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A non-lethal method for studying scorpion venom gland transcriptomes, with a review of potentially suitable taxa to which it can be applied

Freek J. Vonk[1,2,3©], Mátyás A. Bittenbinder[1,2©], Harald M. I. Kerkkamp[1,3©], Dwin G. B. Grashof[4], John P. Archer[4], Sandra Afonso[4], Michael K. Richardson[3], Jeroen Kool[2], Arie van der Meijden[4]*

1 Naturalis Biodiversity Center, Leiden, The Netherlands, 2 Faculty of Sciences, Division of BioAnalytical Chemistry, Department of Chemistry and Pharmaceutical Sciences, Amsterdam Institute of Molecular and Life Sciences, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands, 3 Animal Science and Health Cluster, Institute of Biology Leiden, Leiden University, Leiden, The Netherlands, 4 CIBIO-InBIO, Biopolis, Universidade do Porto, Porto, Portugal

© These authors contributed equally to this work.
* mail@arievandermeijden.nl

Abstract

Scorpion venoms are mixtures of proteins, peptides and small molecular compounds with high specificity for ion channels and are therefore considered to be promising candidates in the venoms-to-drugs pipeline. Transcriptomes are important tools for studying the composition and expression of scorpion venom. Unfortunately, studying the venom gland transcriptome traditionally requires sacrificing the animal and therefore is always a single snapshot in time. This paper describes a new way of generating a scorpion venom gland transcriptome without sacrificing the animal, thereby allowing the study of the transcriptome at various time points within a single individual. By comparing these venom-derived transcriptomes to the traditional whole-telson transcriptomes we show that the relative expression levels of the major toxin classes are similar. We further performed a multi-day extraction using our proposed method to show the possibility of doing a multiple time point transcriptome analysis. This allows for the study of patterns of toxin gene activation over time a single individual, and allows assessment of the effects of diet, season and other factors that are known or likely to influence intraindividual venom composition. We discuss the gland characteristics that may allow this method to be successful in scorpions and provide a review of other venomous taxa to which this method may potentially be successfully applied.

Introduction

Venoms are complex mixtures of bioactive compounds called toxins that have evolved on more than 30 different occasions in the animal kingdom [1,2]. Toxins are often highly specific in their activity and can induce a wide range of pharmacological effects [2–6]. They can act by

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binding to ion-channels for example, by destroying cellular components or by disrupting metabolic pathways, which may lead to paralysis, haematological disruptions, tissue necrosis and pain. Venoms have been studied for a long time in order to better understand their physiological effects from a standpoint of development of treatment of envenomation. However, the high specificity and potency of certain toxins renders them useful as experimental tools or as candidates for the development of novel therapeutics [1,7–12]. The field of transcriptomics has changed our understanding of the diversity and composition of animal venoms in the last decades. Venom transcriptome research focusses primarily on studying venom composition through mRNA-transcripts and their relative expression levels [11,13–16]. The main advantage of using transcriptomics over proteomics is the fact that the transcriptome data allows the study of patterns of gene activation through read counts, and gene evolution through the coding sequences.

Scorpions are, apart from snakes, the most widely studied group of venomous animals when it comes to their venom [4,14,17–20]. This is because scorpion stings are considered a public health problem in (sub-)tropical countries, with global estimates surpassing 1.2 million cases of envenoming resulting in more than 3,250 fatalities a year [5,17]. Scorpion venoms are also studied as candidates of pharmaceutically active molecules with potential drug applications [21–24]. Scorpion venoms are complex mixtures of proteins, peptides and small molecular compounds, with small peptides and proteins targeting ion channels being most prevalent [4,5]. Because of their high specificity for these ion channel proteins, scorpion toxins are being studied for their potential use as therapeutics. As these ion channel proteins are major drug targets, scorpion venoms could be an interesting source for novel candidates in the venoms-to-drugs pipeline [21–23]. Scorpion venoms have been studied via proteomic approaches within the last two decades, but with the emergence of next generation sequencing (NGS) technologies, transcriptome studies have increased in popularity [14,25–35]. The fact that the costs of next generation sequencing have decreased tremendously in recent years has revolutionized 'omics'-studies [11,36–44]. The current method for obtaining a scorpion venom gland transcriptome is based on sacrificing the animal to extract the venom gland from the telson (the "stinger" at the end of the tail-like metasoma, containing the venom glands) or to homogenise the entire telson. Although this method of venom gland sequencing allows an in-depth analysis of the expressed genes in the venom gland, it has its limitations. One of the major limitations is the fact that this only allows analysis at a single time point, making it impossible to study the intraindividual variation caused by ontogenetic stage, season or diet [45,46]. Homogenisation of the telson also includes tissues not involved in venom production, such as the muscles surrounding the glands, and the cuticle, making the sequencing less target-specific. Furthermore, it faces the ethical drawbacks and resource depletion of having to sacrifice the animal [47].

In this study, we have performed mRNA-extraction from the scorpion venom itself, which can be done by using venom that is obtained by electro-stimulation without harming the animal, followed by standard sequencing and analysis techniques. This was done without amplification of mRNA-transcripts in order to avoid bias in the read counts. The novel technique allows for multiple time point transcriptomes from a single individual. This means that we can look at patterns of gene activation over time in the same individual, and assess the effects of diet, season and other factors that are known or likely to affect intraindividual venom composition [45,46]. This study compares venom-derived transcriptomes to the traditional whole-telson transcriptomes in order to show that they are similar. In addition, we will show an extraction at an earlier stage in the gland replenishment to show the possibility of doing a multiple time point extraction using this new method.

Methods

Tissue samples

The tissue and venom samples were obtained from a captive specimen of *Heterometrus laoticus* Couzijn, 1981, obtained from Vietnam through the pet trade. The specimen was maintained in the laboratory as described in [48]. Electrostimulation to obtain venom was performed by applying a square wave with an amplitude of 18V and a 10% duty factor at a frequency of 45Hz through saline-wetted electrodes positioned at the 2nd and 5th metasomal segments. We found this method to be scorpion-friendly, contrary to methods that result in high current through the tissues, such as those employing higher voltages or constant current (see S1 File for a schematic of the device and accompanying code).

To ensure active transcription of venom genes, the venom glands were first emptied by electrostimulation. This venom was discarded. Venom was extracted again 4 days after. The venom from this second extraction was frozen in liquid nitrogen and stored at -80˚C until library preparation. The specimen was then fed and not disturbed for 2 weeks. Venom was then extracted again to stimulate transcription by emptying the venom glands, and this venom was discarded. The venom that was extracted 2 days later was frozen in liquid nitrogen and stored at -80˚C until library preparation. After another rest period of 2 weeks, venom was extracted and discarded again. Five days after this extraction, the specimen was anaesthetised using isoflurane, and frozen in liquid nitrogen. The telson and chela were removed, and stored separately at -80˚C until library preparation.

cDNA library construction

The RNA extractions were done using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. Then, RNA quantification was performed using the Qubit RNA BR (Broad-Range) Assay Kit (Thermo Fisher). The RNA quality was assessed by doing a RIN test using a Tapestation 2200 (Agilent). Library prep was done using the TruSeq RNA Library Prep Kit v2 (Illumina).

DNA sequencing and bioinformatics analysis

The RNA samples were sequenced on an Illumina Hiseq 1500. All samples were given a unique index sequence with read lengths of ~ 280 bp. Sequences were pair-ended (2×125 bp). The venom samples, chela and telson were sequenced separately resulting in four transcriptomes in total. To annotate the telson transcriptomes a custom pipeline was constructed, largely in BioPython (version 1.70), a module for Python (version 3.6.4). This custom bioinformatics pipeline follows six steps leading to full annotation of the transcriptomes: (i) The pipeline calculates the coverage by: average read length (150) * read count of the transcript / length of the transcript. With this formula the coverage of a single transcript is normalised by its size, making the transcript coverage comparable to each other. (ii) The pipeline removes orthologues between the telson and chela transcriptomes by performing a BLASTn with the following parameters: e-value = 1e¬-50; output format = 6; max subject sequences = 1; minimal percentage identity = 99%; minimal percentage coverage = 95%. This removes most housekeeping transcripts from the telson transcriptome. Since no venom or toxin genes are likely to be expressed in the chela, all toxin transcripts, together with some physiological transcripts not expressed in the chela, are kept in the telson transcriptome. (iii) For every transcript left in the transcriptome an open reading frame (ORF) is predicted to increase speed, accuracy and relevance of the next steps. (iv) BLASTp annotation. The fourth step is the actual annotation using BLASTp and the previously created annotated database filled with both physiological and

toxin arthropod proteins. The ORF of every transcript is blasted against the annotated database, using the parameters: e-value 1e-5; output format = 6; max subject sequences = 1. The transcripts are then labelled based on the label of their BLASTp hit or considered "unidentified". (v) Transcript ORF have to be uploaded to SignalP to predict their signal peptides with the SignalP sensitivity set on "Sensitive" [49]. Since this requires manual input, this step was interchanged with step vi to reduce the amount of manual work. (vi) The last step of this pipeline uses all previously gathered data to label the transcripts. Transcripts with a coverage value lower than 5 were removed, since those transcripts have a higher chance of being misassembled and are assumed to be insignificant in the venom of the scorpion. Then all transcripts that were found to have an orthologue in the chela transcriptome were labelled as "physiological". Finally, the remaining transcripts were labelled according to the label of their BLASTp hit. The transcripts labelled as a member of a toxin family or labelled as "other toxin" were considered part of the venom.

Results

In this study we introduce a new method for the generation of venom gland transcriptomes by using extracted scorpion venom from the Vietnam forest scorpion (*Heterometrus laoticus*) (Fig 1). We extracted venom at two days after the start of venom replenishment (V2d) and at four days after the start of venom replenishment (V4d). From these two samples we extracted the mRNA and using Illumina sequencing we generated two venom transcriptomes (Fig 1). Next, using the old method by extracting mRNA from tissue, we generated a telson transcriptome of five days after the start of venom replenishment (T5d) (Fig 2). These venom gland transcriptomes were BLASTed and processed using our python pipeline [35]. Our extractions of the venom gland, the chela, V2d and V4d resulted in 1.32, 0.208, 0.30, 0.678 μg of RNA respectively. However, since we did not standardize the tissue/venom volume used in the extractions, these quantities do not correspond to the RNA quantity in the original tissue or venom.

When we label genes belonging to a toxin family as "toxin", other genes as "physiological" and the remaining unidentified transcript as "unknown", we find that of the transcripts in the V2d transcriptome 5% are labelled "toxin", 55% are labelled "physiological" and 40% are considered "unknown", whereas in the V4d transcriptome 20% are labelled "toxins", 36% are labelled "physiological" and 44% are considered "unknown". Lastly, in the T5d transcriptome 13% of the transcript are labelled as "toxins", 58% are labelled "physiological" and 30% are considered "unknown" (Fig 2). When comparing the individual toxin families present in the venom transcripts, we find that the majority consists of "other toxins" (i.e., venom components not belonging to a major toxin family), followed by potassium-channel binding toxins (KTxs), and phospholipase A$_2$s (PLA$_2$s) (Fig 2). The V2d transcriptome, is mostly made up of "other toxins" (65.5%), followed by κ-KTxs (13.1%), β-KTxs (9.3%) and γ-KTxs (7.2%) (Fig 2). The majority of the V4d transcriptome consists of κ-KTxs (44.7%), followed by γ-KTxs (18.8%), "other toxins" (15.8%) and PLA$_2$s (6.7%) (Fig 2). Lastly, the T5d transcriptome is largely comprised of κ-KTxs (38.6%), followed by "other toxins" (24.4%), γ-KTxs (11.0%) and PLA$_2$s (8.9%) (Fig 2). The total number of read counts for each transcriptome analysis (sum read per contig) consisted of: 42,616,910 reads (V2d), V4d: 37,077,624 reads (V4d), 48,491,348 reads (T5d).

Discussion

This paper describes a new way of generating a scorpion venom gland transcriptome, without sacrificing the animal, using RNA found in extracted venom. This permits the study of the
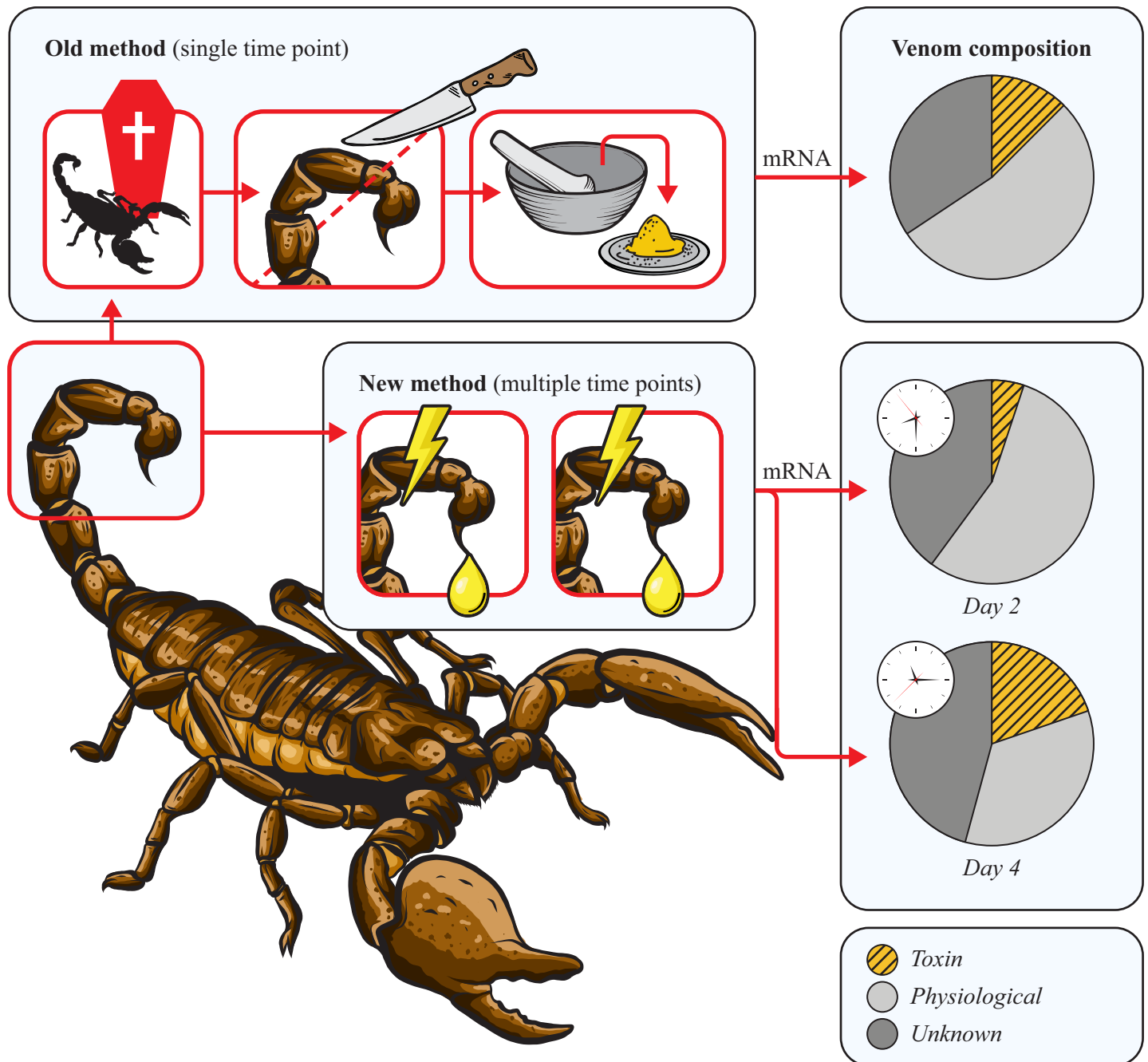
Fig 1. Schematic overview of the old method and the here presented new method for the generation of venom gland transcriptomes. Comparison between the 'traditional' method and our newly developed method for the extraction of mRNA from the Vietnam forest scorpion (*Heterometrus laoticus*). The traditional method involves sacrificing the animal to extract the venom gland from the telson or to homogenize the entire telson in order to collect mRNA, whereas the newly developed method includes mRNA-extraction from scorpion venom, which overcomes the need of harming the animal.

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transcriptome at various time points within a single individual. We have used our method to generate two venom gland transcriptomes suitable for toxin analysis. In order to make a comparison between the two methods, we looked at the V4d and T5d results as these are most similar in point of venom replenishment. Ideally, it would have been more accurate to compare the venom gland transcriptome data (T5d) with the venom transcriptome at the same number
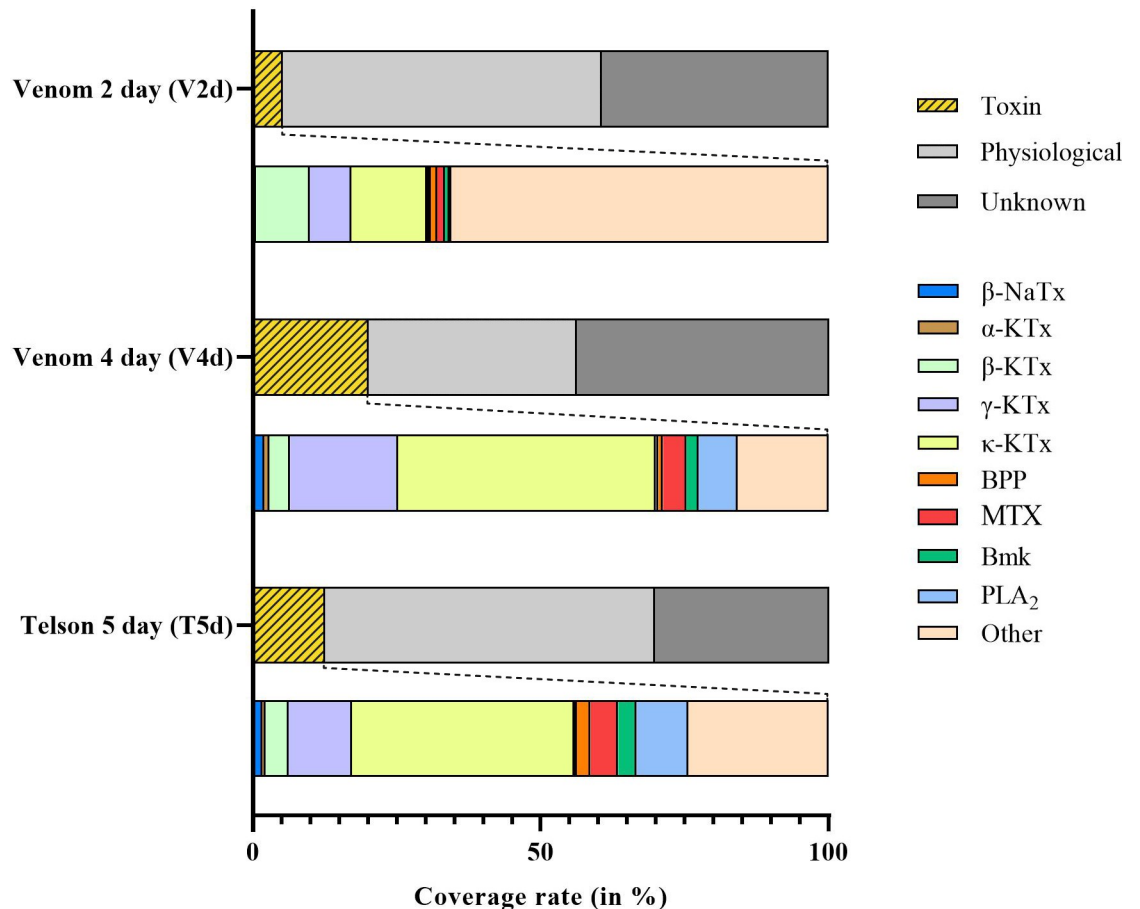
Fig 2. Relative expression levels of transcripts in the venom gland transcriptomes at different timepoints. The graphs show expression rates for venom gland transcriptomes two days after start of venom replenishment (V2d) and four days after the start of venom replenishment (V4d) relative to the whole telson transcriptome five days after the start of venom replenishment (T5d). In the upper bar graphs the transcripts are labelled as "toxin", "physiological" and "unknown" and are shown as a percentage of total expression. The lower graphs represent the relative expression levels across toxin families within the "toxin" transcripts in the venom gland transcriptomes. Abbreviations: NaTx (sodium-channel binding toxin); KTx (potassium-channel binding toxins); ClTx (chlorotoxin); BPP (bradykinin-potentiating peptide); MTX (Maurotoxin); BmK (*Buthus martensii* Karsch-toxin); PLA$_2$ (phospholipase A$_2$). Note: Some toxins are found at such low expression rates that these differentiated from the bar graphs, therefore these are not represented in the legend. These include α-NaTx, calcium-channel binding toxin and Kunitz-type toxin. Host defense proteins were classified under "other".

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of days after venom extraction. However, at day four and five, the venom replenishment is operating fully, as this is in the middle of the duration reported for venom gland replenishment. At this point, the gland is not yet filled, and production of peptides should be maximal. Since it takes time to start the production of the many peptides and glycoproteins that make up scorpion venom, we expect the largest differences in gene activation to lie in the first days of venom replenishment. This is why we expected the difference between day two and four to be larger than between day four and five, as indeed it seems to be. Nevertheless, our results show that this method shows differences in gene activation at different time points after gland depletion, and that these venom transcriptomes are equally informative as the traditional gland transcriptomes.

When comparing their relative toxin composition, we find that the V4d venom transcriptome mostly consists of κ-KTxs, γ-KTxs, "other toxins" and PLA$_2$s whereas the T5d

transcriptome is made up primarily by κ-KTxs, "other toxins", γ-KTxs and PLA₂s (Fig 2). To further compare this, we aligned the protein sequences of κ-KTx and γ-KTx families (S1 and S2 Figs). Here we find that for the 33 κ-KTx sequences found in T5d, only three have no corresponding V4d sequence (S1 Fig). Furthermore, when looking at the expression of the individual sequences, we find that for sequences with high expression rate in the T5d transcriptome that their V4d counterpart also tends to have a higher expression rate (S1 and S2 Figs). This suggests that both methods result in similar expression rates in terms of toxin family, toxin sequences and individual sequence expression. However, there are some differences. These are possibly due to different time of extraction (and thus the difference in venom replenishment) or it is because of the method itself that these variations between transcriptomes occur.

Another concern with the proposed method is that as the mRNA is exposed to the extracellular environment of the venom gland, there is the possibility of RNA-degeneration. In order to examine the extent of RNA degeneration caused by the venom we did a contig comparison between V4d and T5d toxin transcripts. We aligned toxin transcripts with high expression of the major venom families and looked at the percentage of similarity for the overlapping parts. We found that although there are some differences, they are minor, as the similarity percentage is between 96% and 100% (S1 Table). This suggests there may be some RNA degeneration and although it is minimal, it is something that should be taken into account when considering using this method.

The generation of two venom transcriptomes of a single individual enabled us to compare 'early' venom replenishment to 'late' venom replenishment. V2d has a much lower relative amount of toxin transcripts compared to V4d, which may be explained by the fact that venom production had just started. When looking at the individual toxin families (Fig 2) we see that "other toxins" form the main component in the V2d transcriptome, representing 57% of toxin production. This is followed by κ-KTx (18%) and γ-KTx (11%), which are the main components (50% and 18% respectfully) in the V4d. This suggests that at two days the venom production is still getting started. Note that since expression is not standardized against housekeeping genes, these numbers should be interpreted with caution. The proposed method enables new studies into not just the gene activation at different time points of venom replenishment, but also the possible effects of diet or season on the venom production using repeated measurements in a single individual. Thereby this method allows the study of intraindividual variation and further overcomes the ethical drawbacks of having to sacrifice the animal.

This method is based on the fact that mRNA-transcripts for the toxins present in the venom can be used to perform transcriptomic analysis. Venom gland histology plays an important role in the possibility of performing transcriptomics on the mRNA-transcripts present in the venom. Gland products are secreted by exocrine glands onto an epithelial surface, as opposed to products of endocrine glands that are released into the blood stream [50–54]. Three types of exocrine venom glands are recognised, depending on how the venom is being secreted (Fig 3). The first type are the merocrine glands. With this type of secretion, the venom is released through exocytosis without part of the gland cells being lost or damaged. However, cytoplasm and cellular debris can still enter the gland lumen, although only when cells rupture or die [50,54]. These gland types are found in centipedes, heteropterans, reptiles and some mammals (Fig 3, Table 1) [51,55–58]. The second type of glands have an apocrine secretion, in which parts of the cell bud off, producing membrane-bound vesicles containing the cytoplasm of the cell [50,54]. This cytoplasm contains both the venom components as well as cytoplasm containing various cell-specific components such as mRNA-transcripts. This secretion type is found in scorpions, spiders, hymenopterans (i.e. wasps, bees and ants), and the enigmatic platypus (Fig 3, Table 1) [13,59–66]. The third secretion type, in which the accumulation of secretion (i.e., venom components) in the cytoplasm of the secretory cells cause disintegration
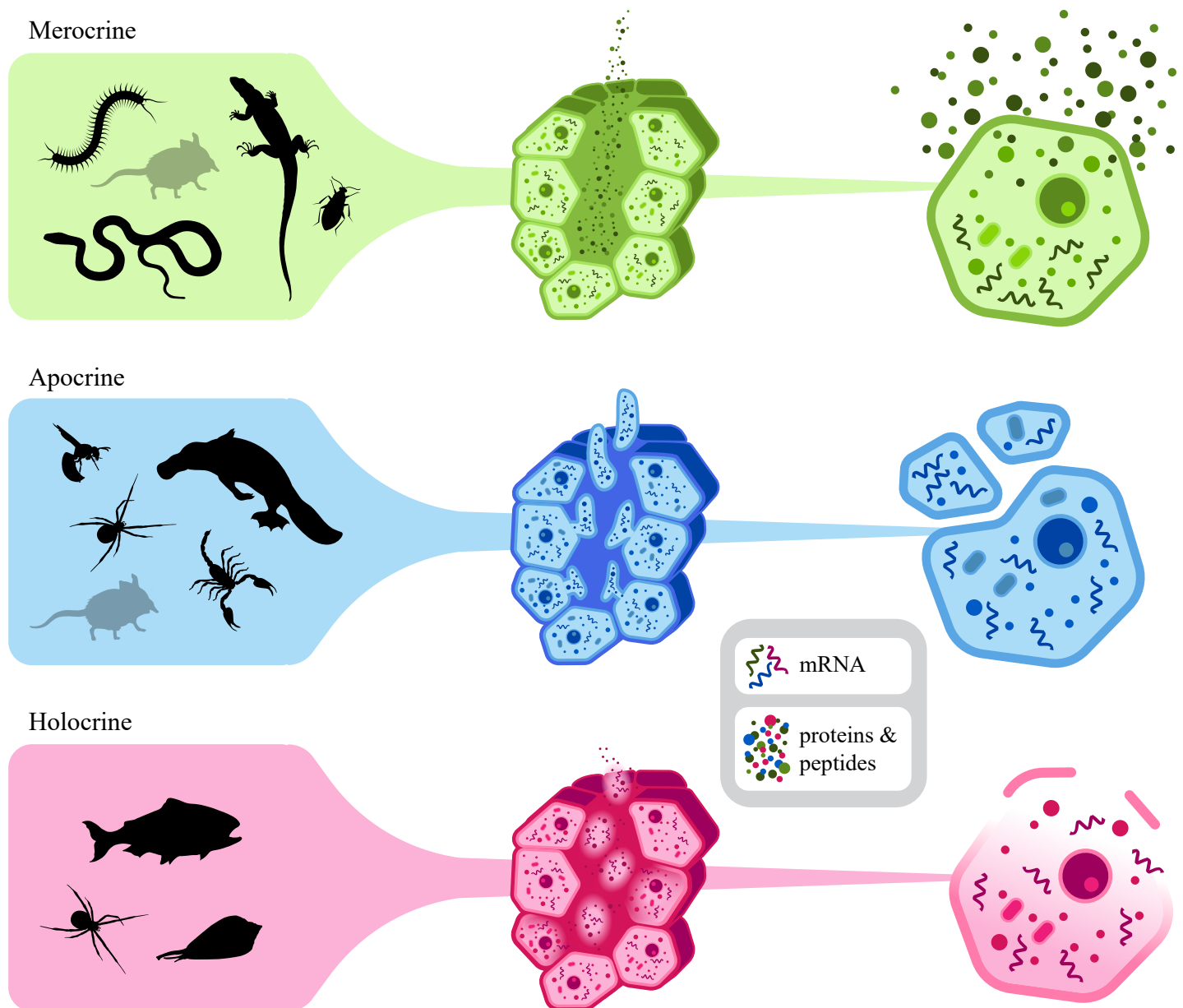
Fig 3. Schematic overview of the three secretion methods in the venom glands across the animal kingdom. The left panel shows the various venomous lineages, clustered based on the method of secretion of their venom-producing cells. For some lineages the gland type depends on the species and therefore these are represented multiple times in this figure. Note that some clades are shown in grey, as literature does not provide sufficient information to properly categorize these based on their method of venom secretion. The middle column of this figure shows a cartoon of excretory cells surrounding the gland lumen and right part of the figure schematically illustrates how venomous secretions are being produced by a single cell (see text for further details).

of the entire cell, is referred to as holocrine secretion. The venom secretions which have accumulated in the cell are released into the gland lumen by rupture of the cell membrane. This type of secretion also delivers cytoplasm, cellular organelles and nuclear cell products into the lumen of the gland, including mRNA [50,51,54,67]. These types of glands can be found in the venom glands of cone snails, certain spider species and teleost fish (Fig 3, Table 1) [59,60,68–77]. Spiders utilise both apocrine and holocrine secretion mechanisms, depending on the species [13,60,61,78]. The secretion method in the venom glands of mammals is dependent on the

Table 1. Overview of different methods of venom gland secretion across all venomous lineages.

Type of gland	Cytoplasm released?	Animal groups	References
Merocrine	No	Centipedes	[57,83–85]
		Heteropterans (assassin bugs, giant water bugs)	[51,58,86]
		Reptiles (snakes, lizards)	[55,56,74]
		Mammals (insectivores)	[44,79]
Apocrine	Yes	Scorpions	[59,87–89]
		Spiders	[13,60,61,78]
		Hymenopterans (bees, ants, wasps)	[62–64]
		Mammals (platypus)	[66,80]
Holocrine	Yes	Cone snails	[68,69,73]
		Spiders	[13,60,61,78]
		Teleost fish	[70–72,74,90,91]
Miscellaneous		Cephalopods*	[43,65,92]
		Cartilaginous fish (stingrays, venomous sharks, chimaeras)*	[67,93–95]
		Mammals (slow lorises, pygmy lorises)**	[81,82]

The various venomous lineages are categorized based on the secretion mechanism in the venom gland. Note that for some taxa the gland type depends on the species and therefore these are represented multiple times in this table. For some clades literature does not provide sufficient information to properly categorize them based on their method of secretion. These have been listed under "miscellaneous".

*No convincing distinction can be made on present literature

**The venom of lorises is a combination of merocrine and apocrine secretions.

clade to which these mammals belong. Venomous insectivores (i.e. solenodons and shrews) have evolved an oral venom system that closely resembles the submaxillary salivary glands, suggesting a merocrine secretion type [44,79]. In male platypuses on the contrary, venom is produced in glands on an extratarsal spur on each hind leg. These glands are likely to have been derived from modified apocrine sweat glands [66,80]. Lastly, the venom secretion of slow and pygmy lorises is rather unique, as these venoms comprise a combination of saliva (merocrine secretion) and fluid from the brachial gland (apocrine secretion) [81,82].

For some venomous clades, literature is lacking or in some cases even contradictory when it comes to classifying these groups based on their method venom secretion. Therefore, no convincing distinction can be made based on present literature. This is the case for the venom secretion mechanisms of cephalopods and cartilaginous fish. Consequently, categorising these clades based on secretion mechanisms should be done with caution, as some gland types are considered apocrine in certain papers, whereas other papers classify these as holocrine. In some cases, the presence of degenerating secretory cells might have been erroneously interpreted as proof for holocrine secretion, whereas the actual mode of secretion is apocrine or even merocrine. This may be the case in the glands of scorpions and cartilaginous fish [59,74,77,87,89]. The same misinterpretation was probably also present in some studies of the ultrastructure of centipede venom glands, in which merocrine secretory glands were wrongfully interpreted as being holocrine [57,83–85].

Animals carrying venom glands with merocrine cells deliver the venom components through exocytosis instead of releasing cytoplasm into the lumen [55,56]. This might explain why mRNA-yields from snake venom are low [96,97]. The excretion type found in these animals makes it impossible to perform NGS of the venom gland without amplification of the mRNA-transcripts. Chen *et al.* managed to sequence mRNA from amphibian skin and snake venom using rtPCR [98,99]. These studies were followed up by studies that were using NGS methods to amplify mRNA in snake venom [96,97]. These studies concluded that snake

venom does not contain sufficient transcripts for NGS sequencing without amplification. Such amplification could introduce unwanted bias in the read counts, making this method less feasible to quantify relative expression rates. Apocrine and holocrine secretion mechanisms on the contrary will potentially yield sufficient mRNA from the cytoplasm in the venom, which would make them suitable for transcriptomic analysis without amplification. In theory, all venoms that are being produced in apocrine or holocrine glands are potentially suitable for transcriptomic analysis. These excretory types will potentially yield mRNA from the cytoplasm in the venom. This paves the way for looking into the variety in venom composition, both intraspecifically and within the same individual. For scorpion venoms for example, we know that the composition exhibits a level of plasticity that can be influenced in response to environmental and behavioural factors [45,46]. Further, the process of venom replenishment is assumed to be an asynchronous process, with certain components being produced at different stages and at varying rates. With this new method, patterns of gene activation and venom production can be followed over time within the same individual, allowing us to study seasonal, ontogenetic and stress-related variation. Although this study focused on venom gland secretions primarily, poisonous animal lineages with apocrine or holocrine secretion methods (e.g., amphibians) might be similarly harnessed to generate the transcriptome of genome-derived compounds (i.e. proteins and peptides) in the poison glands [74–77,100].

Supporting information

S1 Fig. Alignment of κ-KTx contigs with their expression percentage. Overview of the alignments of the κ-KTx contigs from: Whole telson transcriptomes five days after start of venom replenishment (Heterometrus_VG); venom gland transcriptomes two days after start of venom replenishment (Heterometrus_2Day); venom gland transcriptomes four days after the start of venom replenishment (Heterometrus_4Day).
(DOCX)

S2 Fig. Alignment of γ-KTx contigs with their expression percentage. Overview of the alignments of the γ-KTx contigs from: Whole telson transcriptomes five days after start of venom replenishment (Heterometrus_VG); venom gland transcriptomes two days after start of venom replenishment (Heterometrus_2Day); venom gland transcriptomes four days after the start of venom replenishment (Heterometrus_4Day).
(DOCX)

S1 Table. Percentage of basepair similarity in overlapping parts of indicated T5d and V4d contigs.
(DOCX)

S1 File. A. Schematic overview of the scorpion venom extraction device. Schematic of the electrostimulator used to extract venom. This schematic includes an indicator LED, an on-off switch and a switch to choose between an amplitude of 9V and 18V, which all may be omitted for simplicity. The potentiometer is used to select frequency. The parallel 47Ω resistors limit the current to the scorpion. A switch may be added to either lead to the scorpion for fine control of the stimulus. Terminals at the metasoma should be wetted with a drop of saline solution to improve contact. B. Code for the Arduino microcontroller.
(DOCX)

Acknowledgments

Author Contributions

Conceptualization: Arie van der Meijden.

Data curation: Harald M. I. Kerkkamp, John P. Archer.

Formal analysis: Harald M. I. Kerkkamp, Dwin G. B. Grashof, John P. Archer.

Funding acquisition: Arie van der Meijden.

Investigation: Sandra Afonso, Arie van der Meijden.

Methodology: John P. Archer, Arie van der Meijden.

Project administration: Mátyás A. Bittenbinder, Arie van der Meijden.

Resources: Freek J. Vonk, Michael K. Richardson, Jeroen Kool, Arie van der Meijden.

Software: Dwin G. B. Grashof.

Supervision: Freek J. Vonk, Michael K. Richardson, Jeroen Kool, Arie van der Meijden.

Validation: Harald M. I. Kerkkamp.

Visualization: Mátyás A. Bittenbinder, Harald M. I. Kerkkamp.

Writing – original draft: Freek J. Vonk, Mátyás A. Bittenbinder, Harald M. I. Kerkkamp.

Writing – review & editing: Freek J. Vonk, Mátyás A. Bittenbinder, Jeroen Kool, Arie van der Meijden.

References

1. King GF. Venoms as a platform for human drugs: translating toxins into therapeutics. Expert Opin Biol Ther. 2011 Nov; 11(11):1469–84. https://doi.org/10.1517/14712598.2011.621940 PMID: 21939428

2. Casewell NR, Wüster W, Vonk FJ, Harrison RA, Fry BG. Complex cocktails: The evolutionary novelty of venoms. Trends Ecol Evol. 2013; 28(4):219–29. https://doi.org/10.1016/j.tree.2012.10.020 PMID: 23219381

3. Fry BG, Scheib H, van der Weerd L, Young B, McNaughtan J, Ramjan SFR, et al. Evolution of an Arsenal. Mol Cell Proteomics [Internet]. 2008; 7(2):215–46. Available from: http://www.mcponline.org/lookup/doi/10.1074/mcp.M700094-MCP200. PMID: 17855442

4. Gopalakrishnakone P, Possani LD, Schwartz EF, Rodríguez De La Vega RC. Scorpion venoms. Scorpion Venoms. 2015. 1–575 p.

5. Isbister GK, Bawaskar HS. Scorpion envenomation. N Engl J Med. 2014; 371(5):457–63. https://doi.org/10.1056/NEJMra1401108 PMID: 25075837

6. Gutiérrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, Warrell DA. Snakebite envenoming. Nat Rev Dis Prim. 2017; 3:17063. https://doi.org/10.1038/nrdp.2017.63 PMID: 28905944

7. Harvey AL. Natural products in drug discovery. Drug Discov Today. 2008; 13(19–20):894–901. https://doi.org/10.1016/j.drudis.2008.07.004 PMID: 18691670

8. Vetter I, Davis JL, Rash LD, Anangi R, Mobli M, Alewood PF, et al. Venomics: A new paradigm for natural products-based drug discovery. Amino Acids. 2011; 40(1):15–28. https://doi.org/10.1007/s00726-010-0516-4 PMID: 20177945

9. Harvey AL. Toxins and drug discovery. Toxicon. 2014; 92:193–200. https://doi.org/10.1016/j.toxicon.2014.10.020 PMID: 25448391

10. Himaya SWA, Lewis RJ. Venomics-accelerated cone snail venom peptide discovery. Int J Mol Sci. 2018; 19(3):1–12. https://doi.org/10.3390/ijms19030788 PMID: 29522462

11. Wilson D, Daly NL. Venomics: A mini-review. High-Throughput. 2018; 7(3). https://doi.org/10.3390/ht7030019 PMID: 30041430

12. Otvos RA, Still KBM, Somsen GW, Smit AB, Kool J. Drug Discovery on Natural Products: From Ion Channels to nAChRs, from Nature to Libraries, from Analytics to Assays. SLAS Discov. 2019; 24 (3):362–85. https://doi.org/10.1177/2472555218822098 PMID: 30682257

13. Langenegger N, Nentwig W, Kuhn-nentwig L. and Novel Strategies in Transcriptomic and. 2019.

14. Laustsen AH, Solà M, Jappe EC, Oscoz S, Lauridsen LP, Engmark M. Biotechnological trends in spider and scorpion antivenom development. Toxins (Basel). 2016; 8(8).

15. Manzoni C, Kia DA, Vandrovcova J, Hardy J, Wood NW, Lewis PA, et al. Genome, transcriptome and proteome: The rise of omics data and their integration in biomedical sciences. Brief Bioinform. 2018; 19(2):286–302. https://doi.org/10.1093/bib/bbw114 PMID: 27881428

16. Calvete JJ, Sanz L, Angulo Y, Lomonte B, Gutiérrez JM. Venoms, venomics, antivenomics. FEBS Lett [Internet]. 2009; 583(11):1736–43. Available from: https://doi.org/10.1016/j.febslet.2009.03.029 PMID: 19303875

17. Chippaux JP, Goyffon M. Epidemiology of scorpionism: A global appraisal. Acta Trop. 2008; 107 (2):71–9. https://doi.org/10.1016/j.actatropica.2008.05.021 PMID: 18579104

18. Kasturiratne A, Wickremasinghe AR, De Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, et al. The global burden of snakebite: A literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Med. 2008; 5(11):1591–604. https://doi.org/10.1371/journal.pmed. 0050218 PMID: 18986210

19. Gopalakrishnakone P, Calvete JJ. Venom Genomics and Proteomics. Venom Genomics and Proteomics. 2016. https://doi.org/10.1016/j.cbd.2016.08.002 PMID: 27636656

20. Post Y, Puschhof J, Beumer J, Kerkkamp HM, de Bakker MAG, Slagboom J, et al. Snake Venom Gland Organoids. Cell [Internet]. 2020; 180(2):233–247.e21. Available from: https://doi.org/10.1016/j. cell.2019.11.038 PMID: 31978343

21. Soroceanu L, Manning TJ, Sontheimer H. Modulation of Glioma Cell Migration and Invasion Using Cl − and K + Ion Channel Blockers. J Neurosci. 1999 Jul; 19(14):5942–54. https://doi.org/10.1523/ JNEUROSCI.19-14-05942.1999 PMID: 10407033

22. Lyons SA, O'Neal J, Sontheimer H. Chlorotoxin, a scorpion-derived peptide, specifically binds to gliomas and tumors of neuroectodermal origin. Glia. 2002; 39(2):162–73. https://doi.org/10.1002/glia. 10083 PMID: 12112367

23. Rapôso C. Scorpion and spider venoms in cancer treatment: state of the art, challenges, and perspectives. J Clin Transl Res. 2017; 3(2):233–49. PMID: 30873475

24. Tanner MR, Pennington MW, Chamberlain BH, Huq R, Gehrmann EJ, Laragione T, et al. Targeting KCa1.1 channels with a scorpion venom peptide for the therapy of rat models of rheumatoid arthritis. J Pharmacol Exp Ther. 2018; 365(2):227–36. https://doi.org/10.1124/jpet.117.245118 PMID: 29453198

25. Schwartz EF, Diego-Garcia E, Rodríguez de la Vega RC, Possani LD. Transcriptome analysis of the venom gland of the Mexican scorpion Hadrurus gertschi (Arachnida: Scorpiones). BMC Genomics. 2007 Jan; 8:119. https://doi.org/10.1186/1471-2164-8-119 PMID: 17506894

26. Morgenstern D, Rohde BH, King GF, Tal T, Sher D, Zlotkin E. The tale of a resting gland: transcriptome of a replete venom gland from the scorpion Hottentotta judaicus. Toxicon. 2011 May; 57(5):695–703. https://doi.org/10.1016/j.toxicon.2011.02.001 PMID: 21329713

27. Ma Y, He Y, Zhao R, Wu Y, Li W, Cao Z. Extreme diversity of scorpion venom peptides and proteins revealed by transcriptomic analysis: implication for proteome evolution of scorpion venom arsenal. J Proteomics. 2012 Mar; 75(5):1563–76. https://doi.org/10.1016/j.jprot.2011.11.029 PMID: 22155128

28. Rendón-Anaya M, Delaye L, Possani LD, Herrera-Estrella A. Global transcriptome analysis of the scorpion Centruroides noxius: new toxin families and evolutionary insights from an ancestral scorpion species. PLoS One. 2012 Jan; 7(8):e43331. https://doi.org/10.1371/journal.pone.0043331 PMID: 22912855

29. De Oliveira UC, Candido DM, Coronado Dorce VA, Junqueira-De-Azevedo IDLM. The transcriptome recipe for the venom cocktail of Tityus bahiensis scorpion. Toxicon. 2015 Mar; 95:52–61. https://doi. org/10.1016/j.toxicon.2014.12.013 PMID: 25553591

30. Quintero-Hernández V, Ramírez-Carreto S, Romero-Gutiérrez MT, Valdez-Velázquez LL, Becerril B, Possani LD, et al. Transcriptome analysis of scorpion species belonging to the Vaejovis genus. Wicker-Thomas C, editor. PLoS One. 2015 Feb; 10(2):e0117188. https://doi.org/10.1371/journal. pone.0117188 PMID: 25659089

31. Zhang L, Shi W, Zeng XC, Ge F, Yang M, Nie Y, et al. Unique diversity of the venom peptides from the scorpion Androctonus bicolor revealed by transcriptomic and proteomic analysis. J Proteomics [Internet]. 2015; 128:231–50. Available from: https://doi.org/10.1016/j.jprot.2015.07.030 PMID: 26254009

32. Rokyta DR, Ward MJ. Venom-gland transcriptomics and venom proteomics of the black-back scorpion (Hadrurus spadix) reveal detectability challenges and an unexplored realm of animal toxin diversity. Toxicon. 2017 Mar; 128:23–37. https://doi.org/10.1016/j.toxicon.2017.01.014 PMID: 28115184

33. Deng Y, Gu J, Yan Z, Wang M, Ma C, Zhang J, et al. De novo transcriptomic analysis of the venomous glands from the scorpion Heterometrus spinifer revealed unique and extremely high diversity of the

venom peptides. Toxicon. 2018 Mar; 143:1–19. https://doi.org/10.1016/j.toxicon.2017.12.051 PMID: 29305080

34. Ward MJ, Ellsworth SA, Rokyta DR. Venom-gland transcriptomics and venom proteomics of the Hentz striped scorpion (Centruroides hentzi; Buthidae) reveal high toxin diversity in a harmless member of a lethal family. Toxicon. 2018 Feb; 142:14–29. https://doi.org/10.1016/j.toxicon.2017.12.042 PMID: 29248469

35. Grashof DGB, Kerkkamp HMI, Afonso S, Archer J, Harris DJ, Richardson MK, et al. Transcriptome annotation and characterization of novel toxins in six scorpion species. BMC Genomics. 2019 Dec; 20 (1):645. https://doi.org/10.1186/s12864-019-6013-6 PMID: 31409288

36. Vonk FJ, Casewell NR, Henkel C V., Heimberg AM, Jansen HJ, McCleary RJR, et al. The king cobra genome reveals dynamic gene evolution and adaptation in the snake venom system. Proc Natl Acad Sci [Internet]. 2013; 110(51):20651–6. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.1314702110.

37. Castoe TA, De Koning APJ, Hall KT, Card DC, Schield DR, Fujita MK, et al. Erratum: The Burmese python genome reveals the molecular basis for extreme adaptation in snakes (Proceedings of the National Academy of Sciences of the United States of America (2013) 110, 51, (20645–20650) https://doi.org/10.1073/pnas.1314475110) Proc Natl Acad Sci U S A. 2014; 111(8):3194.

38. Tasoulis T, Isbister GK. A review and database of snake venom proteomes. Toxins (Basel). 2017; 9 (9). https://doi.org/10.3390/toxins9090290 PMID: 28927001

39. Calvete JJ. Snake venomics: From the inventory of toxins to biology. Toxicon [Internet]. 2013; 75:44–62. Available from: https://doi.org/10.1016/j.toxicon.2013.03.020 PMID: 23578513

40. Suryamohan K, Krishnankutty SP, Guillory J, Jevit M, Schröder MS, Wu M, et al. The Indian cobra reference genome and transcriptome enables comprehensive identification of venom toxins. Nat Genet [Internet]. 2020; 52(1):106–17. Available from: https://doi.org/10.1038/s41588-019-0559-8 PMID: 31907489

41. Zhou Y, Shearwin-Whyatt L, Li J, Song Z, Hayakawa T, Stevens D, et al. Platypus and echidna genomes reveal mammalian biology and evolution. Nature [Internet]. 2021;(December 2019). Available from: https://doi.org/10.1038/s41586-020-03039-0 PMID: 33408411

42. Margres MJ, Rautsaw RM, Strickland JL, Mason AJ, Schramer TD, Hofmann EP, et al. The Tiger Rattlesnake genome reveals a complex genotype underlying a simple venom phenotype. Proc Natl Acad Sci U S A. 2021; 118(4):1–12. https://doi.org/10.1073/pnas.2014634118 PMID: 33468678

43. Fingerhut LCHW Strugnell JM, Faou P Labiaga ÁR, Zhang J Cooke IR. Shotgun Proteomics Analysis of Saliva and Salivary Gland Tissue from the Common Octopus Octopus vulgaris. J Proteome Res. 2018; 17(11):3866–76. https://doi.org/10.1021/acs.jproteome.8b00525 PMID: 30220204

44. Casewell NR, Petras D, Card DC, Suranse V, Mychajliw AM, Richards D, et al. Solenodon genome reveals convergent evolution of venom in eulipotyphlan mammals. Proc Natl Acad Sci U S A. 2019; 116(51):25745–55. https://doi.org/10.1073/pnas.1906117116 PMID: 31772017

45. Carcamo-Noriega EN, Possani LD, Ortiz E. Venom content and toxicity regeneration after venom gland depletion by electrostimulation in the scorpion Centruroides limpidus. Toxicon [Internet]. 2019; 157(October 2018):87–92. Available from: https://doi.org/10.1016/j.toxicon.2018.11.305 PMID: 30468759

46. Gangur AN, Smout M, Liddell MJ, Seymour JE, Wilson D, Northfield TD. Changes in predator exposure, but not in diet, induce phenotypic plasticity in scorpion venom. Proc R Soc B Biol Sci. 2017; 284 (1863).

47. Zamani A, Saaksjarvi IE, Prendini L. Amateur venom-extraction business may hasten extinction of scorpions. Arachnol Mitteilungen. 2021; 61(1):20–3.

48. Coelho P, Kaliontzopoulou A, Rasko M, van der Meijden A. A 'striking' relationship: scorpion defensive behaviour and its relation to morphology and performance. Funct Ecol. 2017; 31(7):1390–404.

49. Petersen TN, Brunak S, Von Heijne G, Nielsen H. SignalP 4.0: Discriminating signal peptides from transmembrane regions. Nat Methods. 2011; 8(10):785–6. https://doi.org/10.1038/nmeth.1701 PMID: 21959131

50. Farkaš R. Apocrine secretion: New insights into an old phenomenon. Biochim Biophys Acta—Gen Subj. 2015; 1850(9):1740–50. https://doi.org/10.1016/j.bbagen.2015.05.003 PMID: 25960390

51. Swart CC, Felgenhauer BE. Structure and function of the mouthparts and salivary gland complex of the giant waterbug, Belostoma lutarium (Stål) (Hemiptera: Belostomatidae). Ann Entomol Soc Am. 2003; 96(6):870–82.

52. Schiefferdecker P. Die Hautorgane des Menschen und der Säugertiere, Ihre biologische und rassenanatomische Bedeutung sowie die Muscularis sexualis. Biol. Centralbl. 37, no. 11; 1917. p. 534–62.

53. Schiefferdecker P. Uber morphologische Sekretionserscheinungen in den ekkrinen Hautdrüsen des Menschen. Arch Dermatol Syph. 1921; 132(1):130–2.

54. Schaumburg Lever G, Lever WF. Secretion from human apocrine glands: an electron microscopic study. J Invest Dermatol [Internet]. 1975; 64(1):38–41. Available from: https://doi.org/10.1111/1523-1747.ep12540893 PMID: 1110304

55. Mackessy SP. Morphology and ultrastructure of the venom glands of the northern pacific rattlesnake Crotalus viridis oreganus. J Morphol. 1991; 208(1):109–28. https://doi.org/10.1002/jmor.1052080106 PMID: 29865511

56. Mackessy SP, Baxter LM. Bioweapons synthesis and storage: The venom gland of front-fanged snakes. Zool Anz. 2006; 245(3–4):147–59.

57. Ménez A, Zimmerman K, Zimmerman S, Heatwole H. Venom apparatus and toxicity of the centipede Ethmostigmus rubripes (Chilopoda, Scolopendridae). J Morphol. 1990; 206(3):303–12. https://doi.org/10.1002/jmor.1052060307 PMID: 2280408

58. Walker A. Personal communication to MA Bittenbinder. 2021.

59. Soliman BA, Shoukry NM, Mohallal ME, Fetaih HAW, khaled HS. Fine structure of the stinger, histology and histochemistry of the venom gland in the scorpion Androctonus amoreuxi (Buthidae). J Basic Appl Zool [Internet]. 2013; 66(2):41–6. Available from: http://dx.doi.org/10.1016/j.jobaz.2013.03.001.

60. Dos Santos VLP, Franco CRC, Viggiano RLL, Da Silveira RB, Cantão MP, Mangili OC, et al. Structural and ultrastructural description of the venom gland of Loxosceles intermedia (brown spider). Toxicon. 2000; 38(2):265–85. https://doi.org/10.1016/s0041-0101(99)00155-5 PMID: 10665807

61. Silva LM, Carvalho Botelho AC, Nacif-Pimenta R, Martins GF, Alves LC, Brayner FA, et al. Structural analysis of the venom glands of the armed spider Phoneutria nigriventer (Keyserling, 1891): Microanatomy, fine structure and confocal observations. Toxicon. 2008; 51(4):693–706. https://doi.org/10.1016/j.toxicon.2007.12.009 PMID: 18241905

62. Billen J. Morphology and ultrastructure of the dufour's and venom gland in the ant myrmecia gulosa (Fabr.) (Hymenoptera: Formicidae). Vol. 38, Australian Journal of Zoology. 1990. p. 227–40.

63. Kumar NR, Devi A. A study on biochemical composition of the sting gland (poison gland) and the reservoir (poison sac) of the dwarf honey bee Apis florea F. workers. J Appl Nat Sci. 2014; 6(1):101–5.

64. Devi A, Sangeeta, Kumar NR, Kaur J. Honey bee venom and its Composition: Focusing on different Apis species-A review. Artic J Basic Appl Eng Res. 2016; 3(1):96–8.

65. Matus AI. Fine structure of the posterior salivary gland of Eledone cirrosa and Octopus vulgaris. Zeitschrift für Zellforsch und Mikroskopische Anat. 1971; 122(1):111–21. https://doi.org/10.1007/BF00936120 PMID: 4330714

66. Whittington CM, Belov K. Tracing monotreme venom evolution in the genomics era. Toxins (Basel). 2014; 6(4):1260–73. https://doi.org/10.3390/toxins6041260 PMID: 24699339

67. Pedroso CM, Jared C, Charvet-Almeida P, Almeida MP, Neto DG, Lira MS, et al. Morphological characterization of the venom secretory epidermal cells in the stinger of marine and freshwater stingrays. Toxicon. 2007; 50(5):688–97. https://doi.org/10.1016/j.toxicon.2007.06.004 PMID: 17659760

68. Möller C, Vanderweit N, Bubis J, Mari F. Comparative analysis of proteases in the injected and dissected venom of cone snail species. Toxicon. 2013; 65:59–67. https://doi.org/10.1016/j.toxicon.2012.12.014 PMID: 23339854

69. Marshall J, Kelley WP, Rubakhin SS, Bingham JP, Sweedler J V., Gilly WF. Anatomical correlates of venom production in Conus californicus. Biol Bull. 2002; 203(1):27–41. https://doi.org/10.2307/1543455 PMID: 12200253

70. Cameron AM, Endean R. The venom glands of teleost fishes. Toxicon. 1972; 10(3):301–3. https://doi.org/10.1016/0041-0101(72)90018-9 PMID: 5072096

71. Whitear M, Zaccone G, Ricca MB, Cascio P Lo. Fine structure and histochemistry of the venom gland in the Indian stinging catfish (Heteropneustes fossilis). J Zool. 1991; 224(3):479–89.

72. Gopalakrishnakone P, Gwee MCE. The structure of the venom gland of stonefish Synanceja Horrida. Toxicon. 1993; 31(8):979–88. https://doi.org/10.1016/0041-0101(93)90257-j PMID: 8212050

73. Fänge R. Venoms and Venom Glands of Marine Molluscs. 1984;47–62.

74. Roberts KA. An Ultrastructural Survey of the Nuchal Glands of Rhabdophis tigrinus (Serpentes: Colubridae). 2000.

75. Lacombe C, Cifuentes-Diaz C, Dunia I, Auber-Thomay M, Nicolas P, Amiche M. Peptide secretion in the cutaneous glands of South American tree frog Phyllomedusa bicolor: An ultrastructural study. Eur J Cell Biol. 2000; 79(9):631–41. https://doi.org/10.1078/0171-9335-00085 PMID: 11043404

76. De Almeida PG, Felsemburgh FA, Azevedo RA, De Brito-Gitirana L. Morphological re-evaluation of the parotoid glands of Bufo ictericus (Amphibia, Anura, Bufonidae). Contrib to Zool. 2007; 76(3):145–52.

77. Neiva M, Vargas DC, Conceição K, Rádis-Baptista G, Assakura MT, Jared C, et al. Gene expression analysis by ESTs sequencing of the Brazilian frog Phyllomedusa nordestina skin glands. Toxicon [Internet]. 2013; 61(1):139–50. Available from: https://doi.org/10.1016/j.toxicon.2012.10.016 PMID: 23159791

78. Malli H, Kuhn-Nentwig L, Imboden H, Moon M-J, Wyler T. Immunocytochemical localization and secretion process of the toxin CSTX-1 in the venom gland of the wandering spider Cupiennius salei (Araneae: Ctenidae). Cell Tissue Res. 2000; 299(3):417–26. https://doi.org/10.1007/s004419900141 PMID: 10772256

79. Hanf ZR, Chavez AS. A comprehensive multi-omic approach reveals a relatively simple venom in a diet generalist, the northern short-tailed shrew, Blarina brevicauda. Genome Biol Evol. 2020; 12 (7):1148–66. https://doi.org/10.1093/gbe/evaa115 PMID: 32520994

80. Krause WJ. Morphological and histochemical observations on the crural gland-spur apparatus of the echidna (tachyglossus aculeatus) together with comparative observations on the femoral gland-spur apparatus of the duckbilled platypus (ornithorhyncus anatinus). Cells Tissues Organs. 2010; 191 (4):336–54. https://doi.org/10.1159/000252802 PMID: 20224277

81. Anne-Isola Nekaris K, Moore RS, Johanna Rode E, Fry BG. Mad, bad and dangerous to know: The biochemistry, ecology and evolution of slow loris venom. J Venom Anim Toxins Incl Trop Dis. 2013; 19 (1):1–10. https://doi.org/10.1186/1678-9199-19-1 PMID: 23849430

82. Hagey L.R.; Fry B.G.; Fitch-Snyder H. Talking Defensively, a Dual Use for the Brachial Gland Exudate of Slow and Pygmy Lorises. In: Gursky S.L.; Nekaris KAI, editor. Primate Anti-Predator Strategies. Springer; 2013. p. 253–72.

83. Undheim EAB, King GF. On the venom system of centipedes (Chilopoda), a neglected group of venomous animals. Toxicon [Internet]. 2011; 57(4):512–24. Available from: https://doi.org/10.1016/j.toxicon.2011.01.004 PMID: 21255597

84. Rosenberg J, Hilken G. Fine structural organization of the poison gland of Lithobius forficatus (Chilopoda, Lithobiomorpha). Nor J Entomol. 2006; 53(2):119–27.

85. Antoniazzi MM, Pedroso CM, Knysak I, Martins R, Guizze SPG, Jared C, et al. Comparative morphological study of the venom glands of the centipede Cryptops iheringi, Otostigmus pradoi and Scolopendra viridicornis. Toxicon. 2009; 53(3):367–74. https://doi.org/10.1016/j.toxicon.2008.12.010 PMID: 19105969

86. Walker AA, Madio B, Jin J, Undheim EAB, Fry BG, King GF. Melt with this kiss: Paralyzing and liquefying venom of the Assassin bug Pristhesancus plagipennis (Hemiptera: Reduviidae). Mol Cell Proteomics. 2017; 16(4):552–66. https://doi.org/10.1074/mcp.M116.063321 PMID: 28130397

87. Mazurkiewicz JE, Bertke EM. Ultrastructure of the venom gland of the scorpion, Centruroides sculpturatus (Ewing). J Morphol. 1972; 137(3):365–83. https://doi.org/10.1002/jmor.1051370307 PMID: 30366479

88. Keegan HL, Lockwood WR. Secretory epithelium in venom glands of two species of scorpion of the genus Centruroides Marx. Am J Trop Med Hyg. 1971 Sep; 20(5):770–85. https://doi.org/10.4269/ajtmh.1971.20.770 PMID: 4328571

89. Hjelle JT. Anatomy and morphology. In: Biology of Scorpions. 1990. p. 64–160.

90. Khalil AM, Wahsha MA, Abu Khadra KM, Khalaf MA, Al-Najjar TH. Biochemical and histopathological effects of the stonefish (Synanceia verrucosa) venom in rats. Toxicon. 2018; 142:45–51. https://doi.org/10.1016/j.toxicon.2017.12.052 PMID: 29294314

91. Halstead B.W.; Chitwood M.J., Modglin FR. Stonefish stings and the venom apparatus of Synanceja horrida (Linnaeus). Trans Am Microsc Soc. 1956; 75(4):381–97.

92. Cooke I.R; Whitelaw B.; Norman M.; Caruana N.; Strugnell JM. Toxicity in Cephalopods. In: Gopalakirshnakone P.; Malhotra A, editor. Evolution of venomous animals and their toxins [Internet]. Springer; 2017. p. 125–43. Available from: http://www.springer.com/series/13330.

93. Ocampo RR, Halstead BW, Modglin FR. The microscopic anatomy of the caudal appendage of the spotted eagleray, Aëtobatus narinari (Euphrasen), with special reference to the venom apparatus. Anat Rec. 1953; 115(1):87–99. https://doi.org/10.1002/ar.1091150108 PMID: 13016997

94. Halstead BW, Ocampo RR, Modglin FR. A study on the comparative anatomy of the venom apparatus of certain north American stingrays. J Morphol. 1955; 97(1):1–21.

95. Smith DS, Cayer ML, Russell FE. Membrane-limited microtubular aggregates in the venom secreting cells of a stingray. Toxicon. 1974; 12(3):331–5. https://doi.org/10.1016/0041-0101(74)90080-4 PMID: 4458114

96. Modahl CM, Mackessy SP. Full-Length Venom Protein cDNA Sequences from Venom-Derived mRNA: Exploring Compositional Variation and Adaptive Multigene Evolution. Calvete J, editor. PLoS Negl Trop Dis. 2016 Jun; 10(6):e0004587. https://doi.org/10.1371/journal.pntd.0004587 PMID: 27280639

97. Whiteley G, Logan RAE, Leung K-YD, Newberry FJ, Rowley PD, Dunbar JP, et al. Stabilising the Integrity of Snake Venom mRNA Stored under Tropical Field Conditions Expands Research Horizons. Kuch U, editor. PLoS Negl Trop Dis. 2016 Jun; 10(6):e0004615. https://doi.org/10.1371/journal.pntd.0004615 PMID: 27280729

98. Chen T, Bjourson AJ, Orr DF, Kwok H, Rao P, Ivanyi C, et al. Unmasking venom gland transcriptomes in reptile venoms. Anal Biochem. 2002 Dec; 311(2):152–6. https://doi.org/10.1016/s0003-2697(02)00404-9 PMID: 12470674

99. Chen T, Farragher S, Bjourson AJ, Orr DF, Rao P, Shaw C. Granular gland transcriptomes in stimulated amphibian skin secretions. Biochem J. 2003; https://doi.org/10.1042/BJ20021343 PMID: 12413397

100. Conceição K, Bruni FM, Sciani JM, Konno K, Melo RL, Antoniazzi MM, Jared C, Lopes-Ferreira M PD. Identification of bradykinin-related peptides from phyllomedusa nordestina skin secretion using electrospray ionization tandem mass spectrometry after a single-step liquid chromatography. J Venom Anim Toxins incl Trop Dis. 2009; 15(4):633–52.