

Supporting information for:

Revealing the venomous secrets of the spider's web

Figures S1-S8

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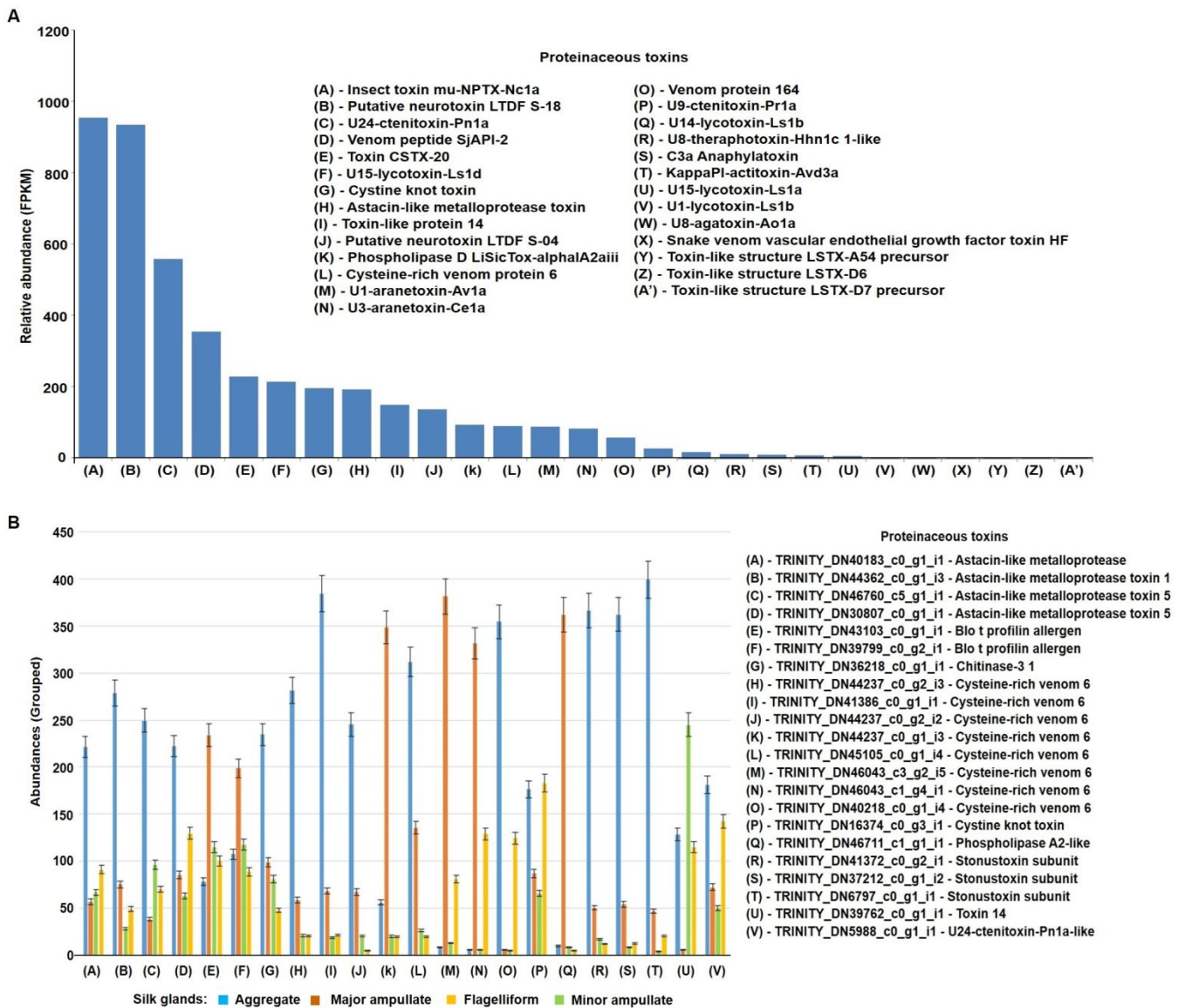


Figure S1. Proteinaceous toxins quantification from *N. clavipes* silk-producing glands. (A) Relative abundance (expressed as FPKM) of the transcripts encoding proteins and/or peptides similar to proteinaceous toxins found in the transcriptome. Bars represent the sum of FPKM for all transcripts according to their annotated toxin function. (B) Quantitative label-free analysis of the proteinaceous toxins-like by gel-free bottom-up proteomics approach. Abundances were based upon precursor/peptide peak intensities.

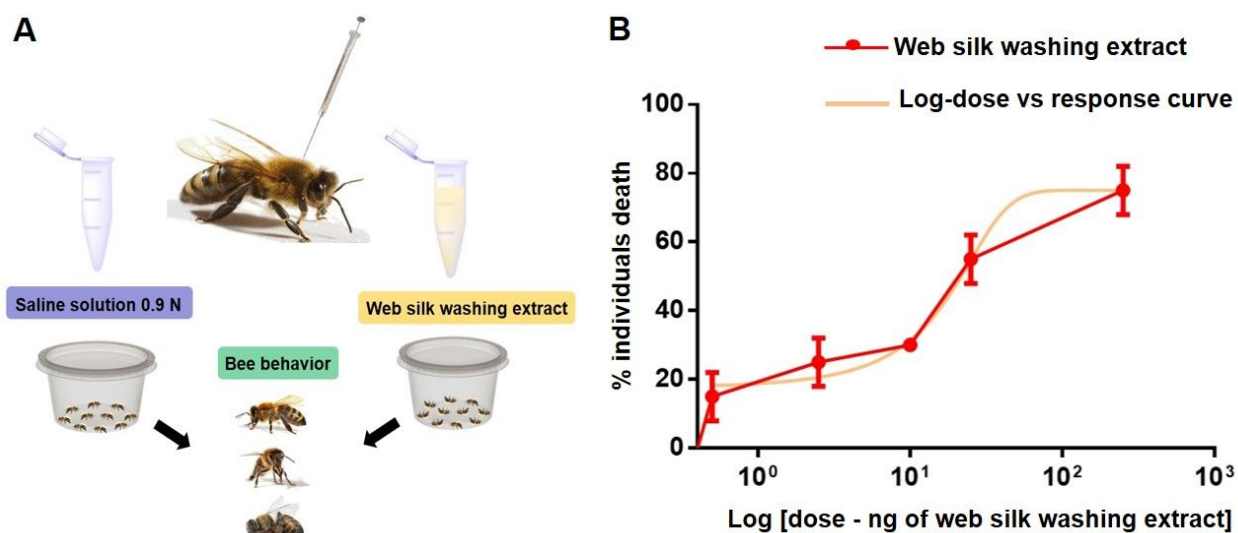


Figure S2. Insect-toxicity bioassays with the *N. clavipes* spider web silk washing extract. (A) Schematic representation of insect-toxicity bioassay. (B) Dose-response curve obtained by the injection of web silk washing extract in the hemocoel of *A. mellifera* bees. Five different doses were injected - 0,5ng, 2,5ng, 10ng, 25ng and 250ng. For the control group was injected saline solution 0.9 N. Bee behavior was observed during 72 hours. DL50 = 21ng; $r^2 = 0,95$.

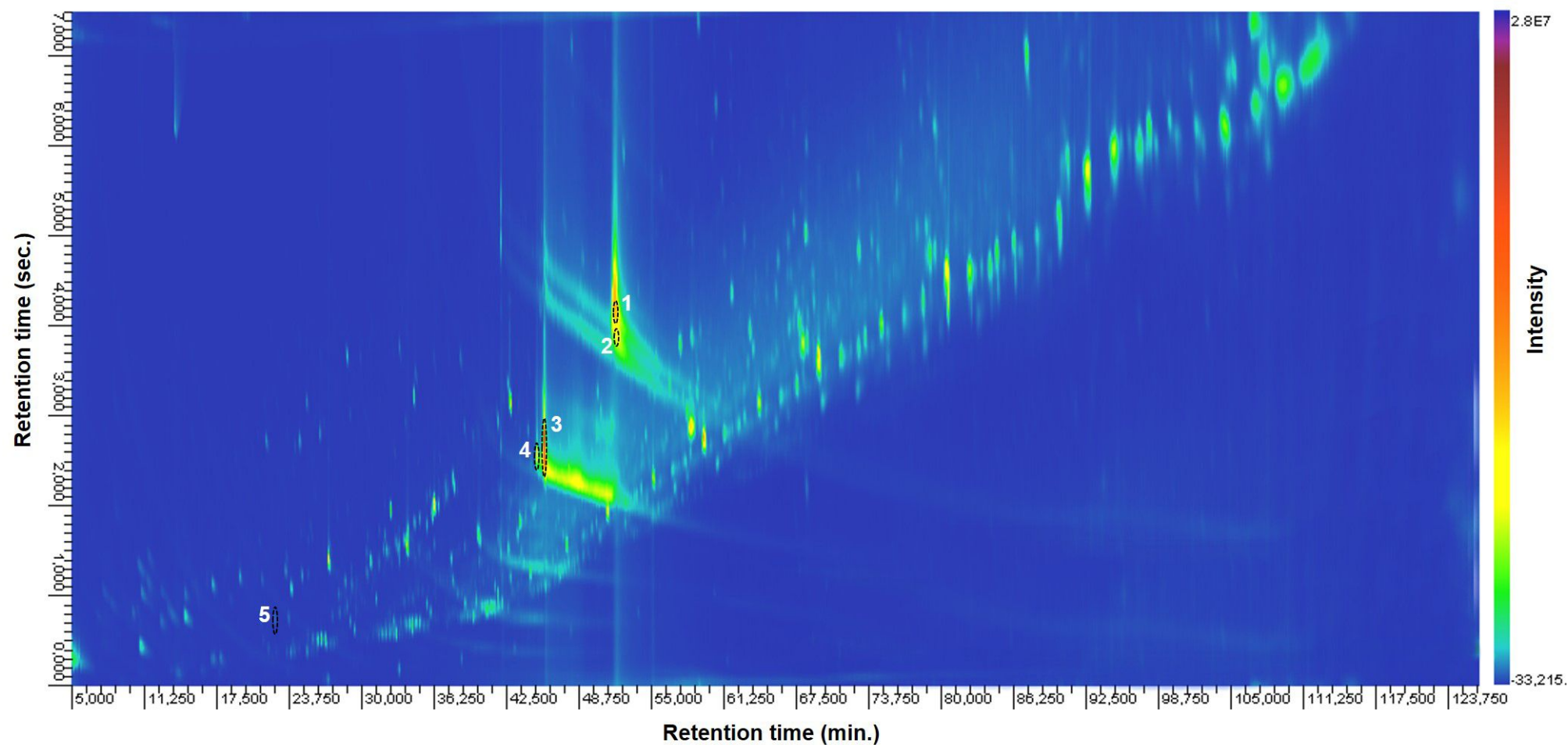


Figure S3. Comprehensive two-dimensional gas chromatography-based metabolomic analysis. Representative profile GCxGC-2D highlighting the fatty acids: 1 - linoleic acid, 2 - oleic acid, 3 - palmitic acid, 4 - palmitoleic acid, and 5 - dodecanoic acid identified in the web silk washing extract in hexane. The fatty acids are marked by a dotted black line in the chromatogram. The detection signals are represented by colours intensity. Colours close to red - most intense signals; and colours close to dark blue - least intense signals.

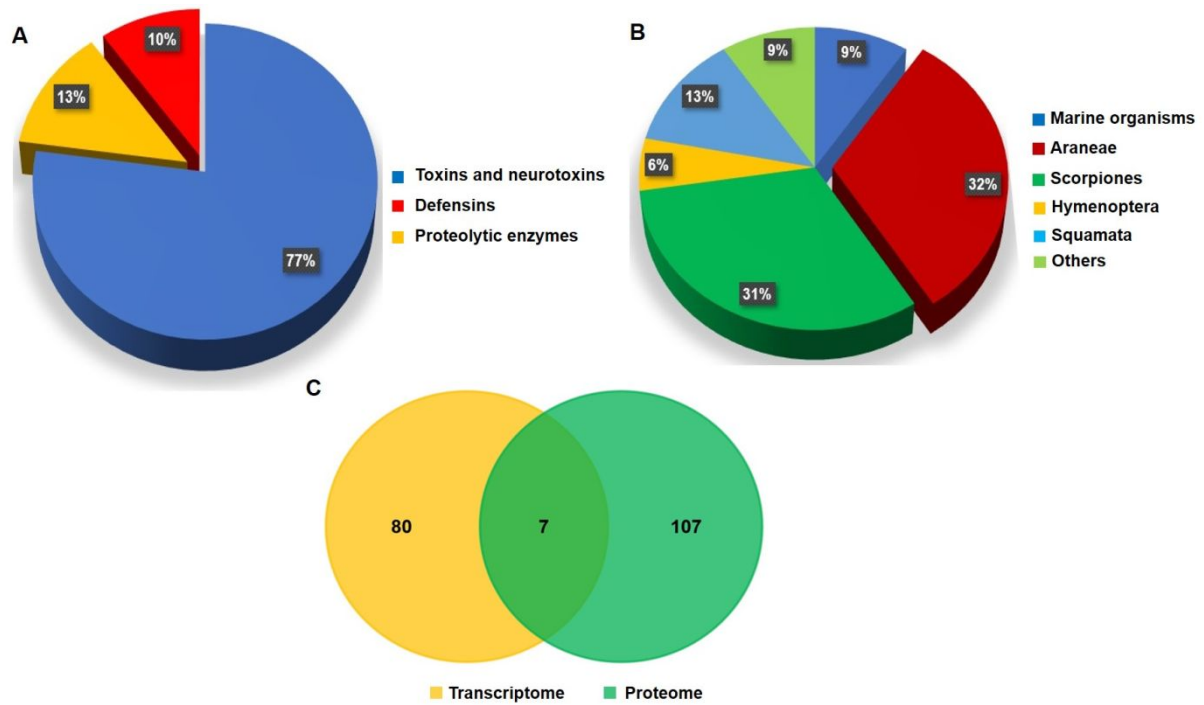


Figure S4. Gel-free shotgun proteomics approach x transcriptome. (A) Total protein distribution into the toxin-like proteins, neurotoxin-like proteins, defensins and proteolytic enzymes functional groups identified in the silk-producing glands and web silk by gel-free shotgun proteomics approach. (B) Total taxonomic distribution of best-match identified protein sequences against databases. (C) Venn diagram showing only shared toxin-like proteins and neurotoxin-like proteins found in proteome and transcriptome. It was considered only non-redundant identifications from taxa Araneae; and only proteins with the same accession code were designated as shared proteins between the two approaches.

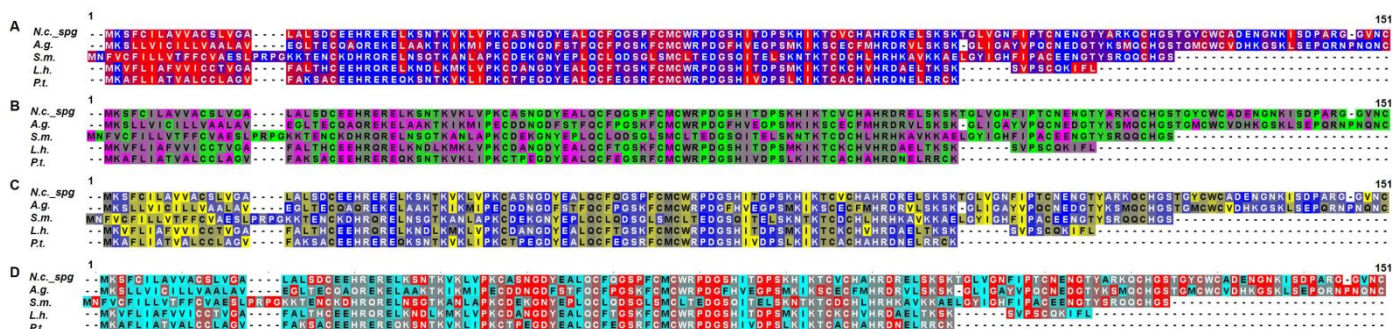


Figure S5. Multiple sequence alignments. Orthologous gene annotated as potential neurotoxin LTDF S-18 (TRINITY_DN80876_c0_g1_i1) that is shared among all five spider species analyzed. *N. c_spg* - *N. clavipes* silk-producing glands, *A. g.* - *A. geniculata*, *L. h.* - *L. hesperus*, *P. t.* - *P. tepidariorum*, and *S. m.* - *S. mimosarum*. (A) Hydrophobicity - the most hydrophobic residues are colored red and the most hydrophilic ones are colored blue. (B) Helix propensity - residues colored in pink demonstrate high propensity and residues colored in green demonstrate low propensity. (C) strand propensity - residues colored in yellow demonstrate high propensity and residues colored in blue demonstrate low propensity. (D) turn propensity - residues colored in red demonstrate high propensity and residues colored in light blue demonstrate low propensity.

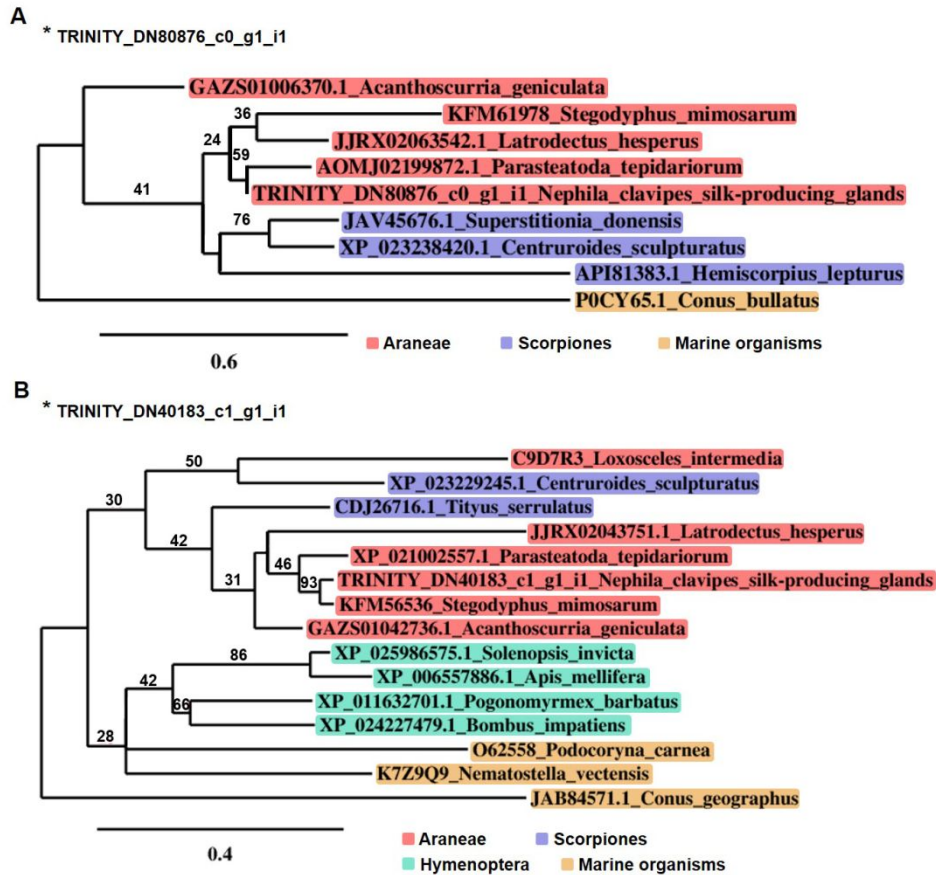


Figure S6. Comparative genomics analysis. (A) Phylogenetic tree based on the amino acid sequence of the orthologous gene annotated as potential neurotoxin LTDF S-18 (TRINITY_DN80876_c0_g1_i1) in the five analyzed spider species (*N. clavipes* silk-producing glands, *A. geniculata*, *L. hesperus*, *P. tepidarium* and *S. mimosarum*), three scorpion species (*Superstitionia donensis*, *Centruroides sculpturatus* and *Hemiscorpius lepturus*) and one marine organism species (*Conus bullatus*). (B) Phylogenetic tree based on the amino acid sequence of the orthologous gene annotated as astacin-like metalloprotease toxin (TRINITY_DN40183_c1_g1_i1) in the five analyzed spider species (*N. clavipes* silk-producing glands, *A. geniculata*, *L. hesperus*, *P. tepidarium* and *S. mimosarum*) and the *Loxosceles intermedia* spider, two scorpion species (*Centruroides sculpturatus* and *Tityus serrulatus*), four Hymenoptera species (*Solenopsis invicta*, *Apis mellifera*, *Pogonomyrmex barbatus* and *Bombus impatiens*) and three marine organism species (*Nematostella vectensis*, *Podocoryna carnea*, and *Conus geographus*).

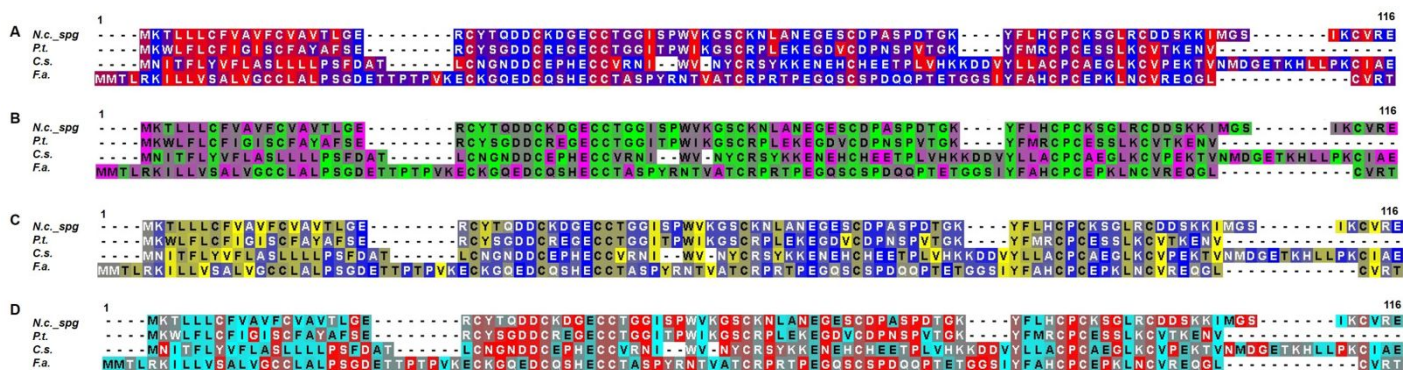


Figure S7. Multiple sequence alignments. Orthologous gene annotated as U8-theraphotoxin-Hhn1c 1-like (TRINITY_DN31416_c0_g1_i1) that is shared among the three taxa. *N. c._spg* - *N. clavipes* silk-producing glands, *P. t.* - *P. tepidarium*, *C. s.* - *Centruroides sculpturatus* and *F. a.* - *Fopius arisanus*. (A) Hydrophobicity - the most hydrophobic residues are colored red and the most hydrophilic ones are colored blue. (B) Helix propensity - residues colored in pink demonstrate high propensity and residues colored in green demonstrate low propensity. (C) strand propensity - residues colored in yellow demonstrate high propensity and residues colored in blue demonstrate low propensity. (D) turn propensity - residues colored in red demonstrate high propensity and residues colored in light blue demonstrate low propensity.

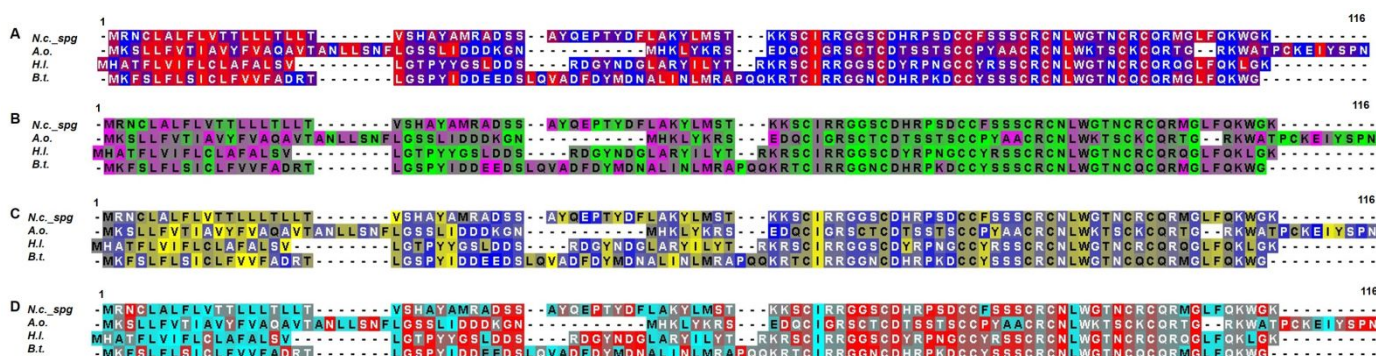


Figure S8. Multiple sequence alignments. Orthologous gene annotated as toxin-like structure AgorTX_B6 (TRINITY_DN44948_c0_g1_i2) that is shared among the three taxa. *N. c._spg* - *N. clavipes* silk-producing glands, *B. t.* - *Bombus terrestris*, *H. l.* - *Hemiscorpius lepturus* and *A. o.* - *Agelena orientalis*. (A) Hydrophobicity - the most hydrophobic residues are colored red and the most hydrophilic ones are colored blue. (B) Helix propensity - residues colored in pink demonstrate high propensity and residues colored in green demonstrate low propensity. (C) strand propensity - residues colored in yellow demonstrate high propensity and residues colored in blue demonstrate low propensity. (D) turn propensity - residues colored in red demonstrate high propensity and residues colored in light blue demonstrate low propensity.