ADDITIONAL FILE 1

Venom variation and evolution in montane pitvipers (Viperidae: Cerrophidion).

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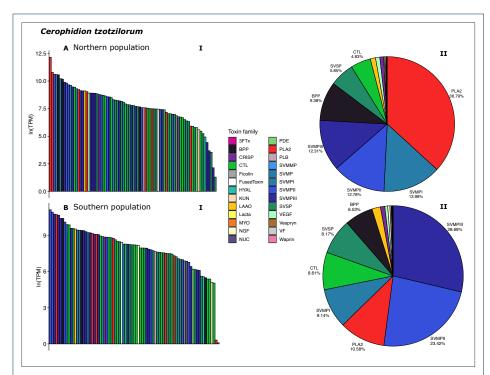


Figure S1. RSEM results for *C. tzotziolorum*. **A.** Average of the northern population; and **B.** Average of the southern population. (I) barplot of the log ranked expression of toxin genes; (II) pie chart of the percent expression of each toxin family for individual populations and for all the individuals.

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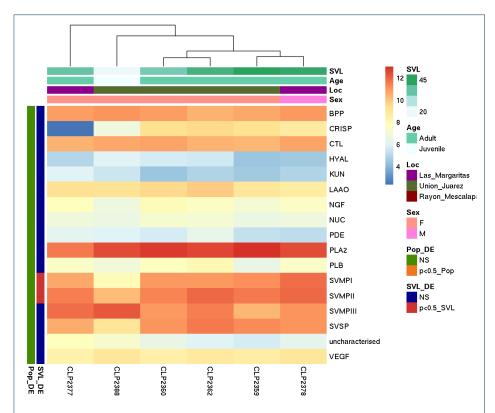


Figure S2. Heatmap showing the log TPM expression of toxin families in C. godmani. In the left columns (Pop & SVL) the orange and red colors respectively represent significant differential expression agreement by both DESeq2 and edgeR (FDR < 0.05).

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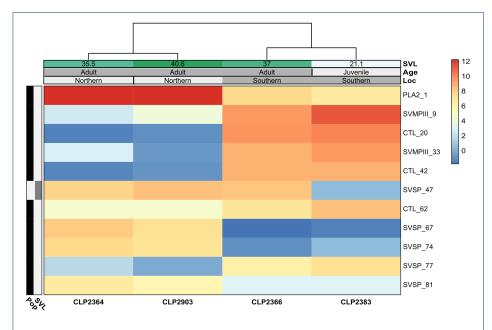


Figure S3. Heatmap showing the log TPM expression of toxins identified as differentially expressed in C. tzotzilorum ordered by the average expression. In the left columns (Pop & SVL) the darker colors represent significant differential expression agreement by both DESeq2 and edgeR (FDR < 0.05).

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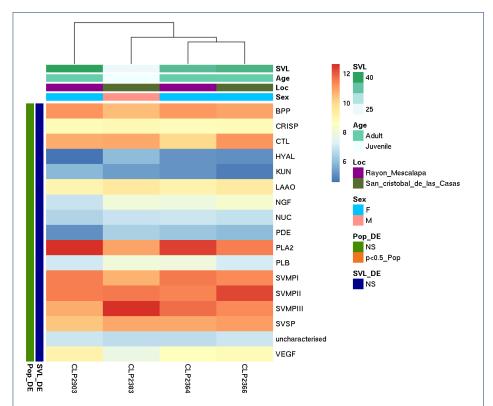


Figure S4. Heatmap showing the log TPM expression of toxin families in C. tzotzilorum. None of the toxin families were significally differently expressed by both DESeq2 and edgeR (FDR < 0.05), However PLA₂s were differentially expressed in DESeq2 by population, and HYAL, SVMPIII, and VEGF were differentially expressed in DESeq2 by SVL.

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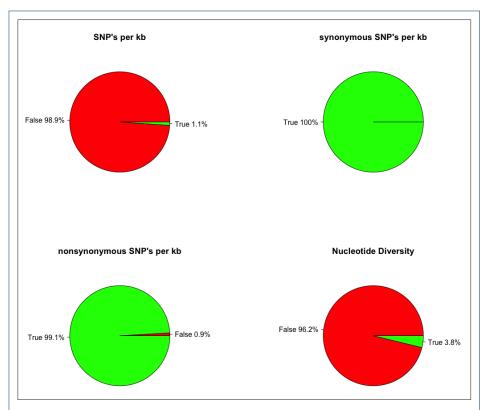


Figure S5. Bootstrap pie charts, with 1000 repetitions of the linear model comparing toxins against nontoxins with equal sample size. True in green (p < 0.05) and false in red (p > 0.05).

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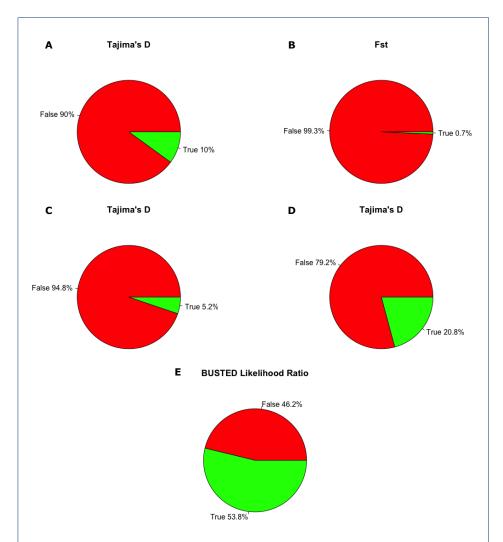


Figure S6. Bootstrap pie charts, with 1000 repetitions of the linear model comparing toxins against nontoxins with equal sample size. True in green (p < 0.05) and false in red (p > 0.05). **A** Tajima's D. **B** F_ST. **C** Synonymous SNPs Tajima's D. **D** Nonsynonymous SNPs Tajima's D. **E** BUSTED model LRT, tested with a non parametric Kruskal Wallis test.

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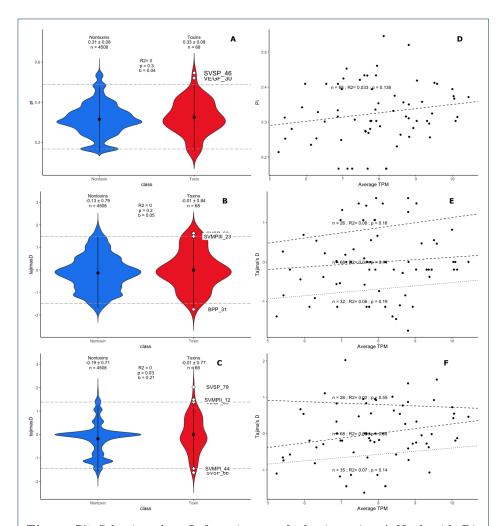


Figure S7. Selection plots. Left: estimates of selection using A Nucleotide Diversity (π) , B Synonymous SNPs Tajima's D, and C Nonsynonymous SNPs Tajima's D for toxins and nontoxins, each with the nontoxin 95th percentile (dotted lines) to identify outlier toxins. The toxin family and the rank based on highest-to-lowest average expression in the transcriptome are displayed for toxins which fall outside the 95th percentile. Right: Linear regressions of the Toxin's mean expression (Average TPM) and estimates of selection including D Nucleotide Diversity (π) , E Synonymous SNPs Tajima's D., and F Nonsynonymous SNPs Tajima's D. For Tajima's D, dotted lines are regressions of all the transcripts (center), just positive values (top) and just negative values (bottom).

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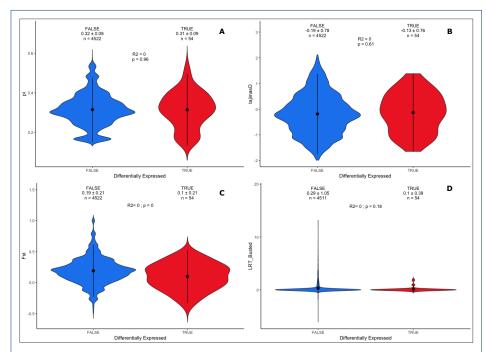


Figure S8. Selection plots. Left: estimates of selection using **A** Nucleotide Diversity (π) , **B** Tajima's D, **C** F_ST , and **D** BUSTED model LRT for non differentially expressed genes (FALSE) and differentially expressed (TRUE).

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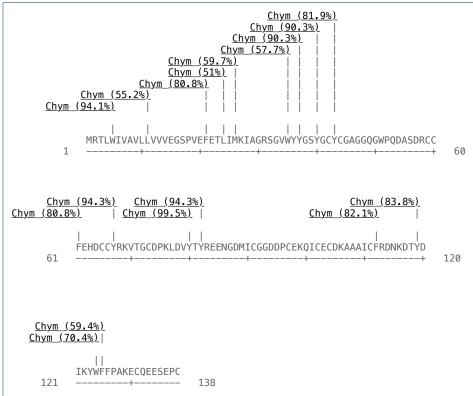


Figure S9. Expasy Peptide Cutter Results ([1]) for Cgodm_PLA2_11. The peptide cutter tool was set to identify Chimotrypsin like cleavege residuals with cut of 50 % probability of cleavage.

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References

1. Expasy Peptide Cutter [webpage]; 2022. Available from: https://web.expasy.org/peptide_cutter/.