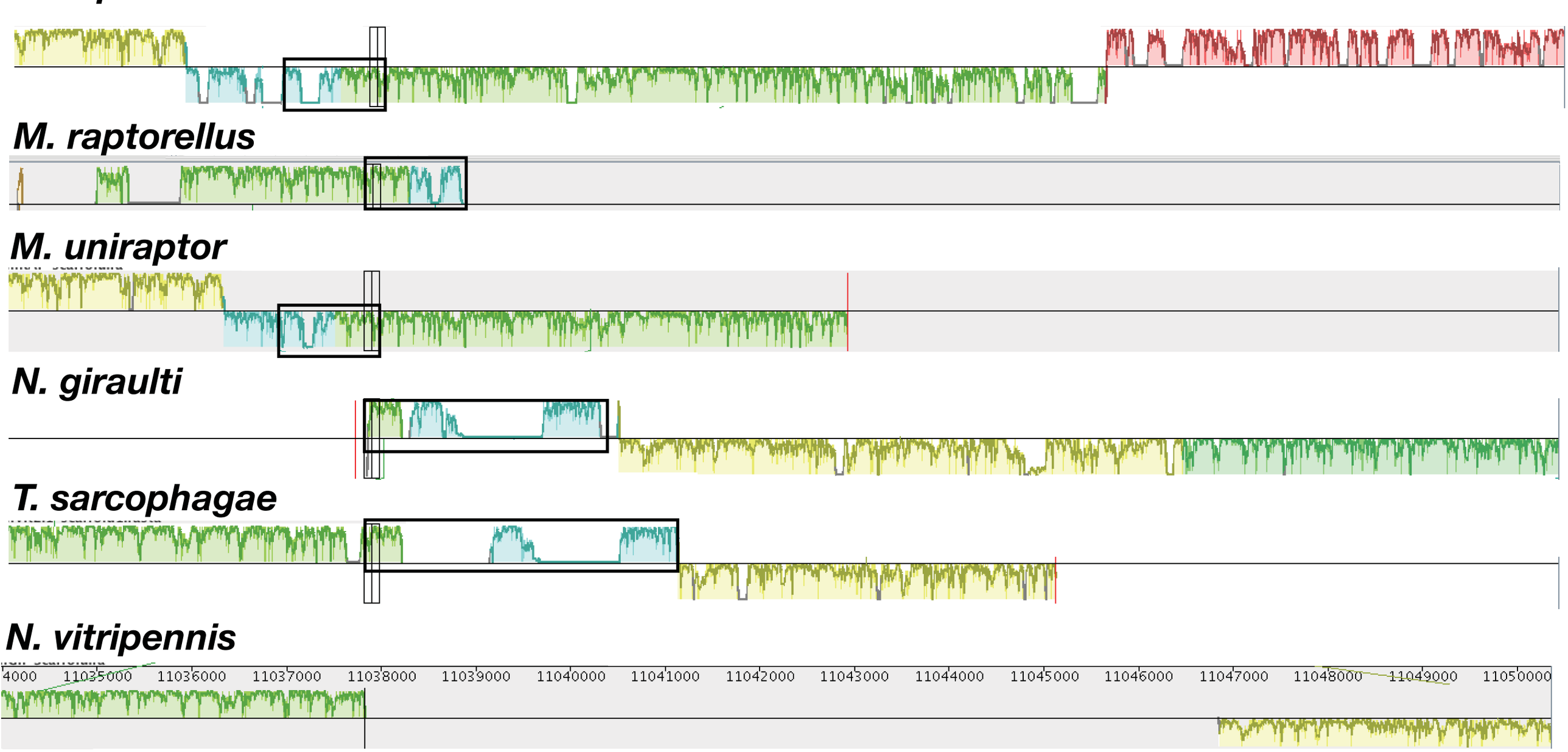
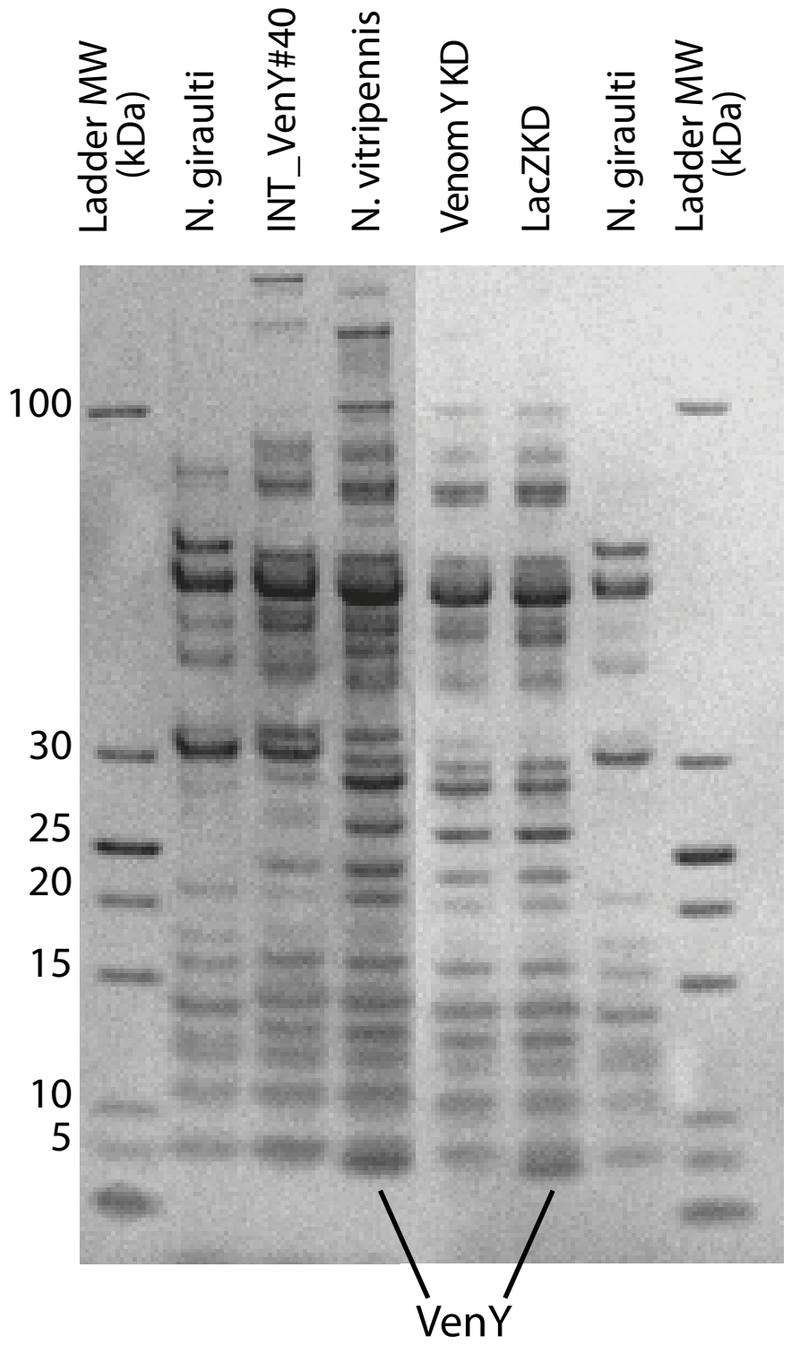
Supplemental Figure 1: Synteny of the VenY genomic region. The genome scaffolds showing the position and direction of $VenY_A$ (yellow bars) and $VenY_B$ (orange bars). The star (*) signifies a nonfunctional gene. Colors represent synteny blocks across genomes.

Supplemental Figure 2: Deletion of VenY region in *N. vitripennis*. A zoomed out view of the genome scaffolds showing the position and direction of VenY region from Sup Fig 1 in black boxes. Colors represent synteny blocks across genomes. Flanking regions of *N. vitripennis* match the other scaffolds, but large indel removed ~2500bp including the VenomY region



Supplemental Figure 3: An SDS PAGE gel showing that VenY is not affected by the LacZ RNAi control. The gel contains size separation of venom reservoir proteins for *N. giraulti*, INT_VenY#40 (an introgression line between *N. giraulti* and *N. vitripennis* that does not contain VenY), whole *N. vitripennis* venom, *N. vitripennis* following VenY knocked down via RNAi, and *N. vitripennis* LacZ RNAi control venom. VenY is the smallest protein visible on the gel, with a molecular weight around 5kDa.



Supplemental Figure 4: Sequenced VenY peptides from whole venom proteomic sequencing aligned to the full predicted VenY protein.