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Exploring the venom gland transcriptome of the Ecuadorian scorpion *Teuthraustes atramentarius*

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INTRODUCTION & AIM

Scorpion venoms are complex mixtures of bioactive molecules, including neurotoxins, cytolytic peptides, enzymes, and other components that target ion channels, membrane receptors, and various physiological pathways. Although many medically important species have been extensively studied, little is known about the molecular composition of venoms in rare or endemic taxa. The scorpion genus Teuthraustes Simon, 1878 (family Chactidae) comprises 27 described species, with 15 recorded from Ecuador. These species exhibit a remarkable concentration in the Andean highlands and Amazonian regions, although the genus also occurs in Colombia, Venezuela, Peru, and Brazil. Specifically, *Teuthraustes atramentarius*, the type species, was originally described from Ecuador and remains endemic to the country's central highlands. Despite its early description, no omics-based studies have investigated its venom composition. Here, we present the first exploration of the venom gland transcriptome of *T. atramentarius*.

METHOD

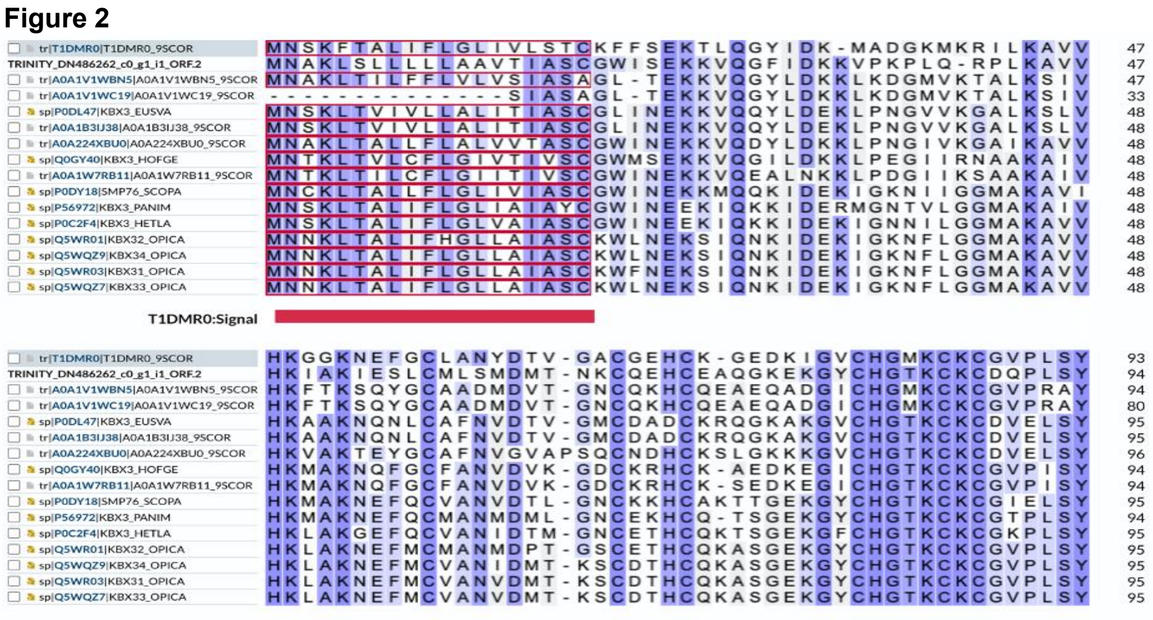
RNA from dissected venom glands of three specimens, following venom induction by electrostimulation, was sequenced using Illumina RNA-Seq. The assembled transcriptome was analyzed with the DeTox pipeline to identify toxin candidates. Top candidates with signal peptides, cysteine patterns, and similarity to known toxins were manually inspected based on best-hit alignments and preliminary phylogenetic analysis

Figure 1. Composite figure illustrating the biological specimen and experimental setup **a)** Frontal view of the scorpion *Teuthraustes atramentarius* used in this study, Electrostimulation apparatus, including a custom-built restraint tube (b) designed for specimen handling and c) venom extraction and electrode terminals adapted for venom extraction (right), **d)** Close-up images of the metasomal segments and telson of *T. atramentarius*, highlighting the anatomical region involved in venom production and inoculation

RESULTS & DISCUSSION

Among 40,083 predicted ORFs, 288 matched ToxProt entries, with seven retained as strong toxin candidates. These included one homologous to Phi-liotoxin-Lw1a, a ryanodine receptor modulator; one Cathepsin D-like aspartic peptidase; two invertebrate defensins; two CAP superfamily cysteine-rich venom proteins (CRVPs); and one Hge-scorpine, a multifunctional peptide with antimicrobial and ion channel-blocking properties. (Main table)

Figure 2



UnitProt analysis with similar sequences of the toxin protein families identified were deployed to prove proximity and allow to discard hits that did not present conservate patterns in the alignment. (Figure 2 and 3)

Figure 3

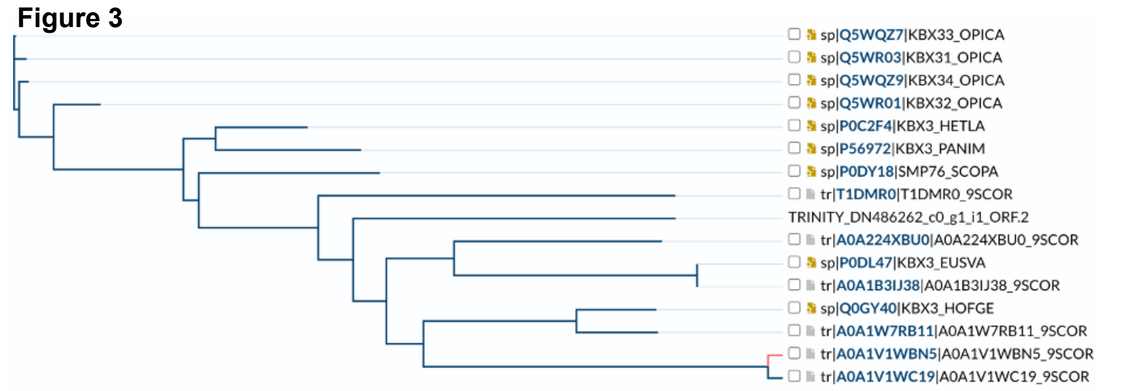


TABLE 1

ID	pfam domains	toxinDB_sseqid	toxinDB_pident	toxinDB_evalue	description
TRINITY_DN486262_c0_g1_i1_ORF.2	Toxin_38	sp|Q0GY40|KBX3_HOFGE	54.7	1.66e-32	Hge-scorpine
***This toxin candidate, matched Hge-scorpine from the scorpion *Hoffmannihadrurus gertschi* (ToxProt: Q0GY40; 54.7% identity, E-value: 1.66e-32), a multifunctional peptide with antimicrobial, haemolytic, and potassium channel-blocking activities. It also aligned with a putative sodium channel toxin from *Megacormus gertschi* (UniProt: A0A224XBU0; E-value: 1.57e-29), consistent with their phylogenetic proximity.**					
TRINITY_DN104230_c3_g1_i2_ORF.3	Asp	sp|Q18DC8|RE12_3_ECHOC	32.3	1.04e-24	Renin (gene Asp-Eoc51)
***This ORF rated as SBDC by DeTox, showed significant homology to a Cathepsin D-like aspartic peptidase 1 from the Brazilian scorpion Tityus serrulatus (UniProt: U6JRM9; 40.1% identity, E-value: 7.09e-39), and to a renin-like toxin included in ToxProt from *Echis ocellatus* (Q18DC8; 32.3% identity, E-value: 1.04e-24), suggesting a closer relationship to the scorpion-derived aspartic peptidase and/or a potential role in venom maturation or proteolytic activation.**					
TRINITY_DN128292_c0_g1_i13_ORF.11	CAP	sp|A0A218QX58|CRVP_TITSE	55.5	7.29e-90	Cysteine-rich venom protein
TRINITY_DN128292_c0_g1_i14_ORF.18	CAP	sp|A0A218QX58|CRVP_TITSE	48.2	1.73e-122	Cysteine-rich venom protein
***These two ORFs were annotated with the Defensin_2 domain and showed homology to a defensin toxin from the Manchurian scorpion Olivierus martensii (syn. Mesobuthus martensii) in ToxProt (P0DQU0; 62.3% and 60.4% identity; E-values: 6.39e-23 and 2.6e-22, respectively). Both sequences also matched an invertebrate defensin (Amercin) from the tick *Amblyomma americanum* (A0F088; 79.5% and 78% identity). These results suggest both sequences are strong candidates for inclusion in the invertebrate defensin toxin family.**					
TRINITY_DN137346_c0_g1_i2_ORF.4	NA	sp|P0DJ08|TXS2B_HORWA	59.4	1.75e-24	Phi-liotoxin-Lw1a
***70 aas ORFs rated as SBC with homology to Phi-liotoxin-Lw1a from the Australian rainforest scorpion *Hormurus waigiensis* (syn. *Liocheles waigiensis*). This toxin is described as a potent modulator of both ryanodine-sensitive calcium-release channels RyR1 and RyR2 with high potency through an opening-mediated mechanism.**					
TRINITY_DN1029950_c0_g1_i1_ORF.3	Defensin_2	sp|P0DQU0|DEF5_MESMA	62.3	6.38e-23	Defensin BmKDfsin5
TRINITY_DN688629_c0_g1_i1_ORF.3	Defensin_2	sp|P0DQU0|DEF5_MESMA	60.4	2.6e-22	Defensin BmKDfsin5
***these ORFs were annotated with the Defensin_2 domain and showed homology to a defensin toxin from the Manchurian scorpion *Olivierus martensii* (syn. *Mesobuthus martensii*) in ToxProt (P0DQU0; 62.3% and 60.4% identity; E-values: 6.39e-23 and 2.6e-22, respectively). Both sequences also matched an invertebrate defensin (Amercin) from the tick Amblyomma americanum (A0F088; 79.5% and 78% identity). These results suggest both sequences are strong candidates for inclusion in the invertebrate defensin toxin family.**					

CONCLUSION

To our knowledge, this study constitutes the first omics-based approach to investigating the venom composition of *T. atramentarius*, revealing seven strong toxin candidates related to known scorpion and animal toxins. However, further research is needed, particularly proteomic validation in venom samples, broader specimen sampling, and integration with proteotranscriptomic and evolutionary approaches.

FUTURE WORK / REFERENCES

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