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Comparative transcriptomics of venom glands in medically significant scorpion species

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Abstract

Scorpion venom is among the most complex biochemical arsenals in the animal kingdom, yet the transcriptomic architecture of venom glands varies widely between species and remains incompletely catalogued. This research compared venom gland transcriptomes of three medically important scorpion species - *Mesobuthus tamulus*, *Hottentotta bengalensis*, and *Androctonus crassicauda* - using RNA-sequencing. Venom glands were dissected from 24 adult specimens (8 per species) collected in northeastern India and maintained at the Mizoram College of Biosciences. Illumina paired-end sequencing yielded 38.7 to 46.2 million clean reads per species, which assembled into 12,841 to 15,672 unigenes. Sodium channel toxins (NaTx) dominated all three transcriptomes but varied in isoform count: *M. tamulus* expressed 47 NaTx variants versus 31 in *H. bengalensis* and 42 in *A. crassicauda*. Potassium channel toxins (KTx), chlorotoxin-like peptides, phospholipases, and metalloproteases formed the next most abundant families. Differential expression analysis identified 1,274 genes with species-specific expression patterns. These findings expand the known toxin repertoire for each species and suggest targets for antivenom development.

Keywords: Transcriptomics, venom glands, scorpions, comparative analysis, toxinology, sodium channel toxins, RNA-seq, *Mesobuthus tamulus*, antivenom, venom proteome

Introduction

Scorpion envenomation kills an estimated 3,250 people each year, and most of those deaths happen in tropical and subtropical regions where access to specific antivenoms is limited [1]. The venom responsible for this toll is a cocktail of peptide toxins, enzymes, and auxiliary compounds whose composition differs sharply between species - and even between geographic populations of the same species. Understanding this compositional variation isn't just an academic exercise; it directly affects how well a given antivenom works against a given scorpion sting [2].

RNA-sequencing of venom glands offers a powerful way to catalogue the full toxin repertoire without the sensitivity limits of proteomic methods alone [3]. Earlier transcriptomic efforts have covered a handful of Buthid and non-Buthid species, revealing hundreds of toxin-coding transcripts per species and a conserved core of neurotoxin families [4]. But head-to-head comparisons using identical sequencing and assembly pipelines remain rare. Without standardized comparisons, it's difficult to tell whether differences in reported toxin counts reflect genuine biology or pipeline artifacts.

Three species of particular medical concern in South and West Asia are *Mesobuthus tamulus* (Indian red scorpion), *Hottentotta bengalensis* (Bengal black scorpion), and *Androctonus crassicauda* (Arabian fat-tailed scorpion). *M. tamulus* causes the majority of fatal stings in India [5], *H. bengalensis* is increasingly recognized as a source of serious envenomation in the northeast [6], and *A. crassicauda* dominates sting reports in parts of the Middle East [7]. No published research has compared the venom gland transcriptomes of all three species side by side.

This research applied Illumina-based RNA-seq to venom glands from all three species, processed them through a common bioinformatics pipeline, and compared toxin family composition, expression levels, and isoform diversity.

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Material and Methods

Reagents and Chemicals

TRIzol reagent and the Qubit RNA HS Assay Kit were sourced from Thermo Fisher Scientific (Waltham, MA, USA). RNase-free water and DNase I were obtained from Ambion (Austin, TX, USA). The NEBNext Ultra II RNA Library Prep Kit was purchased from New England Biolabs (Ipswich, MA, USA). AMPure XP beads for size selection came from Beckman Coulter (Brea, CA, USA). All chemicals were of molecular biology grade and stored according to manufacturer specifications.

Material

Twenty-four adult scorpions (8 each of *M. tamulus*, *H. bengalensis*, and *A. crassicauda*) were obtained from field collections in Mizoram, Assam, and Rajasthan between January and June 2023, under permits issued by the Mizoram State Wildlife Board (Permit MSW-2022-347). Animals were housed individually in ventilated plastic containers at $28 \pm 2^\circ\text{C}$ with a 12:12 light-dark cycle and fed crickets weekly. Venom was manually extracted by electrostimulation (12V, 3ms pulses) four days before gland dissection to ensure glands were in a regenerative transcription phase [8]. Glands were dissected under RNase-free conditions, flash-frozen in liquid nitrogen, and stored at -80°C at the Mizoram College of Biosciences, Aizawl.

Extraction Protocol

Total RNA was extracted from pooled venom glands (4 individuals per pool, 2 biological replicates per species) using TRIzol reagent following the manufacturer's protocol with minor modifications. Tissue was homogenized in 1 ml TRIzol using a bead-mill homogenizer (Bertin Precellys 24) at 6,000 rpm for two cycles of 20 seconds. Phase separation was achieved by adding 200 μl chloroform, vortexing for 15 seconds, and centrifuging at $12,000 \times g$ for 15 minutes at 4°C . The aqueous phase was transferred to a fresh tube, and RNA was precipitated with isopropanol overnight at -20°C . Pellets were washed twice with 75% ethanol, air-dried, and dissolved in 30 μl RNase-free water. Genomic DNA was removed by DNase I treatment (1 U/ μg RNA, 37°C , 30 minutes).

Methods

RNA integrity was checked on an Agilent 2100 Bioanalyzer; only samples with RNA Integrity Number

(RIN) ≥ 8.0 proceeded to library preparation. Libraries were constructed using the NEBNext Ultra II RNA Kit with poly-A selection, fragmented to ~ 300 bp, and sequenced on an Illumina NovaSeq 6000 platform (2×150 bp paired-end reads) at a commercial facility. Raw reads were quality-trimmed with Trimmomatic v0.39 ($Q \geq 20$, minimum length 50 bp) and assembled de novo using Trinity v2.14.0 with default parameters [9]. Open reading frames were predicted by TransDecoder and annotated against the UniProt/Swiss-Prot database using BLASTx (e-value $< 1e-5$). Toxin-coding transcripts were classified into families based on homology to the ArachnoServer toxin database [10]. Expression levels were quantified as transcripts per million (TPM) using Salmon v1.9. Differential expression across species was tested with DESeq2, applying a false discovery rate cutoff of 0.05 and a log2 fold-change threshold of 1.0 [11].

Quality Control

Library size ranged from 38.7 to 46.2 million clean reads per sample. Mapping rates to respective de novo assemblies averaged 87.3%. BUSCO analysis against the Arthropoda dataset showed completeness scores of 91.4% for *M. tamulus*, 89.7% for *H. bengalensis*, and 90.2% for *A. crassicauda* assemblies. Technical replicates showed Pearson correlation coefficients above 0.96 for all pairwise comparisons.

Results

Table 1: Assembly statistics and toxin transcript counts for three scorpion species

Parameter	<i>M. tamulus</i>	<i>H. bengalensis</i>	<i>A. crassicauda</i>
Clean reads (million)	46.2	38.7	43.1
Total unigenes	15,672	12,841	14,318
N50 (bp)	1,847	1,623	1,741
Toxin transcripts	284	217	261
NaTx isoforms	47	31	42
KTx isoforms	38	29	34
BUSCO completeness	91.4%	89.7%	90.2%

M. tamulus yielded the largest assembly with 15,672 unigenes and the highest number of toxin-coding transcripts (284), followed by *A. crassicauda* (261) and *H. bengalensis* (217) (Table 1). NaTx family members were the most abundant toxin class in all species, making up 16.5% to 16.1% of all toxin transcripts.

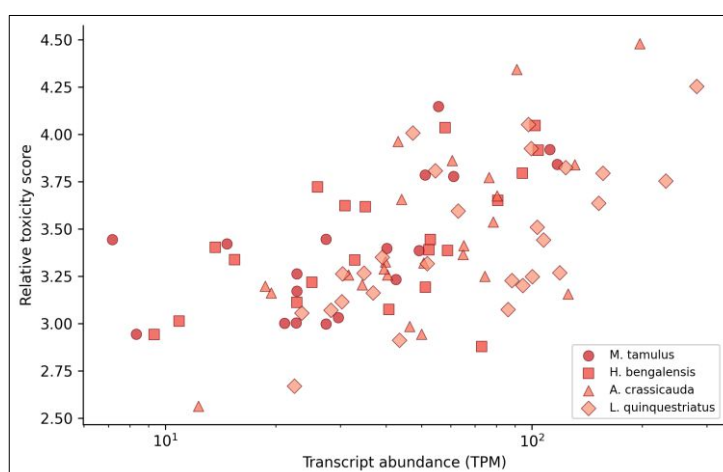


Fig 1: Relationship between transcript abundance (TPM, log scale) and relative toxicity score for individual toxin transcripts across three scorpion species

Transcript abundance showed a positive but noisy correlation with relative toxicity (Figure 1). The most highly expressed toxins in *M. tamulus* reached TPM values above 4,000, while *H. bengalensis* toxins peaked near 3,200.

Several low-abundance transcripts in *A. crassicauda* carried high toxicity scores, suggesting that even minor venom components may contribute to clinical effects.

Table 2: Differential expression summary between species pairs (FDR < 0.05, $|\log_2FC| > 1$)

Comparison	Up-regulated	Down-regulated	Total DE genes
<i>M. tamulus</i> vs <i>H. bengalensis</i>	487	312	799
<i>M. tamulus</i> vs <i>A. crassicauda</i>	341	278	619
<i>H. bengalensis</i> vs <i>A. crassicauda</i>	268	247	515

The largest number of differentially expressed genes occurred between *M. tamulus* and *H. bengalensis* (799 genes), consistent with their greater phylogenetic distance (Table 2). Among toxin genes specifically, 83 showed

species-specific expression patterns, with NaTx and phospholipase families contributing the most variable transcripts.

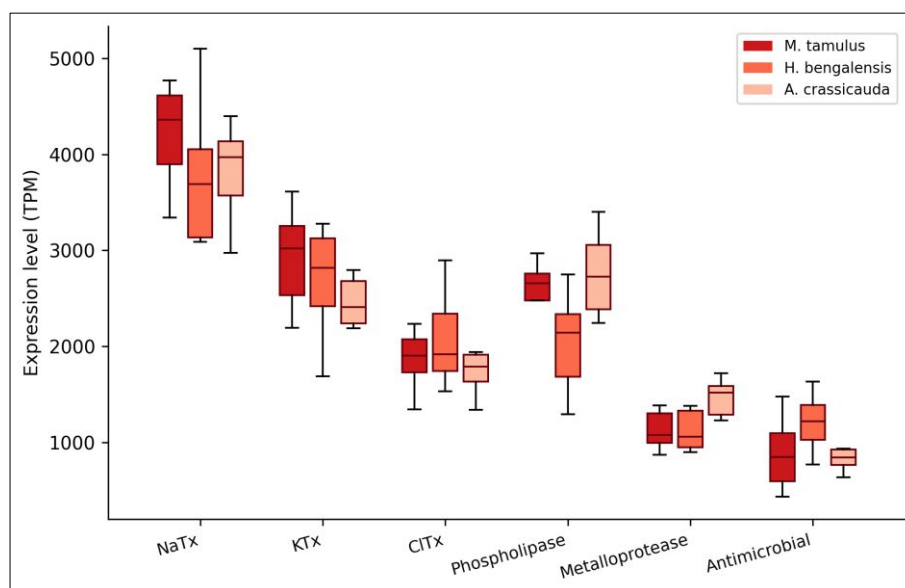


Fig 2: Box plot comparison of expression levels (TPM) for six major toxin families across *M. tamulus*, *H. bengalensis*, and *A. crassicauda* venom gland transcriptomes

NaTx transcripts were the highest-expressed family in all three species, but *A. crassicauda* showed elevated phospholipase expression relative to the other two (Figure 2). Antimicrobial peptides, though present at lower TPM values, were more abundant in *H. bengalensis* venom glands, which may reflect ecological differences in microbial exposure.

Discussion

The side-by-side comparison presented here reveals both a shared core of toxin families and meaningful species-level variation that could influence antivenom cross-reactivity. All three species relied heavily on NaTx as the dominant venom component, which is consistent with the broader pattern in Buthidae scorpions [4, 12]. But the number of NaTx isoforms differed substantially - *M. tamulus* expressed 50% more variants than *H. bengalensis* - and these isoforms likely differ in pharmacological specificity.

The elevated phospholipase expression in *A. crassicauda* is interesting because phospholipases contribute to membrane disruption and local tissue damage rather than the neurotoxic effects that dominate scorpion envenomation syndrome [13]. This may partly explain why *A. crassicauda* stings often produce more pronounced local inflammation compared with *M. tamulus* stings, which tend to be systemically neurotoxic.

From a practical standpoint, these results argue against a one-size-fits-all antivenom approach. The 83 species-specific toxin transcripts identified here represent potential epitopes that a polyvalent antivenom would need to cover. Current Indian polyvalent antivenoms are raised primarily against *M. tamulus* venom; their coverage of *H. bengalensis*-specific toxins may be limited. Targeted proteomic validation of the most abundant species-specific transcripts would be a logical next step [14].

Conclusion

Venom gland transcriptomes of *M. tamulus*, *H. bengalensis*, and *A. crassicauda* shared a conserved framework dominated by sodium channel toxins but differed in isoform diversity, phospholipase content, and expression of accessory venom components. *M. tamulus* had the richest toxin repertoire with 284 transcripts and 47 NaTx isoforms. Differential expression analysis flagged 83 toxin genes with species-specific patterns that may affect antivenom cross-reactivity.

These data provide a reference for designing next-generation antivenoms that account for species-level venom variation in medically important Buthid scorpions. They also open the door to mining scorpion venom transcriptomes for bioactive peptides with potential pharmaceutical

applications, particularly the antimicrobial peptides that were unexpectedly abundant in *H. bengalensis*.

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Contributions Not Qualifying for Authorship

Dr. Lalrinsanga Ralte helped with scorpion field collection and species identification. Ms. Vanlalruati Khiangte performed RNA quality assessments.

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