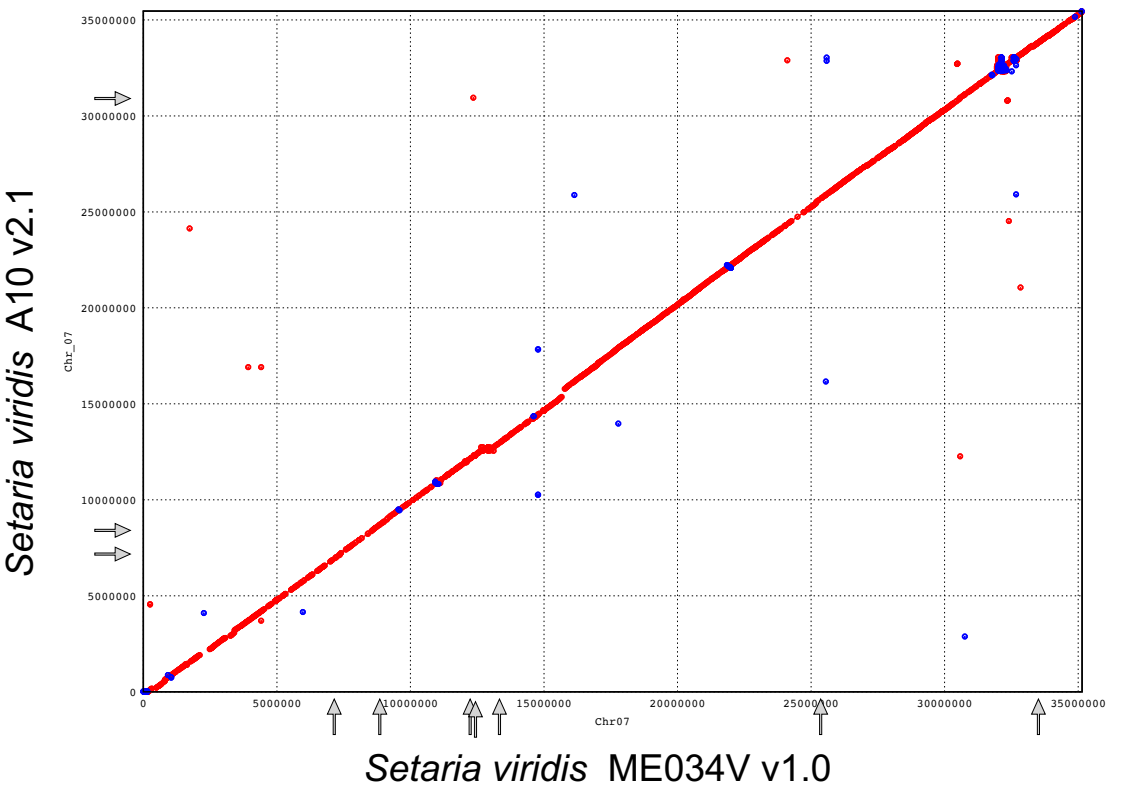



**Figure S3. Chromosome alignments of (top) ME034V to A10.1, (middle) ME034V to *S. italica,* and (bottom) *S. italica* to A10.1 cont.** g) Chromosome 7. Full legend on part a.

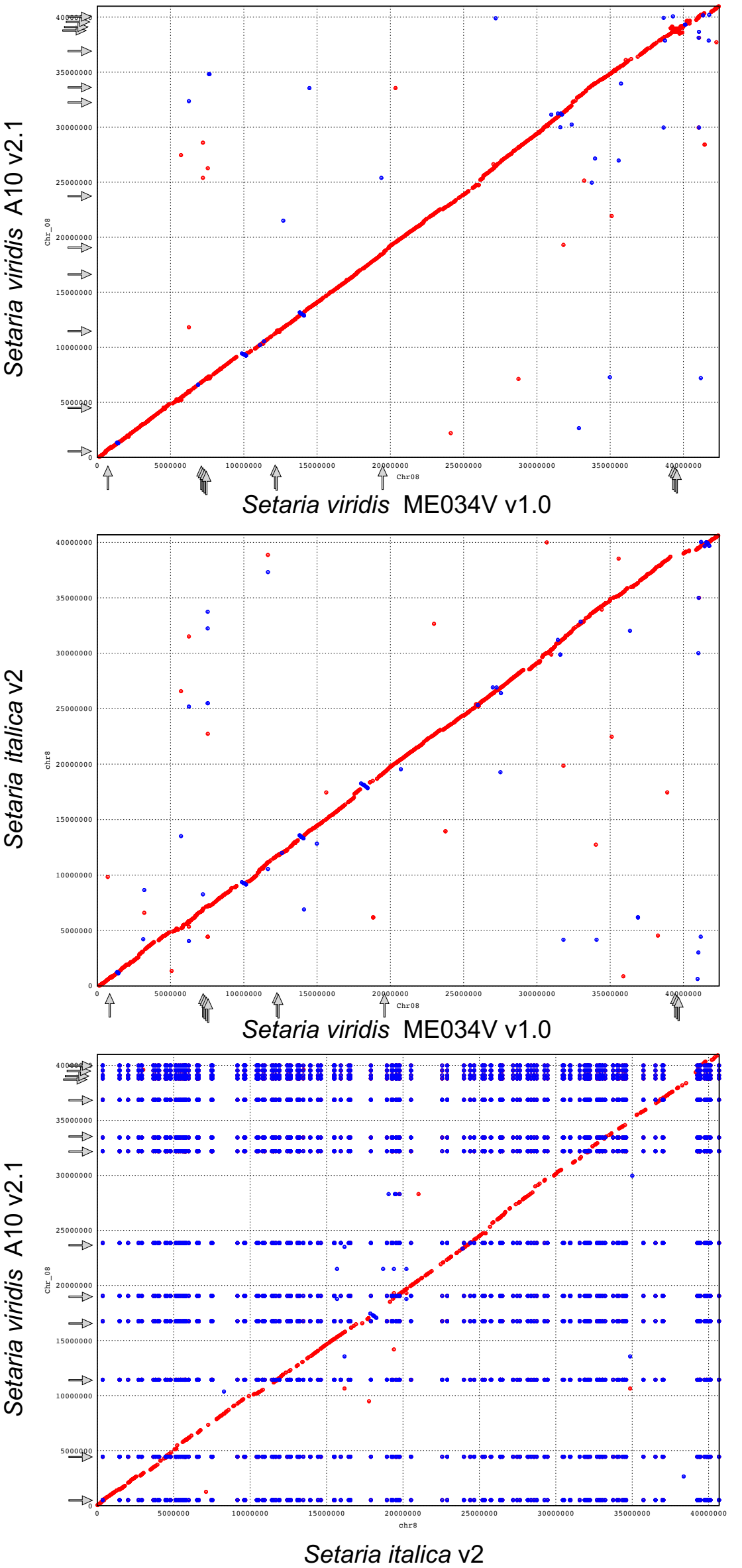



**Figure S3. Chromosome alignments of (top) ME034V to A10.1, (middle) ME034V to *S. italica,* and (bottom) *S. italica* to A10.1 cont.** h) Chromosome 8. Full legend on part a.

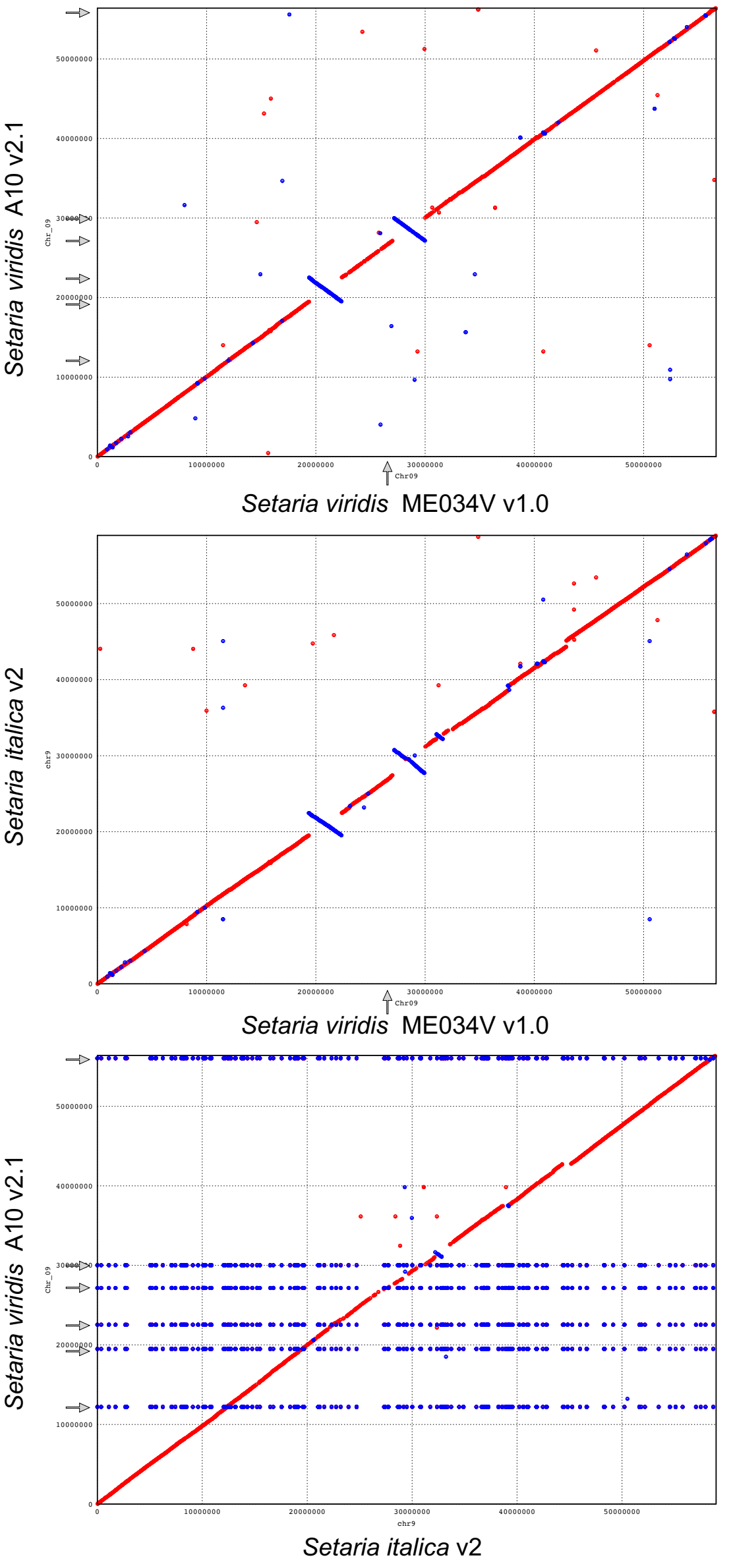



**Figure S3. Chromosome alignments of (top) ME034V to A10.1, (middle) ME034V to *S. italica,* and (bottom) *S. italica* to A10.1 cont.** i) Chromosome 9. Full legend on part a.

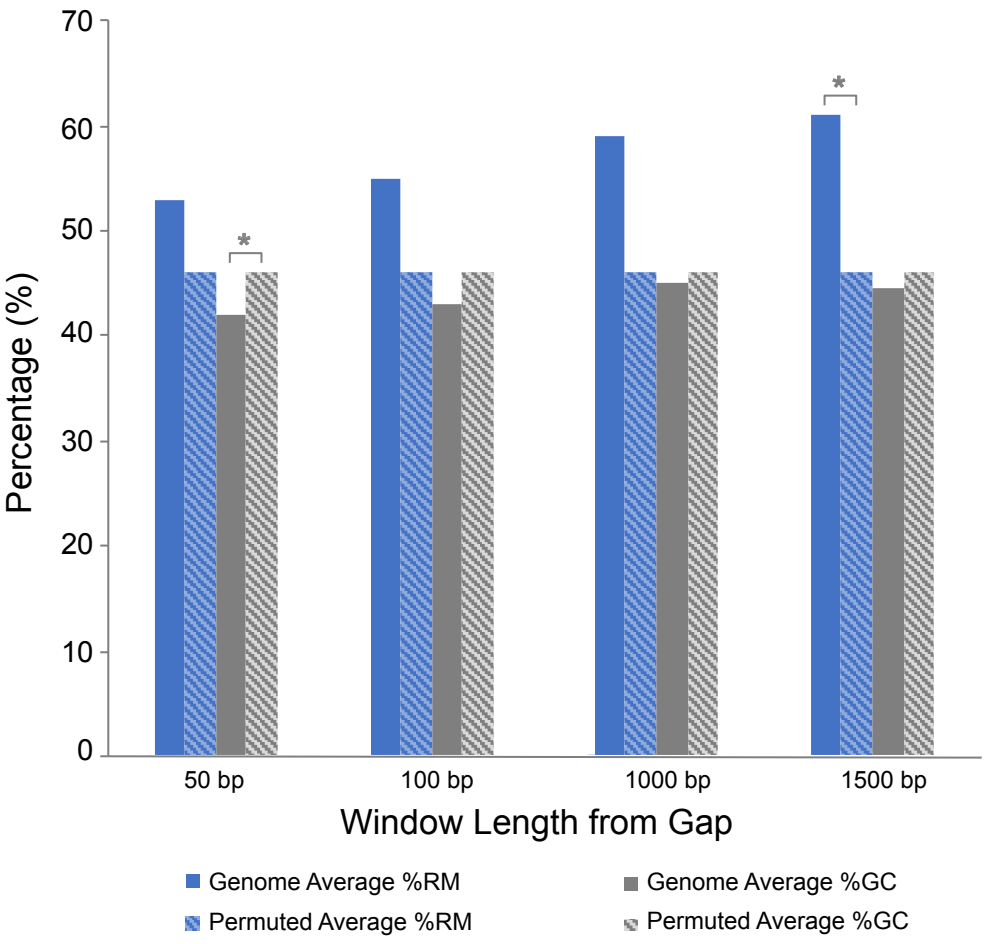



**Figure S4. Statistical enrichment of repeat and low-GC near ME034V assembly gaps.** One thousand randomized permutations of gap positions were generated to compare the true density of repeats and GC content with permuted values. The assessments were made with sequence from 50, 100, 1000, and 1500 bp from the gap flanks. P-values were calculated as the number of instances where the permuted values were either higher (for repeats) or lower (for %GC) than the observed values.

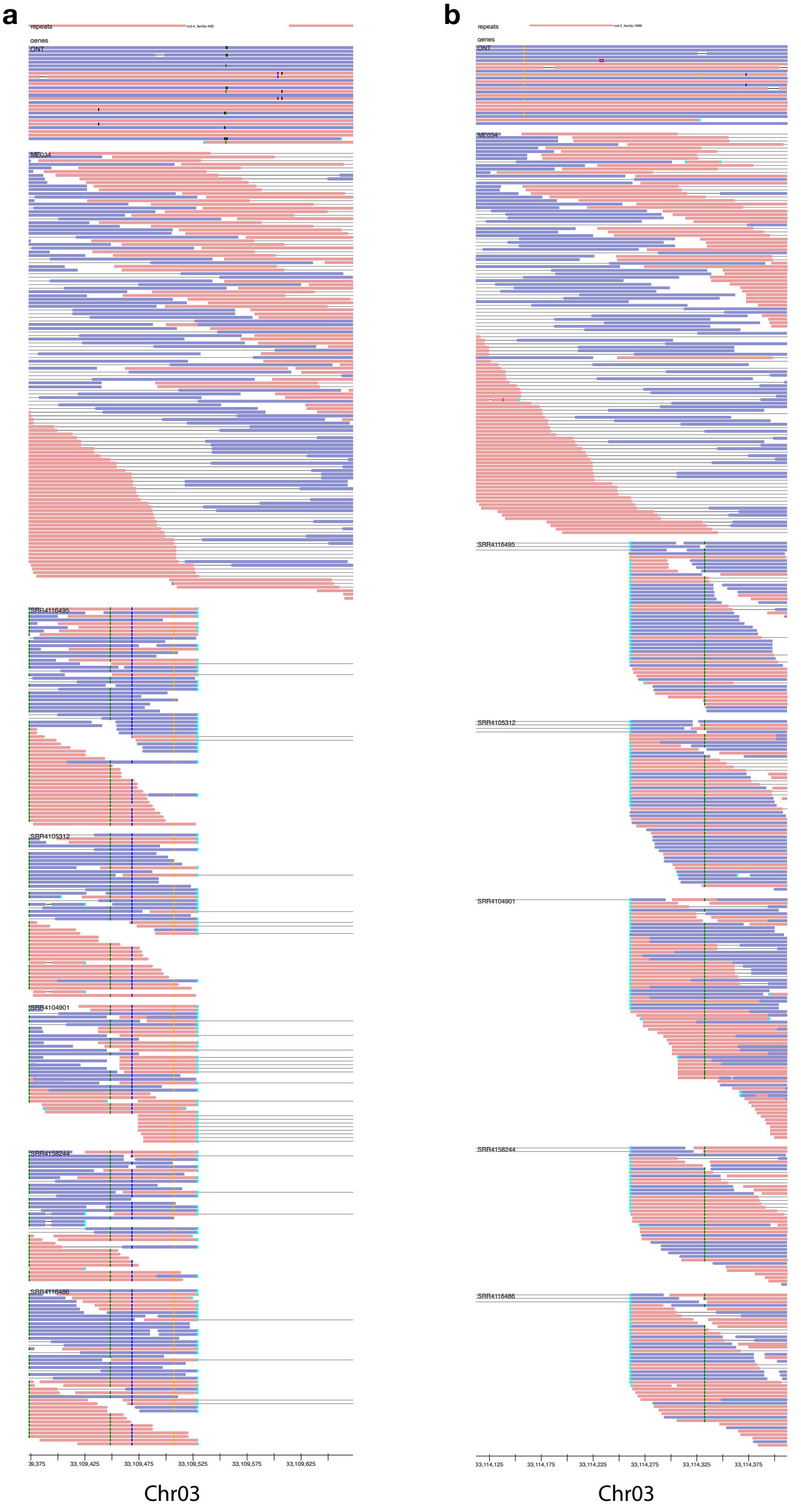



**Figure S5. Read support for the *copia* Insertion (DEL0053315).** A homozygous deletion (DEL00053315) was bioinformatically predicted in multiple *Setaria* samples which correspond to an LTR-rich locus around Chr03:33.2 Mb in ME034V. The left (a) and right (b) flanking regions illustrate alignments of ME034V Oxford Nanopore (ONT) reads, and paired Illumina reads from ME034V, SRR4116495, SRR4105312, SRR4104901 (Table S4). Pairs with too long of an insertion size (denoted by long gray bars connecting reads) and split reads (cyan box at read terminus) are indicated. SNPs are colored boxes of purple, green, dark blue, and orange while gaps are black.

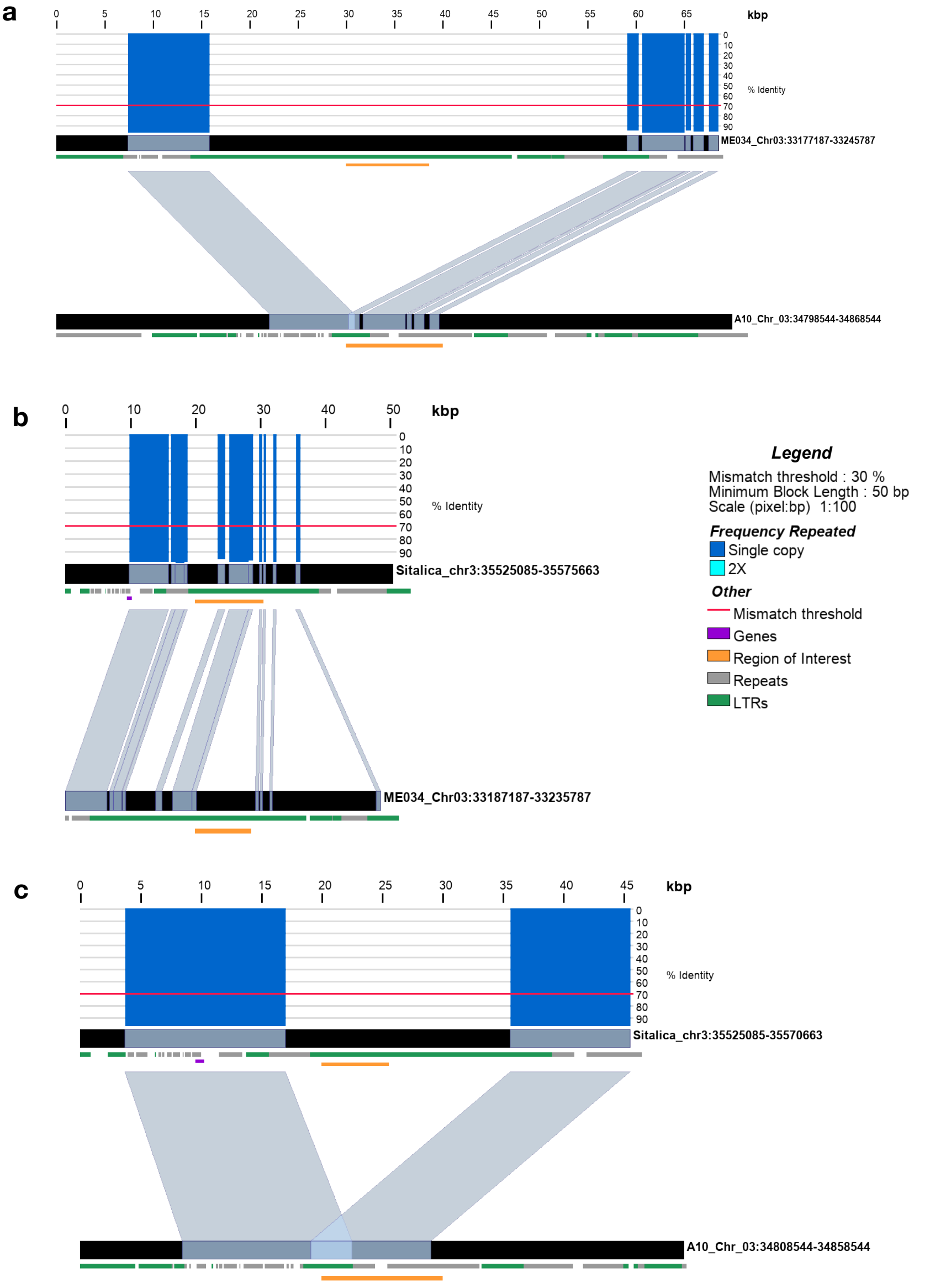



**Figure S6. Genome view of *copia* insertion (DEL0053315).** Synteny alignments between ME034V and A10 (a), ME034V and *S. italica* (b), and *S. italica* and A10 (c) showing the homozygous deletion (DEL00053315) of *copia*-rich region in A10. Blue-grey bars connect the two genomes when DNA sequence with >70% identity is observed (red line indicates threshold). Bars above the top track indicate sequence identity along the chromosomal segment from 0-100%, with the color indicating wither single copy (blue) or double copy (cyan) matches. Green tracks represent LTR elements while gray tracks are all other repeats repeat element classes. Orange tracks indicate the 1:1 homologous region to the predicted deletion site.

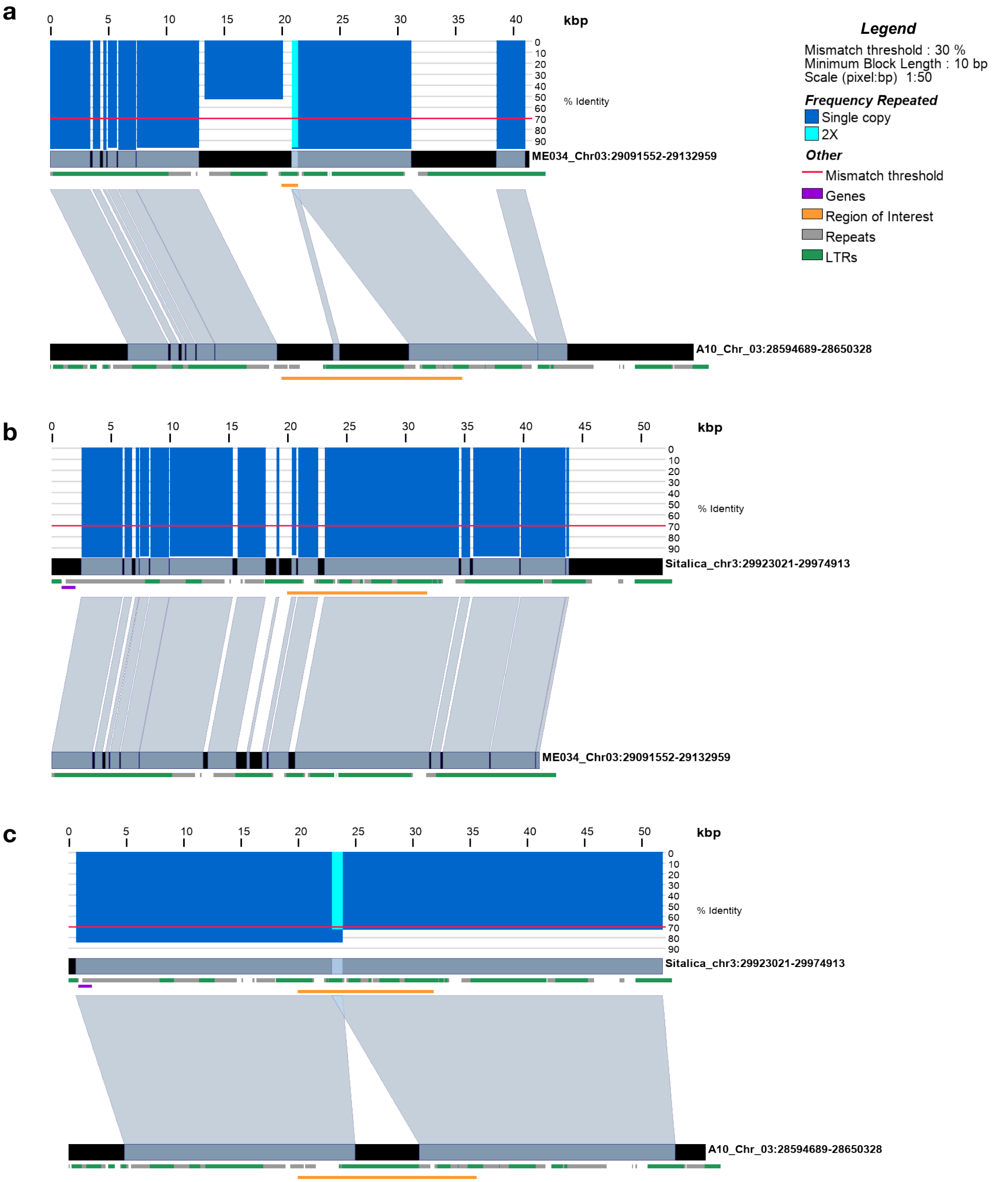



**Figure S7. Genome view of *copia* insertion (DEL00051066).** Synteny alignments between ME034V and A10 (a), ME034V and *S. italica* (b), and *S. italica* and A10 (c) showing a homozygous *copia* insertion unique to the A10 assembly. Blue-grey bars connect the two genomes when DNA sequence with >70% identity is observed (red line indicates threshold). Bars above the top track indicate sequence identity along the chromosomal segment from 0-100%, with the color indicating wither single copy (blue) or double copy (cyan) matches. Green tracks represent LTR elements while gray tracks are all other repeats repeat element classes. Orange tracks indicate the 1:1 homologous region to the predicted deletion site.

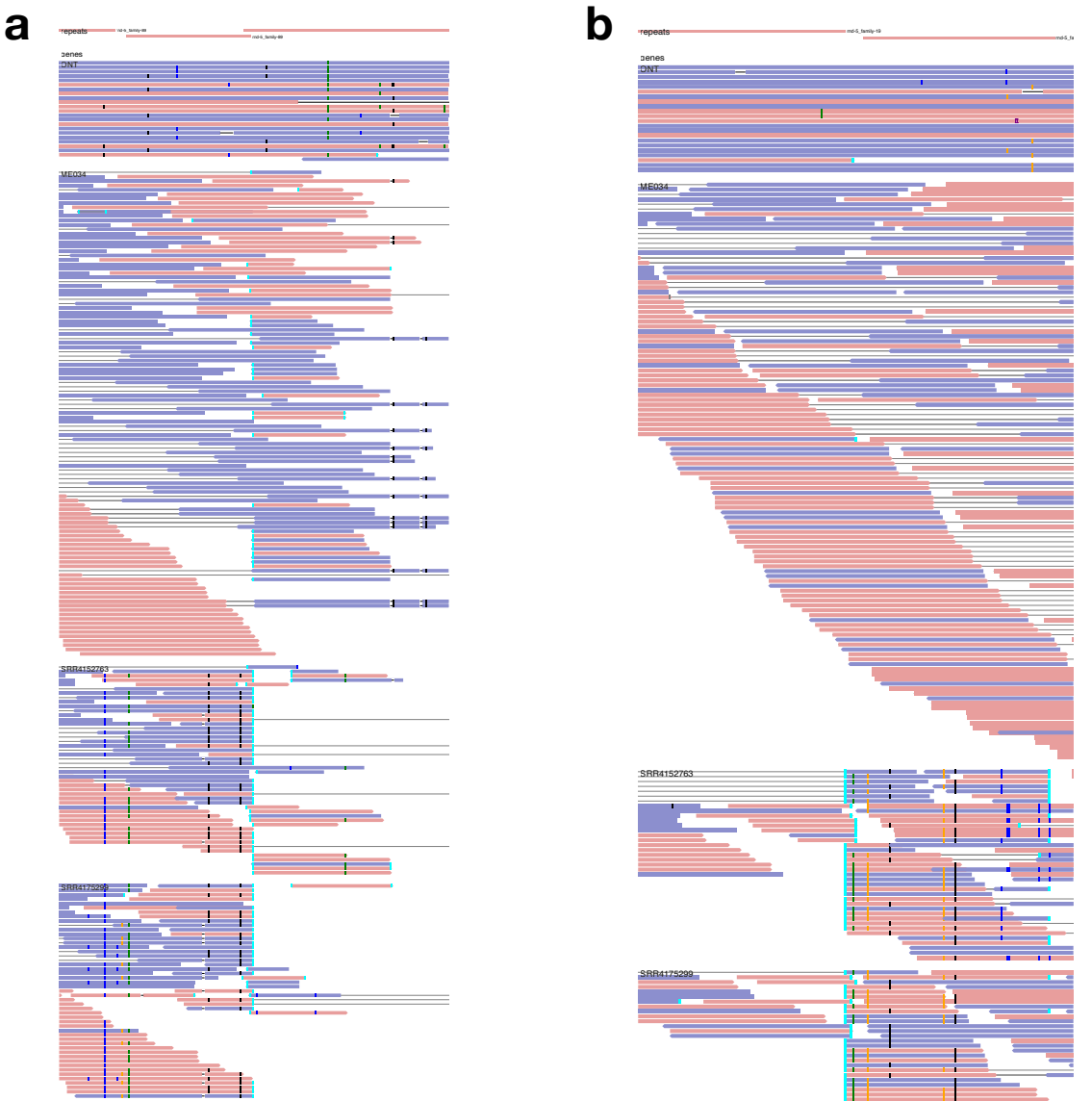



**Figure S8. Read support for *copia* insertion (DEL00051066).** A *copia* insertion in the A10 assembly relative to ME034V and *S. italica* was discovered after assessing the deletion locus DEL00051066. The insertion was identified in multiple *Setaria* samples around Chr03:29.1 Mb in ME034V. Left (a) and right (b) flanking regions illustrate alignments of ME034V ONT reads, ME034V paired Illumina reads, and other *Setaria* cultivars (see label on track for identifier; Table S4). Overlapping split paired reads, indicative of an insertion, are denoted with a bright blue box at the end of the read. SNPs are colored boxes of purple, green, dark blue, and orange while gaps are black.

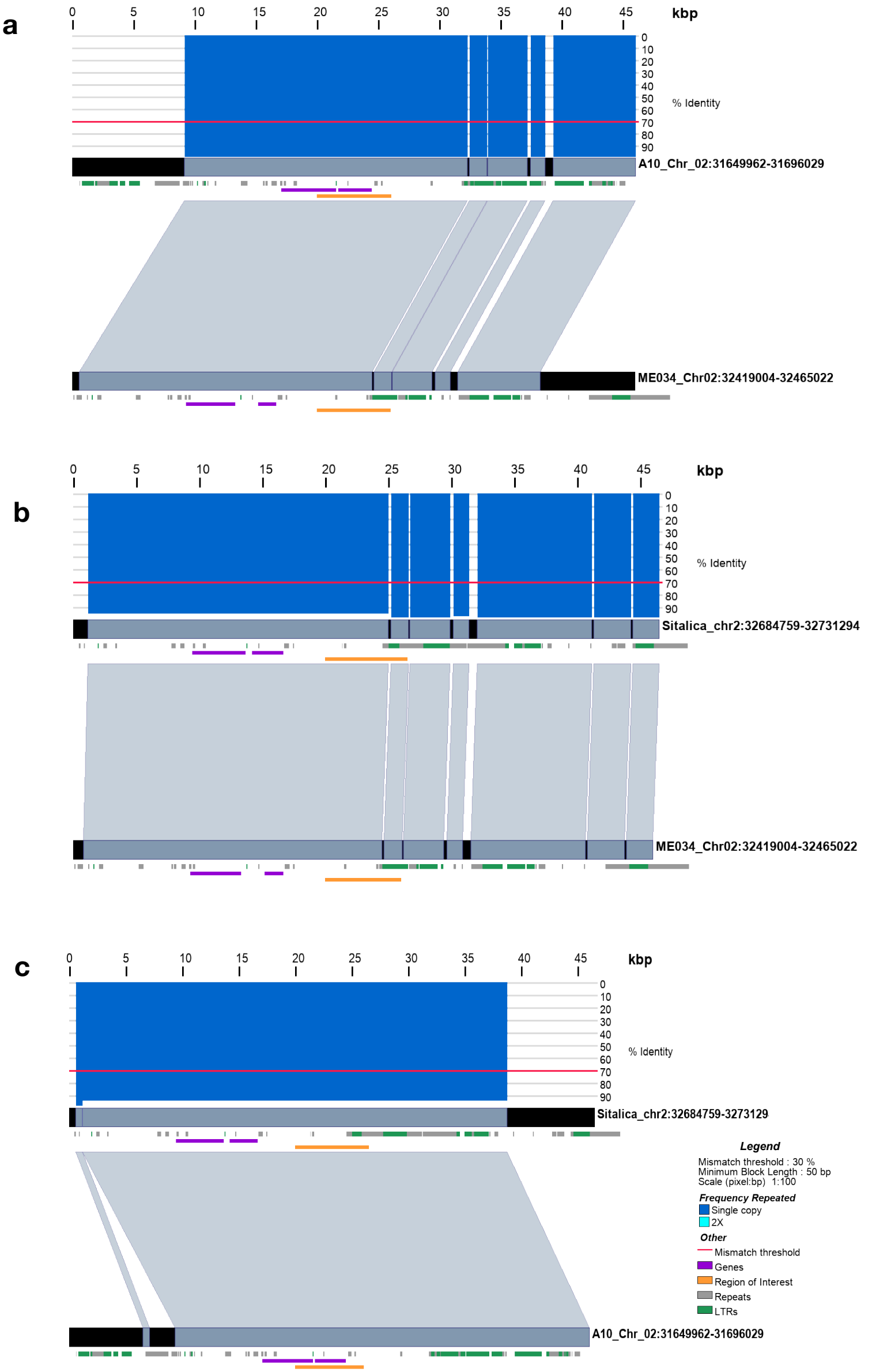



**Figure S9. Genome view of gypsy insertion (DEL00033261).** Synteny alignments between ME034V and A10 (a), ME034V and *S. italica* (b), and *S. italica* and A10 (c) showing the presence of the *gypsy* element in all three genomes. Blue-grey bars connect the two genomes when DNA sequence with >70% identity is observed (red line indicates threshold). Bars above the top track indicate sequence identity along the chromosomal segment from 0-100%, with the color indicating wither single copy (blue) or double copy (cyan) matches. Green tracks represent LTR elements while gray tracks are all other repeats repeat element classes. Orange tracks indicate the 1:1 homologous region to the predicted deletion site.

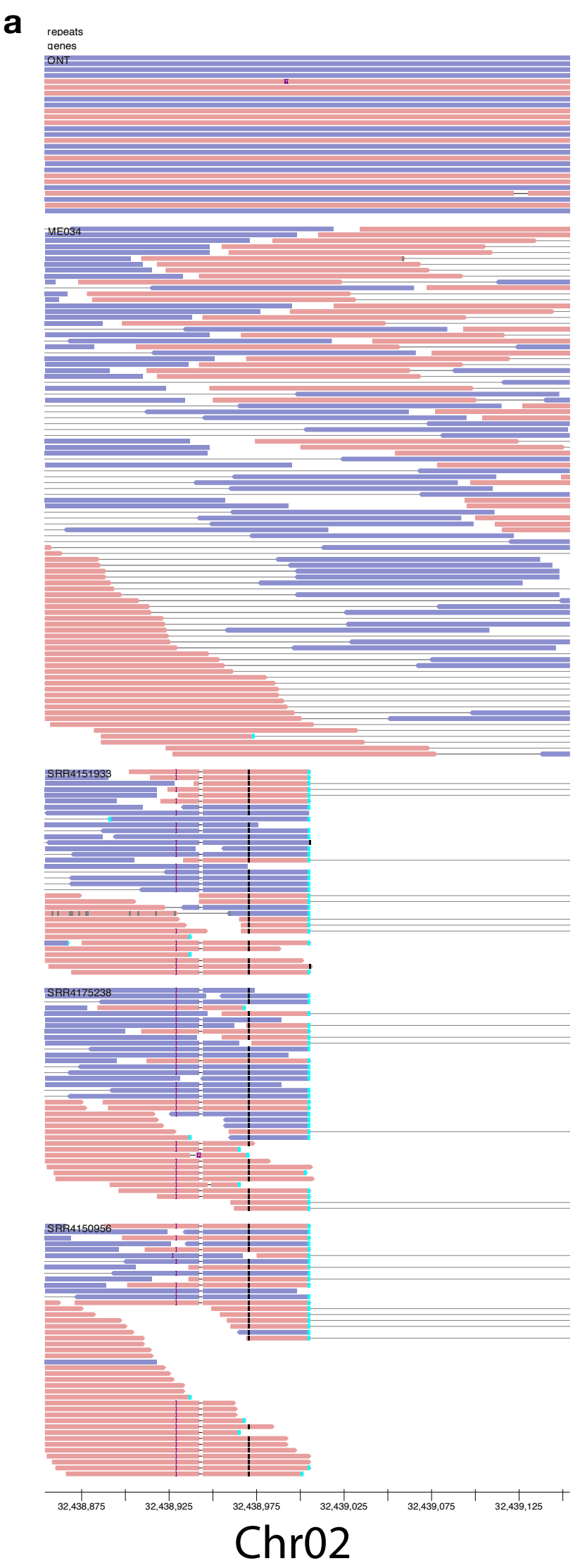




**Figure S10. Read support for *gypsy* insertion (DEL00033261).** A *gypsy* element present in all three *Setaria* assemblies (approximate location Chr02:32.4 Mb in ME034V) is missing in three other *S. viridis* samples SRR4151933 (cultivar Feldman_MF156), SRR4175238 (cultivar Estep_ME018), and SRR4150956 (cultivar Feldman_MF137). Left (a) and right (b) flanking regions illustrate alignments of ME034V ONT reads, ME034V paired Illumina reads, and other *Setaria* cultivars (Table S4). Pairs with too long of an insertion size (denoted by long gray bars connecting reads) and split reads (bright blue box at read terminus) are indicated. SNPs are colored boxes of purple, green, dark blue, and orange while gaps are black.