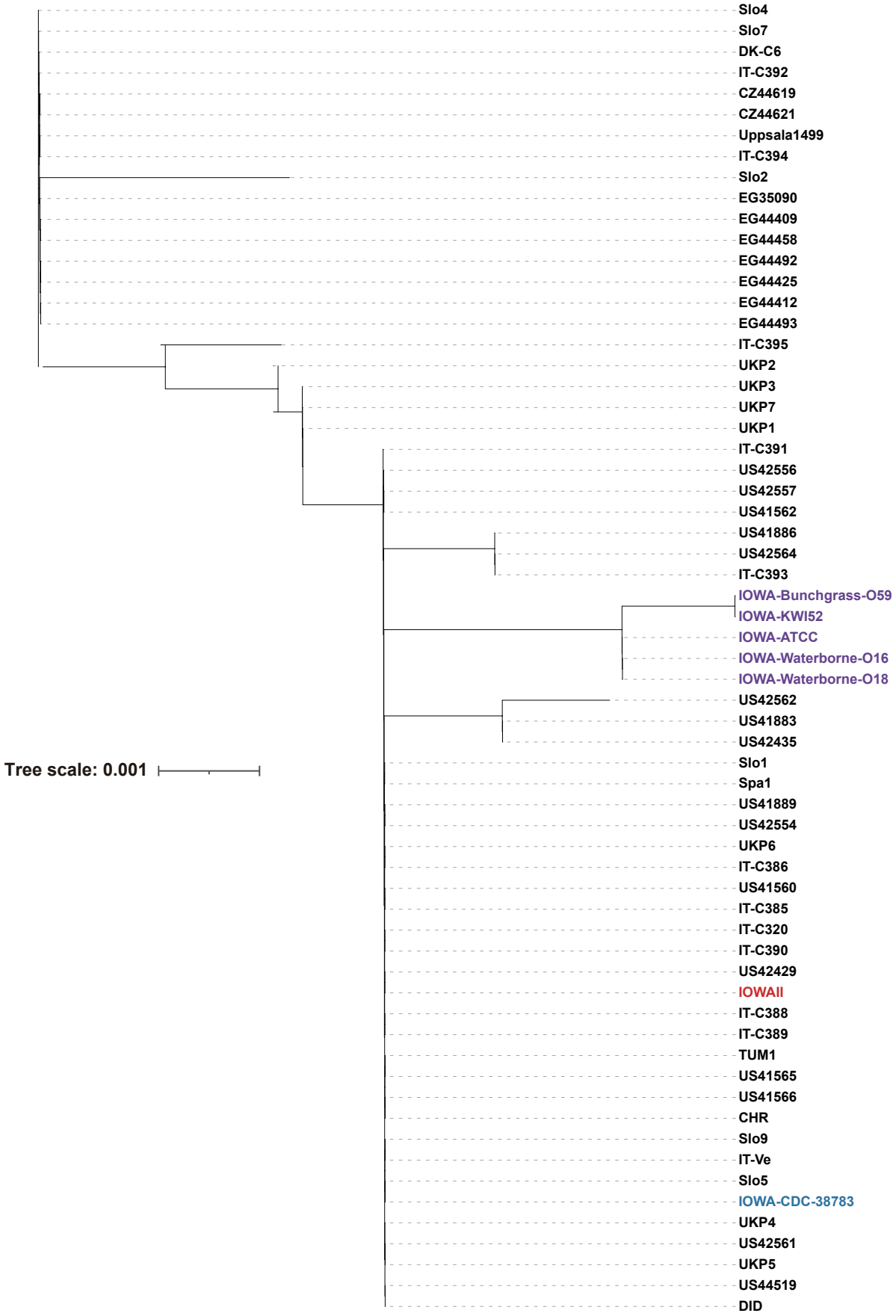
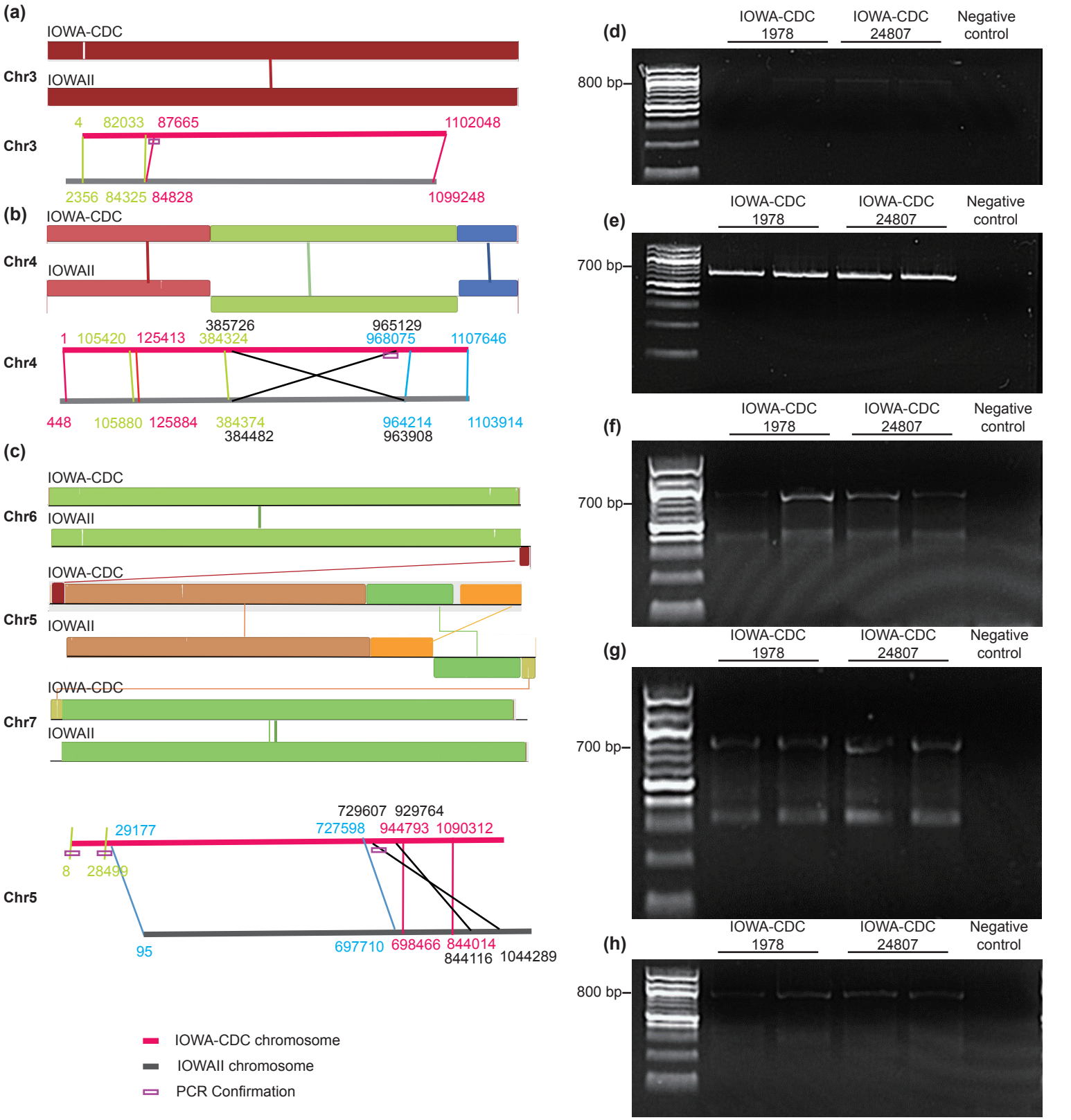
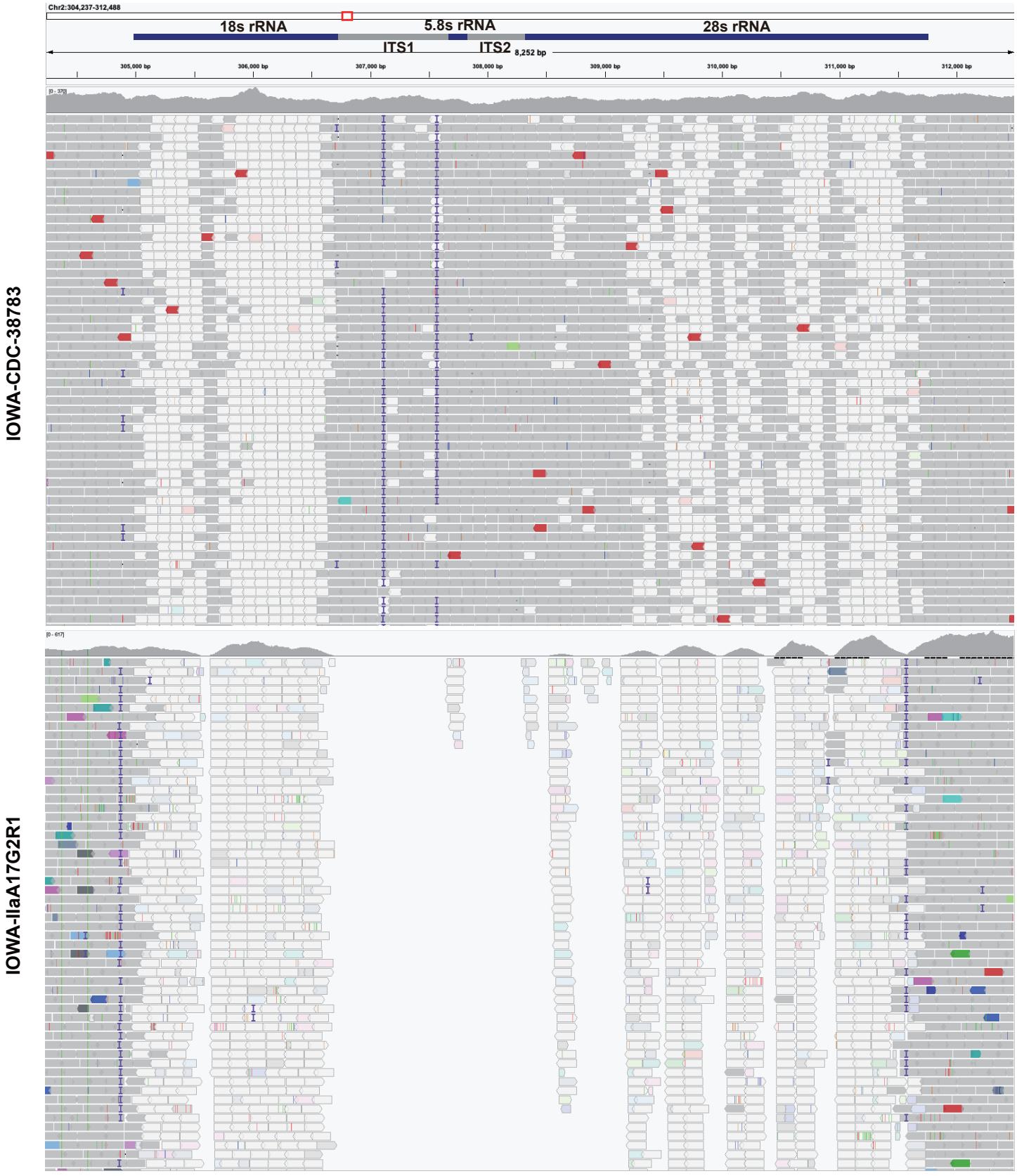


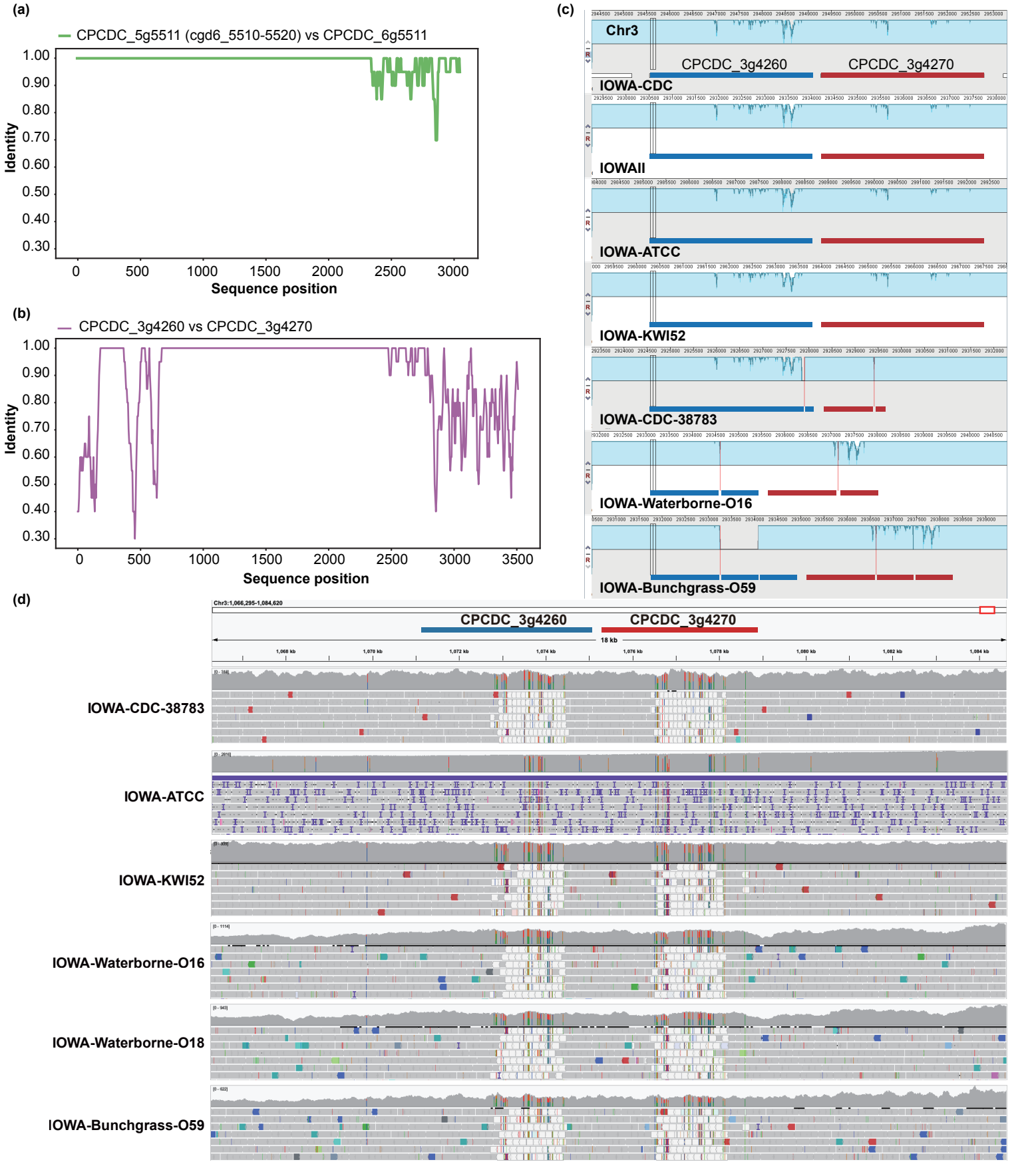
Supplemental Fig. 1. Phylogenetic analysis of the *gp60* gene among 63 isolates. ML analysis was conducted using the GTR+I model. The branch colors represent different subtypes.



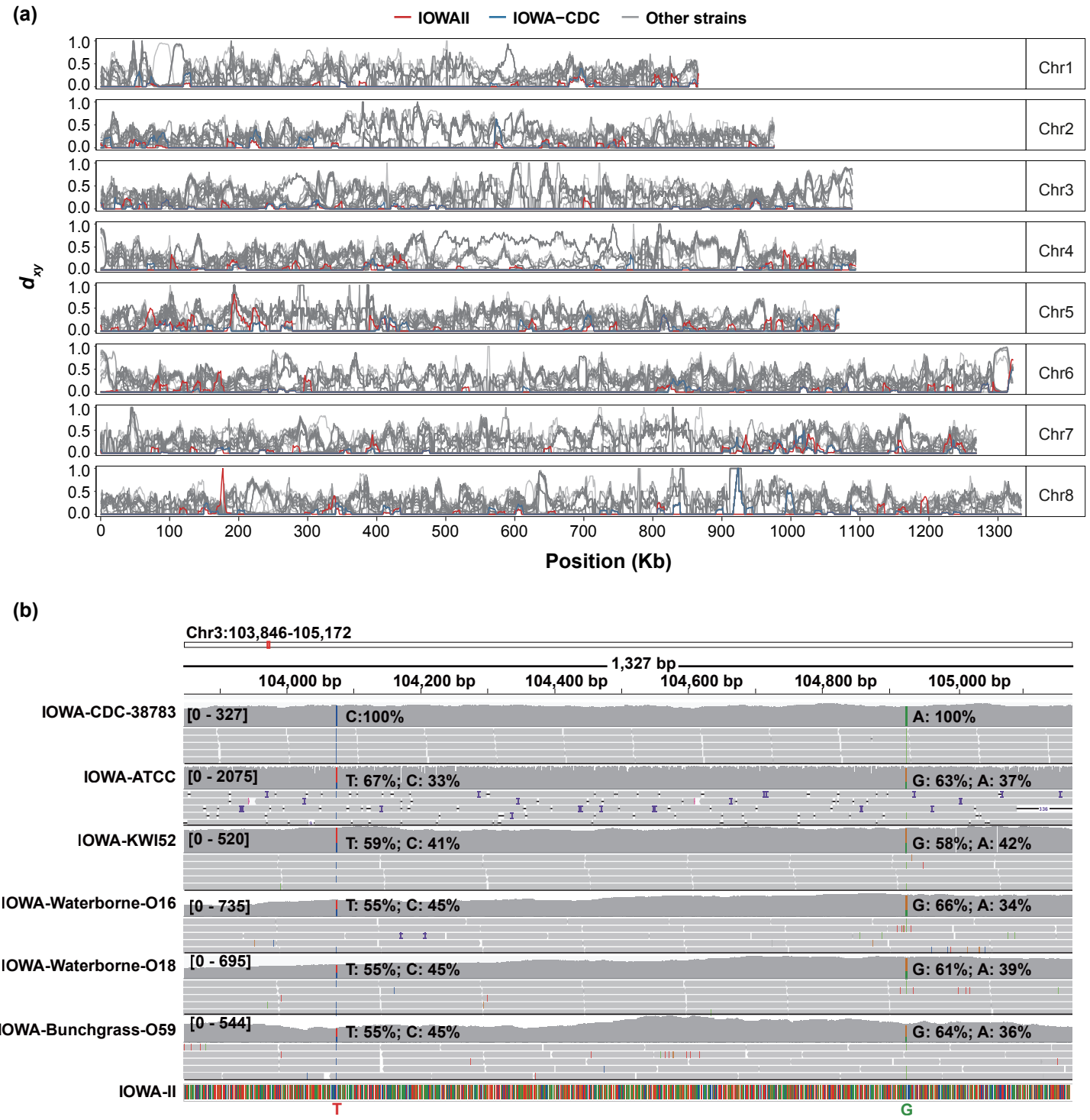
Supplemental Fig. 2. Syntenic relationships between assemblies of *Cryptosporidium parvum* IOWA-CDC and IOWAII. (a-c) Synteny in chromosomes 3, 4, 5, 6, 7, and 8. The syntenic relationship between IOWA-CDC and IOWAII is displayed in the top panel, while the locations of PCR amplicons are shown in the bottom panel (synteny in parallel-colored lines and inversions in crossed black lines). (d-h) PCR validation using C. parvum IOWA-CDC DNA. Lanes 1 to 6 in all gels are the DNA ladder, two replicates of IOWA-CDC-1978, two replicates of IOWA-CDC-24807, and negative control, respectively. (a) 5632 bp insertion in chromosome 3 in the IOWA-CDC genome. (b) Reverse assembly of a middle fragment (579,427 bp) in chromosome 3 in the IOWA-CDC genome. (c) Insertion at the 5' end of chromosome 5 as well as reverse assembly and translocation at the 5' end of chromosome 5 from chromosome 6 and 3' end of chromosome 5 from chromosome 7 in the IOWA-CDC genome. (d) Confirmation of the 5,632 bp insertion at nucleotide position 87,665. Both IOWA-CDC preparations produced the expected 938 bp PCR product. (e) Confirmation of the right joint of the reversely assembled fragment around nucleotide position 965,129. Both I OWA-CDC preparations produced the expected 689 bp PCR product. (f) Confirmation of the 5 kb insertion at the 5' end of chromosome 5 in IOWA-CDC genome. Both IOWA-CDC preparations produced the expected 755 bp PCR product. (g) Confirmation of the insertion around nucleotide position 29,177 of chromosome 5. Both IOWA-CDC preparations produced the expected 759 bp PCR product. (h) Confirmation of the left joint (around nucleotide position 727,598) of the reversely assembled fragment in chromosome 5. Both IOWA-CDC preparations produced the expected 798 bp PCR product.



Supplemental Fig. 3. Sequence differences in the rRNA unit in chromosome 2 between IOWA-CDC and IOWA-IIaA17G2R1 lines through close inspection of the read-mapping result in the region of chromosome 2 from nucleotide position 304,237 to 312,488. All reads of the IOWA lines were mapped to the IOWA-CDC genome. The IGV results of the IOWA-CDC and IOWA-IIaA17G2R1 illustrate here. The numbers in the square brackets show the average sequencing depth. The vertical bars show the percentage of coverage at a locus. Rectangles with light gray borders and transparent fill are reads that can be aligned to multiple locations.



Supplemental Fig. 4. Sequence similarity between paralogous genes and its effect on genome assembly. (a) Sequence identity between CPCDC_5g5511 (cgd6_5510-5520) and CPCDC_6g5511 across the length of the genes. (b) Sequence identity between CPCDC_3g4260 and CPCDC_3g4270 across the length of the genes. (c) Assembly errors at the CPCDC_3g4260 and CPCDC_3g4270 loci in genomes acquired through long-read (the first four panels) and short-read (the next three panels) sequencing. The color blocks (known as Locally Collinear Blocks) are conserved segments of sequences, whereas the inverted white peaks within each block are sequence divergence between the reference genome and the other genome. Assembled chromosomes or contigs are bordered by vertical red lines. The CPCDC_3g4260 and CPCDC_3g4270 genes are indicated by blue and red boxes, respectively. Contig breakages are seen in the two genes in the assemblies acquired through short-read sequencing. (d) Close inspection of the read-mapping at the CPCDC_3g4260 and CPCDC_3g4270 loci. All reads of IOWA lines were mapped to the IOWA-CDC genome. The numbers in the square brackets show the average sequencing depth. The vertical bars show the percentage of coverage at a locus, while colored lines represent the four types of nucleotides. Rectangles with light gray borders and transparent fill are reads that can be aligned to multiple locations.



Supplemental Fig. 5. Presence of introgression of exogenous sequences into the IOWA-IIaA17G2R1 lines. (a) The dxy between IOWA IIaA17G2R1 and other *C. parvum* IIa strains across the eight chromosomes in 10-kb sliding window. (b) Close inspection of the read-mapping result in the region of chromosome 3 from nucleotide position 103,846 to 105,172. All reads of IOWA lines were mapped to the IOWA-II genome. The numbers in the square brackets show the average sequencing depth. The vertical bars show the percentage of coverage at a locus, while colored lines represent the four types of nucleotides.