Specific Downregulation of Spermatogenesis Genes Targeted by 22G RNAs in Hybrid Sterile Males Associated with an X-Chromosome Introgression

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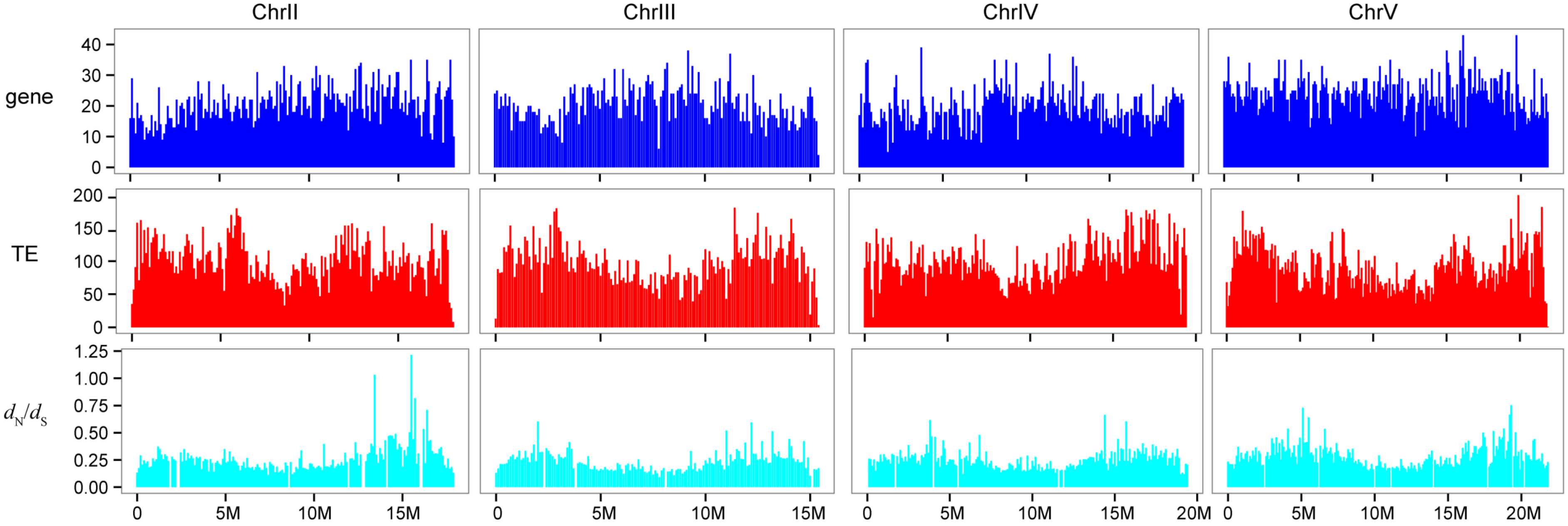
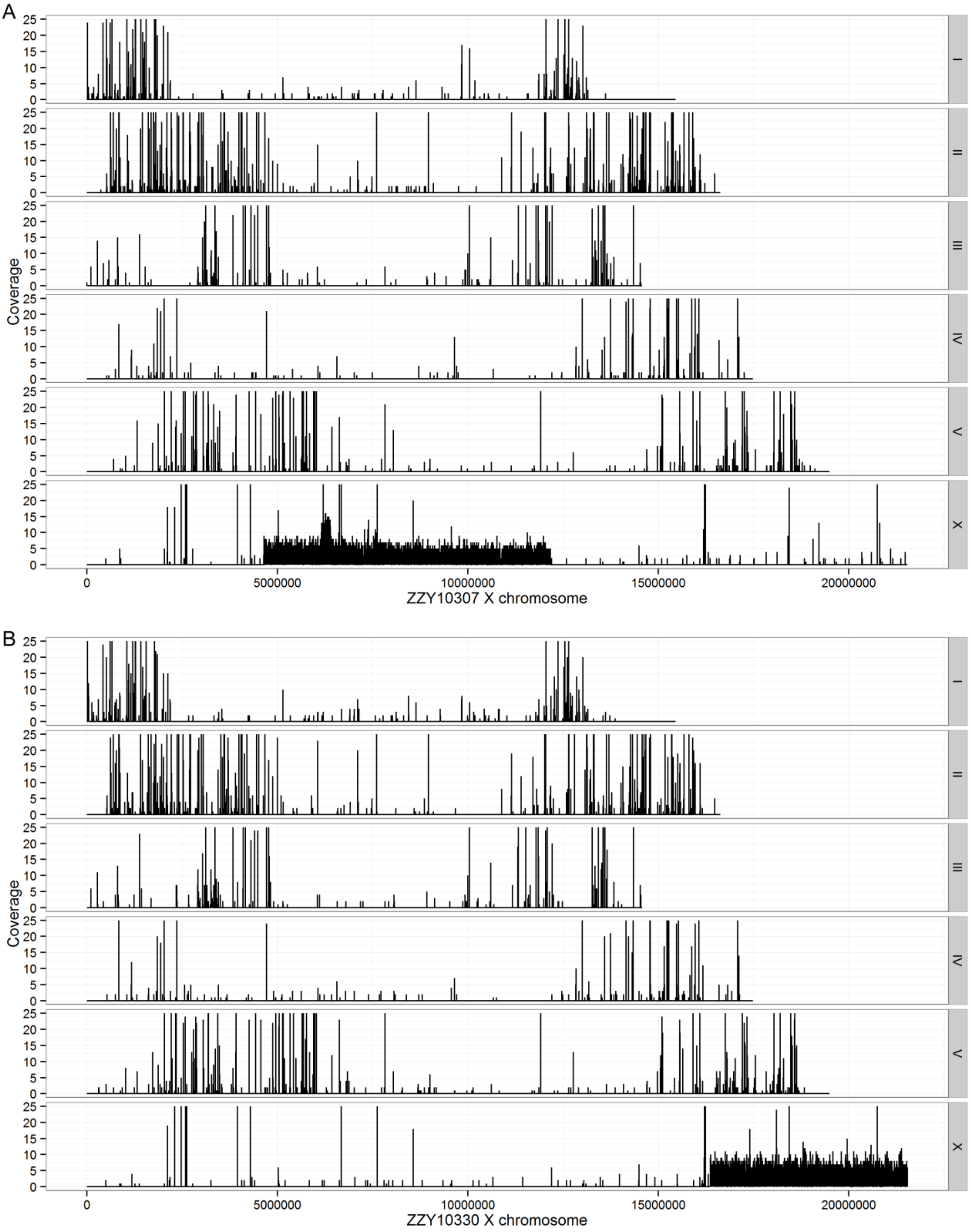
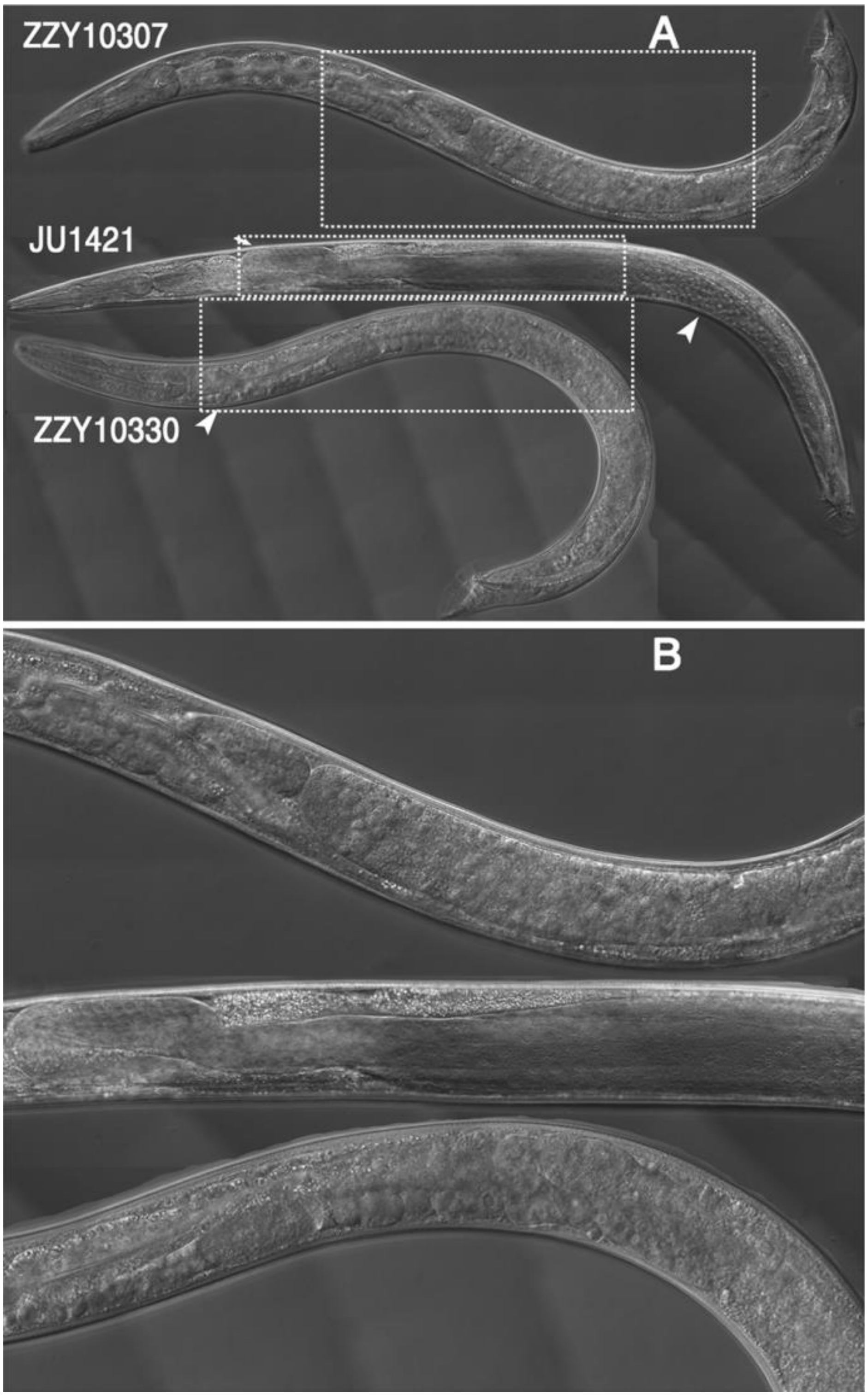
Supplemental Figures (S1-S9)



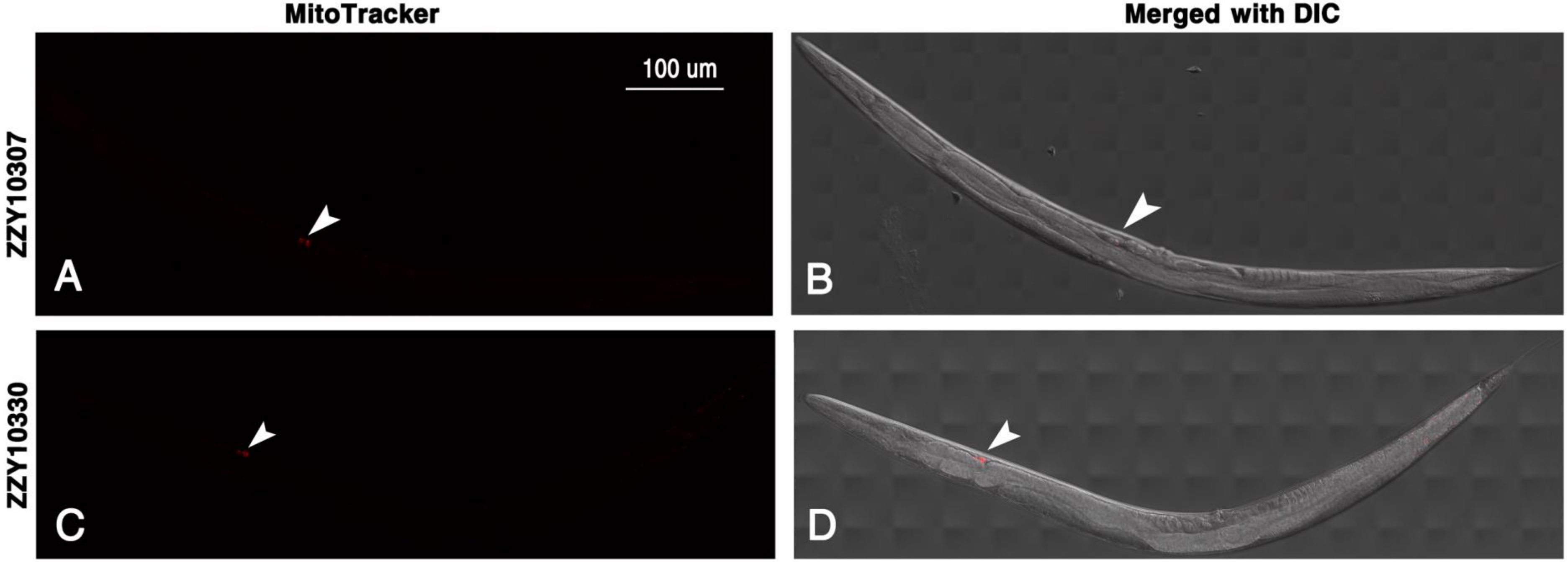
Supplemental Figure 1 Overview of *C. nigoni* genome (Continued from Figure 1). Shown are densities of coding genes (blue), transposable element (TE) (red) and dN/dS ratio (cyan) of orthologous pairs between *C. nigoni* and *C. briggsae* over chromosome II-V, all in a window size of 100 Kbps.



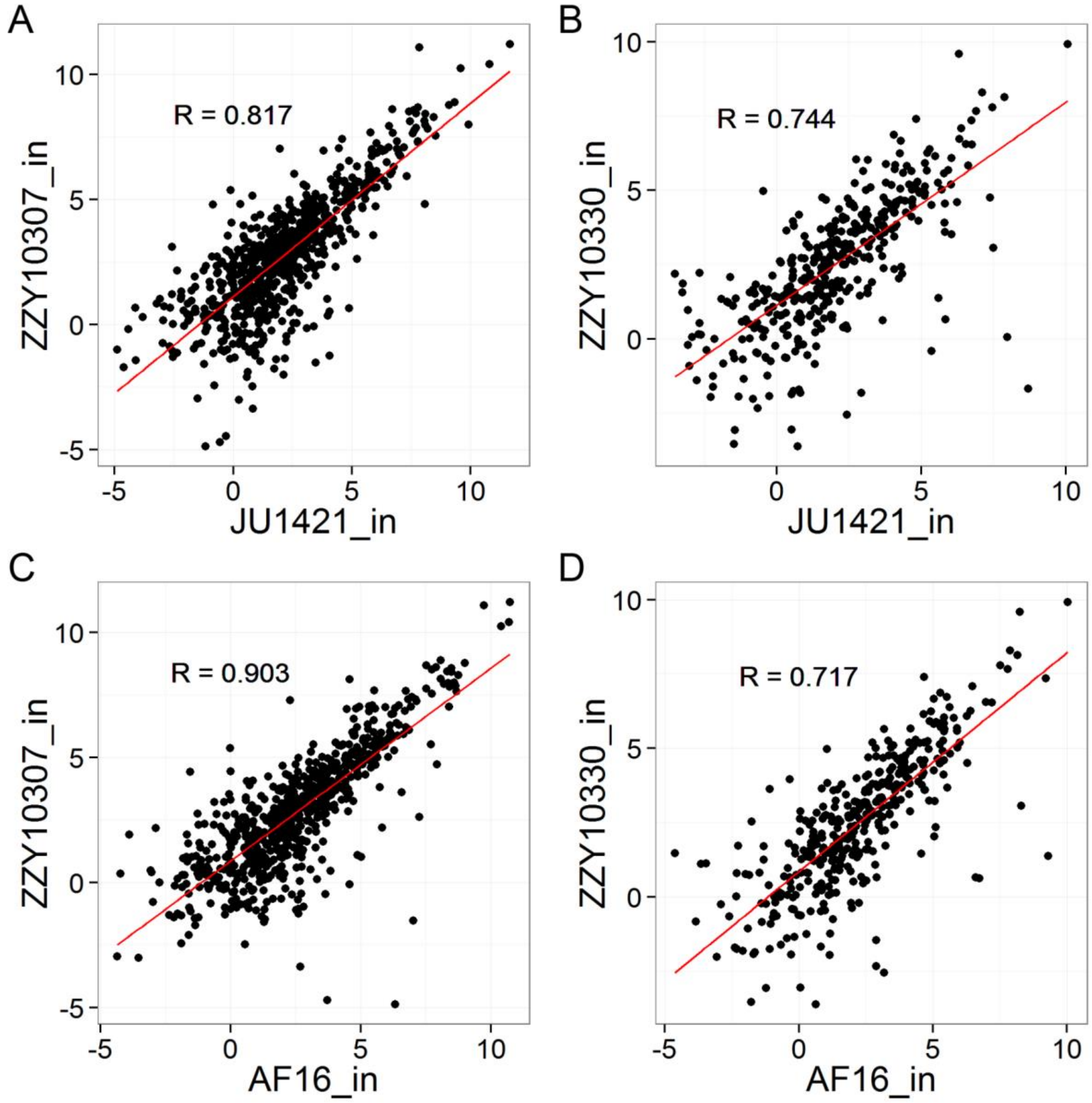
Supplemental Figure 2 Confirmation of introgression boundaries by NGS for ZZY10307 (A) and ZZY10330 (B), each carrying an introgression derived only from *C. briggsae* X chromosome. Shown are genotyping results for autosomes in the same format as that for X chromosome shown in Figure 2. Read coverage (y axis) is shown across *C. briggsae* chromosome coordinates in base pair.



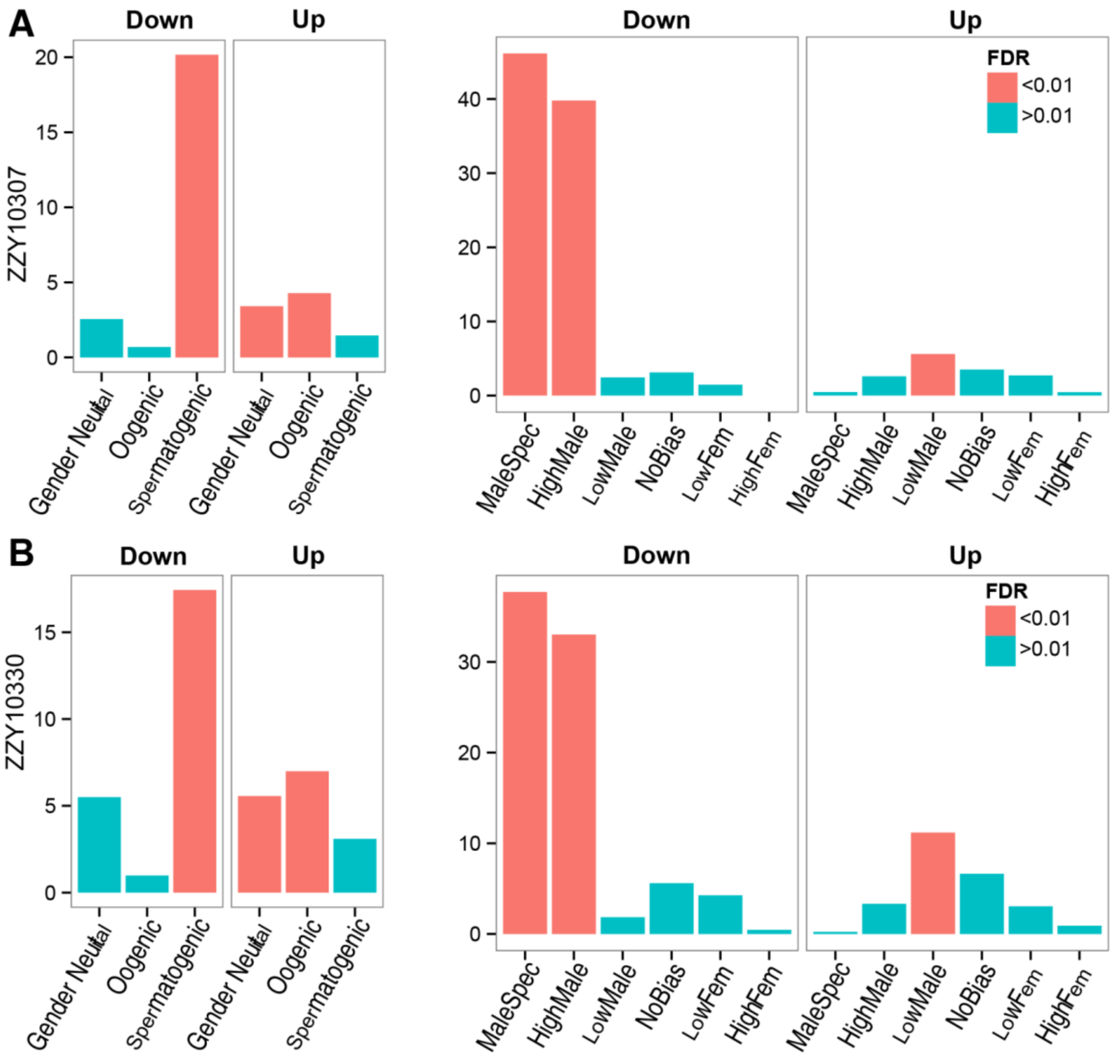
Supplemental Figure 3 DIC micrographs of male germline. (A) Shown are adult male of ZZY10307 (top), *C. nigoni* (JU1421) (middle), andZZY10330 (bottom). A “U” shaped turn in *C. nigoni* germline is indicated with an arrow and sperms with an arrow head. Displacement of sperms is indicated with an arrowhead in ZZY10330. (B) A zoom-in view of partial germline (indicated with dashed rectangle) of ZZY10307 (top), JU1421 (middle) and ZZY10330 (bottom) respectively. Note that germlines in both hybrid are disorganized compared with that of JU1421.



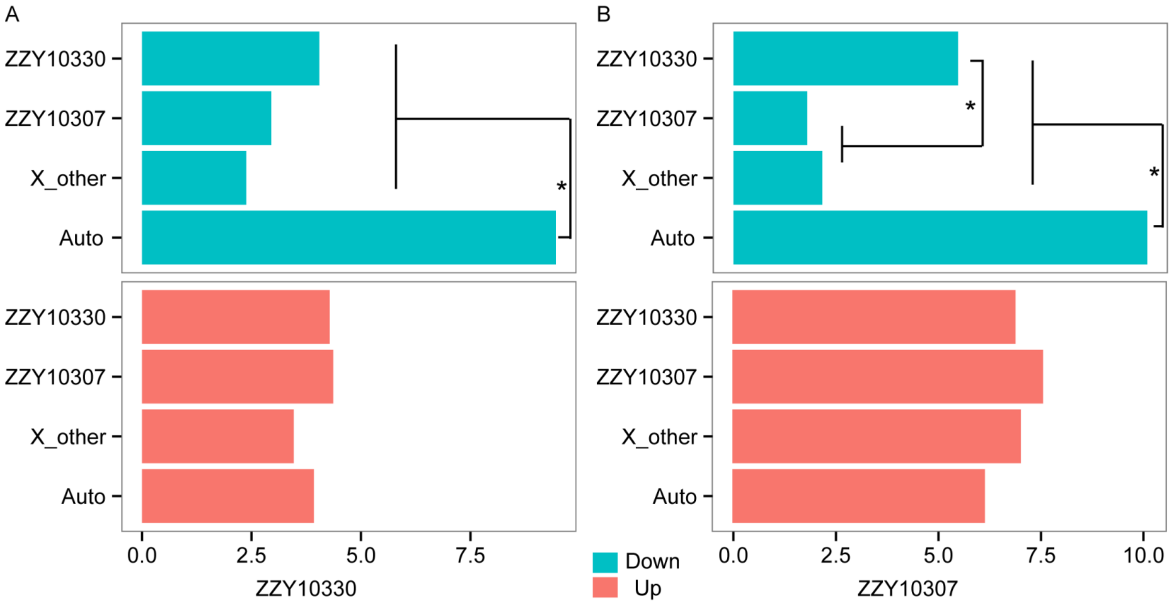
Supplemental Figure 4 Mating test between hybrid sterile male and *C. nigoni* (JU1421) virgin female. (A & B) Micrographs of MitoTracker stained *C. nigoni* females and its superimposition with DIC respectively. Micrographs were taken 24 hours after mating with ZZY10307 males. Transferred sperms were indicated with arrowhead. (C & D) Micrographs of MitoTracker stained *C. nigoni* females and its superimposition with DIC respectively. Micrographs were taken 24 hours after mating with ZZY10330 males. Transferred sperms were indicated with arrowhead.



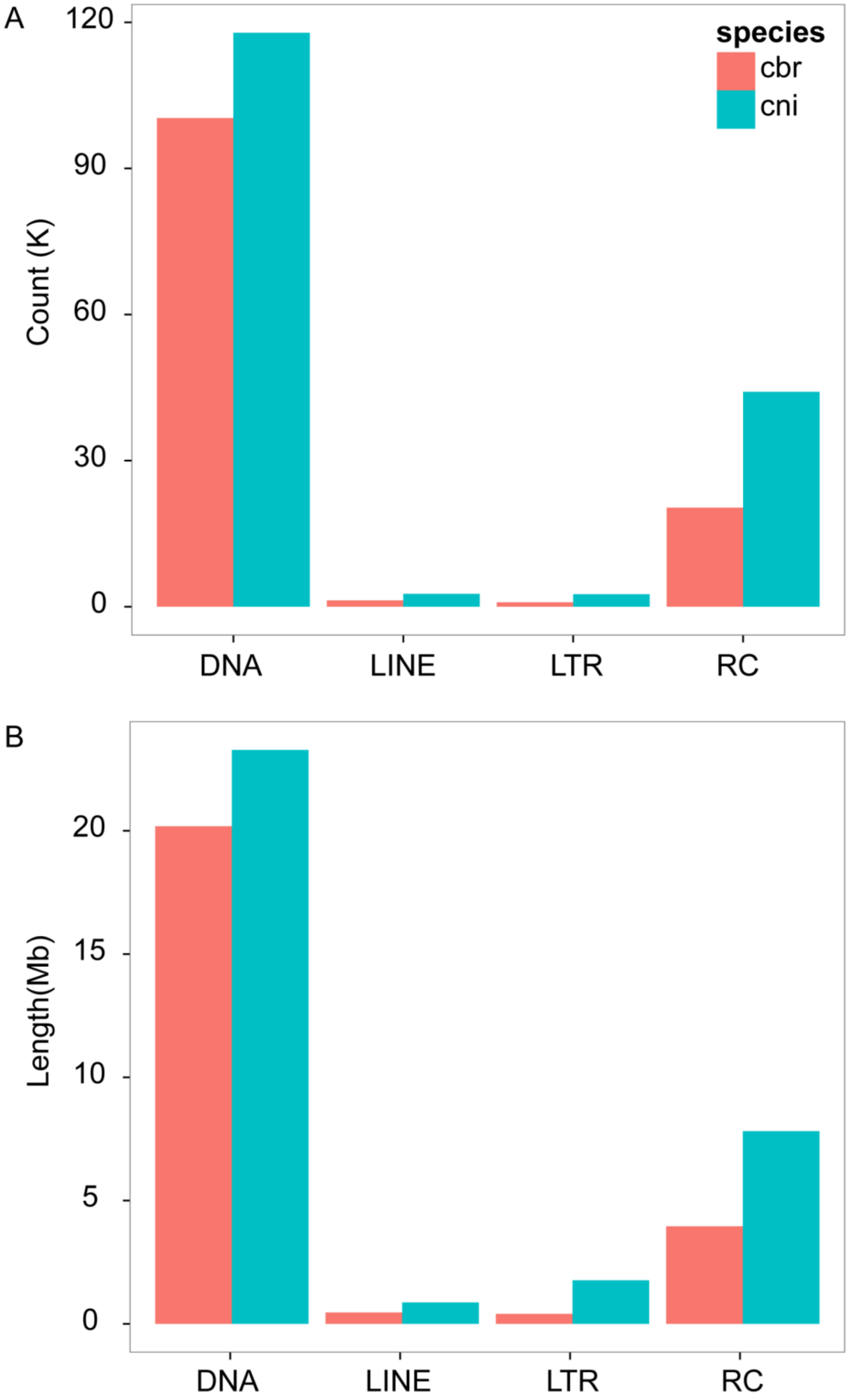
Supplemental Figure 5 Pairwise comparison of expression between genes expressed within (_in) the introgression region on the X chromosome and their orthologous ones in either of parental strains. (A) and (C), Scatterplot showing expression of the genes located within ZZY10307 introgression region and their orthologues in AF16 and JU1421 respectively. (B) and (D), Scatterplot showing expression of the genes located within ZZY10330 introgression region and their orthologues in AF16 and JU1421 respectively.



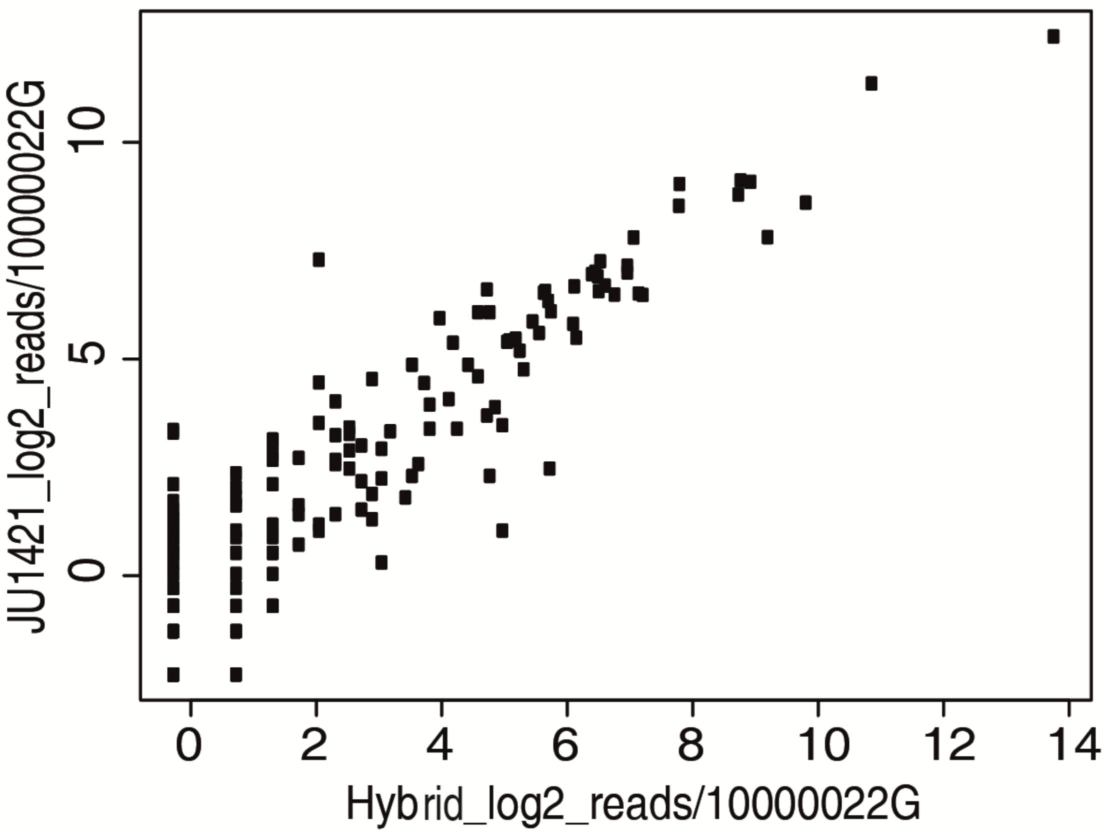
Supplemental Figure 6 Enrichment analysis of misregulated (down- (cyan) and up-regulated (red)) genes in either ZZY10307 or ZZY10330 out of gonad- (left) and sex-specific genes (right) respectively. (A) The enrichment analysis for ZZY10307. (B) The enrichment analysis for ZZY10330. The Y axis indicates the percentage of genes while X axis indicates gene otology defined previously.



Supplemental Figure 7 The down-regulated genes are significantly enriched on autosomes in both hybrid strains as shown in Figure 3E. (A and B) Ratios of down- (green) or upregulated genes (brick) on different part of genome of ZZY10307 and ZZY10330. A significant ratio change was indicated with a * ($p < 0.01$, Fisher's exact test). Expression of the genes within introgression ZZY10330 also shows a significant decrease than other genes on the X chromosome.



Supplemental Figure 8 The copy number (A) and total length (B) of different TE classes identified in *C. briggsae* (cbr, A) and *C. nigoni* genome (cni, B).



Supplemental Figure 9 Scatterplot showing 22G RNAs mapping to individual TEs in hybrid ZZY10330 and wild type JU1421.