Centipede venoms as a source of drug leads

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ABSTRACT

Introduction: Centipedes are one of the oldest and most successful lineages of venomous terrestrial predators. Despite their use for centuries in traditional medicine, centipede venoms remain poorly studied. However, recent work indicates that centipede venoms are highly complex chemical arsenals that are rich in disulfide-constrained peptides that have novel pharmacology and three-dimensional structure.

Areas covered: This review summarizes what is currently know about centipede venom proteins, with a focus on disulfide-rich peptides that have novel or unexpected pharmacology that might be useful from a therapeutic perspective. We also highlight the remarkable diversity of constrained three-dimensional peptide scaffolds present in these venoms that might be useful for bioengineering of drug leads.

Expert opinion: The resurgence of interest in peptide drugs has stimulated interest in venoms as a source of highly stable, disulfide-constrained peptides with potential as therapeutics. Well-studied venomous taxa such as cone snails and snakes have yielded FDA-approved drugs, while ancient invertebrate predators such as spiders and sea anemones have yielded molecules that are currently in preclinical studies and clinical trials, respectively. However, until recently, the paucity of research on centipede venoms made it easy to discount them as a potential source of peptides with therapeutically useful properties. Seminal studies over the past five years have revealed that centipede venoms have exceedingly complex proteomes that are perhaps richer than any other venom in unique disulfide-rich peptide scaffolds. Like most arthropod predators, centipede venoms are rich in peptides that target neuronal ion channels and receptors, but it is also becoming increasingly apparent that many of these peptides have novel or unexpected pharmacological properties with potential applications in drug discovery and development.

KEYWORDS

therapeutic; biologic; peptide drug; drug discovery; bioengineering; centipede venom; venom peptide; venomics; ion channel.

1. Introduction

1.1 The current landscape of venom-derived drugs

There are currently six FDA-approved venom-derived drugs that are used to treat a variety of disorders ranging from hypertension to pain and diabetes [1]. Many more venom-derived molecules that target an even wider range of disorders, including autoimmune disease, stroke and cancer, are in clinical and preclinical development [2-10]. Most of these molecules were derived from just a few well-studied venomous taxa [5,11], and therefore venoms remain an underutilized source of pharmacologically active compounds. For example, it has been estimated that less than 1% of the repertoire of bioactive spider-venom peptides has been sampled, despite spiders being regarded as one of the better studied venomous animals [12]. Venomous animals encompass a phyletic diversity that is hugely underappreciated, which has left entire groups of independently evolved venomous animals almost completely unstudied [13,14]. Centipedes are one such group. Despite their use for centuries in traditional medicine [15-17], centipede venoms remain poorly studied from a biochemical and pharmacological perspective, and consequently they are underappreciated as a potential source of drug leads.

It has recently been shown that centipede venoms are a rich source of peptides with unusual structural and pharmacological diversity [16,18]. While peptides have emerged over the past decade as an attractive source of new therapeutics [19], naturally occurring peptides often suffer from intrinsic weaknesses such as poor stability and bioavailability that limit their potential as drugs [20]. In contrast, venom peptides often have remarkable chemical, thermal and biological stability due to their compact three-dimensional structures that are typically reinforced by numerous disulfide bonds [4,21,22]. Moreover, the intrinsic properties that allow venom peptides to exert their function—namely potency, selectivity, stability, and solubility—are highly desirable traits for therapeutics.

1.2. Centipedes: old and venomous

With the possible exception of scorpions, centipedes (Class Chilopoda) are the oldest lineage of venomous terrestrial predators [23], with a fossil record dating back 420 million years. Not to be confused with their non-venomous sister-class, the millipedes (Class Diplopoda), centipedes are the main predatory group within Myriapoda. They are a highly successful group of venomous animals with approximately 3,500 extant species, making them more speciose than snakes and scorpions [5]. Extant centipedes are classified into five orders: Scutigeromorpha (house centipedes), Lithobiomorpha (stone centipedes), Geophilomorpha (earth centipedes), Scolopendromorpha (popular in the pet trade, often referred to as scolopenders, and includes giant species) and Craterostigmomorpha (Figure 1). With the exception of Craterostigmomorpha, which is represented by only two species occurring in New Zealand and Tasmania, these orders are distributed worldwide across all continents except Antarctica.

Centipedes are almost exclusively predators, and most species are nocturnal. Although they prey upon a wide diversity of invertebrates, with some larger species feeding on vertebrates, most centipede diets consist primarily of arthropods, which they incapacitate using venom injected through a pair of powerful pincer-like appendages called forcipules. Venom is expelled from the glands via a cuticular duct through a pore located near the tip on the outer curvature of the forcipules. Each gland is composed of a collection of distinct secretory units, each of which comprises 3–4 cells [24]. Recent mass spectrometry imaging studies have revealed that peptide toxins are produced in a heterogeneous fashion within the venom gland, with distinct zip codes for each peptide [25,26].

1.3. Centipede venoms are complex chemical arsenals

Centipedes are notorious for delivering painful defensive bites [24]. However, despite their size and the frequency of encounters with humans, very little is known about centipede venom [18,24]. What we *do* know, however, suggests that centipedes are an excellent source of novel bioactive molecules. The great age of their lineage, the deep evolutionary divergences between living orders, and the large number of extant species, create a phylogenetic breath of independently evolving venoms that greatly exceeds that of most venomous taxa that have already yielded drugs, including reptiles, cone snails, and scorpions. Moreover, the majority of non-enzyme protein families described from centipede venom appear to be unique to centipedes, and consequently they are likely to include novel peptides and proteins with therapeutically useful biological functions [18].

The ancestral centipede venom contained a mix of high molecular weight proteins (HMWPs) and low molecular weight peptides, with further expansion of the venom cocktail in subsequent lineages [18]. The venom peptides, which are of most interest from a drug discovery perspective, are extraordinarily diverse [25,27-32]. Undheim et al. [30] classified centipede venom peptides into 31 phylogenetically distinct families of scutigerotoxins (SCUTX1–3) and scoloptoxins (SLPTX1–28). Remarkably, 24 of these families are comprised of cysteine-rich peptides with 1–6 putative disulfide bonds, the majority of which show no similarity to known peptides and do not contain any recognizable structural or functional domains. Thus, centipede venoms appear to be an excellent source of *novel* peptides.

2. Novel disulfide-rich peptide scaffolds in centipede venom

2.1 Centipede venoms are a rich source of novel structural scaffolds

Among the properties that make venoms attractive as a potential source of therapeutics is the abundance of disulfide-rich peptides [5,33]. The internal cross-bracing provided by covalent disulfide bonds creates a rigid structural framework that provides resistance to chemical and thermal denaturation as well as degradation by proteases [4,21,22,34,35]. Although peptides of similar structure can harbor a diversity of interesting pharmacological properties, the characterization of new

disulfide frameworks is of particular interest from a drug discovery perspective as they are more likely to exhibit novel pharmacology [36] and expand the repertoire of structural templates for bioengineering purposes [37-41].

Centipede venoms appear to be a richer source of novel disulfide-rich peptide scaffolds than other venoms studied to date. There are 24 phylogenetically distinct disulfide-rich peptide scaffolds that are currently recognized in centipede venoms: SCUTX1–2, SLPTX1–20, SLPTX26, and SLPTX28 [18]. Remarkably, 19 of these bear no obvious resemblance to the sequence or cysteine framework of any known disulfide-rich peptide. Several of these peptide-toxin families show substantial variation in terms of length, primary structure, and cysteine-pattern [30]. For example, members of SLPTX11 range in size from 4 to 20 kDa, and contain anywhere from 2 to 9 disulfide bonds. Consistent with this diversity of size and cysteine framework, the masses of centipede venom peptides do not follow a bimodal distribution (Figure 2) as has been observed for venom peptides from spiders and scorpions [42]. This is consistent with centipede venoms containing a much greater diversity of three-dimensional peptide scaffolds than scorpions and spiders which are both dominated by a single peptide fold (i.e., cystine-stabilised α/β defensin fold [33] and inhibitor cystine knot fold [43,44], respectively).

Despite the diversity of novel disulfide-rich peptide scaffolds present in centipede venoms, the Protein Data Bank (PDB) currently contains structures for only four centipede venom peptides. Moreover, these structures belong to just two toxin families, SLPTX3 and SLPTX4, and hence they provide a mere glimpse of the structural diversity present in centipede venoms.

2.2 The SLPTX3 fold: weaponization of a hormone

The first reported structure of a centipede venom peptide was that of μ-SLPTX$_3$-Ssm6a (henceforth just Ssm6a), a three-disulfide, 46-residue peptide from the Chinese red-headed centipede *Scolopendra subspinipes mutilans* (Figure 3a,b) [35]. The all-helical 3D structure of Ssm6a revealed that it is derived from a family of ubiquitous ecdysozoan peptide hormones known as the ion transport peptide/crustacean hyperglycemic hormone (ITP/CHH) family [35]. The 3D structure of ITP/CHH peptides is comprised of five helices (α1 to α5), of which three are inter-connected by disulfide bonds (α1–α3, α2–α3, and α2–α4) [45]. However, in weaponized ITP/CHH peptides—that is, ITP/CHH peptides that are found in venoms *and* exert a toxic role—the fifth helix has been removed to create helical arthropod-neuropeptide-derived (HAND) toxins [35] (Figure 3b–d). This derivation, which rids HAND toxins of the only helix not stabilized by disulfide bonds, appears to provide these peptides with greatly increased stability compared to the ancestral hormone, and consequently this helix ablation was likely a key innovation during weaponization of the hormone [35].

Remarkably, HAND toxins have arisen on at least two separate occasions within the last 150 million years—once in spiders of the genus *Eratigena (*previously *Tegenaria*) and once in the centipede genus *Scolopendra*. However, centipede HAND toxins have undergone greater radiation than those in spiders, with the HAND-form accounting for the vast majority of molecular diversity in the SLPTX3 family [29-31]. In centipede HAND toxins, one turn of α3 was excised to remove the residues between cysteines 4 and 5, thereby creating a highly compact and ultrastable peptide fold [21,35]. For example, no degradation of Ssm6a was observed when the peptide was incubated for one week in human plasma *in vitro*, and the peptide was shown to be highly resistant to thermal denaturation in 4 M urea; remarkably, the midpoint of the thermal unfolding transition (T_m) was even very high (71˚C) in 8 M urea [21]. Hence, the HAND peptide scaffold appears to be an ideal structural template for engineering therapeutically useful biological functions, as has been done successfully with inhibitor cystine knot toxins [40,41].

2.3 The SLPTX4 fold: Rho-toxin

Rho-toxin (RhTx) is a 27-residue peptide (Figure 4a) isolated from the venom of *S. subspinipes mutilans* [46]. It contains four cysteines with a pattern typical of the SLPTX4 family. RhTx folds into a relatively compact structure held together by two disulfide bridges (C1–C3, C2–C4) that anchors a highly disordered N-terminal tail to the structured C-terminal half of the peptide (Figure 4b). All charged residues are restricted to this C-terminal ordered region, and they are located on the same face of the folded toxin.

It is interesting to note the marked differences between the three-dimensional scaffolds of the SLPTX3 and SLPTX4 peptide families despite their superficially similar neurotoxic functions. The SPLTX3 fold is comprised entirely of α helices (Figure 3b–d) while the SPLTX4 fold is largely devoid of regular secondary structure (Figure 4b). Moreover, whereas the SPLTX3 fold is compact and rigid, the SLPTX4 fold as exemplified by RhTx is less compact and, at least in the N-terminal region, structurally dynamic. Considering the immense structural diversity underlying the pharmacological activities exhibited by centipede venom peptides, it is apparent that centipede venoms are not only a promising source of *natural* bioactive peptides but also a rich toolbox for bioengineering of therapeutic leads.

3. Novel pharmacological activities in centipede venom peptides

The venoms of spiders and scorpions are rich in peptide neurotoxins that target the insect neuromuscular junction and central nervous system. These peptide neurotoxins primarily target presynaptic ion channels involved in electrical signaling and neurotransmitter release, including voltage-gated sodium (Na_V), calcium (Ca_V) and potassium (K_V) channels [47-56]. As for arachnids, centipede venoms also appear to be rich in peptide neurotoxins that target voltage-gated ion channels: for example, numerous centipede-venom peptides have been isolated that inhibit Na_V, Ca_V, and K_V channels in rat dorsal root ganglion sensory neurons [28,29,57]. Small, non-reticulated peptides with antimicrobial activity have also been isolated from centipede venoms [58,59]. While some of these peptides may be interesting from a drug discovery perspective, similar pharmacological activities have been noted previously for venom peptides from a wide variety of venomous taxa. Thus, in the following sections we focus on novel pharmacological activities that have so far been reported only for centipede venoms, or activities that one might not expect to find in centipede venoms. In Table 1 we summarize the complete range of pharmacological activities that have been reported for centipede venom peptides.

3.1 Sneaking up on a target: SSD609 inhibits KCNQ1 via interaction with the auxiliary subunit

Venom peptides that modulate the activity of voltage-gated ion channels typically interact with either: (i) the pore region of the channel, acting much like a cork in a bottle to impede ion flow; or (ii) the voltage-sensor domain in order to alter the voltage-dependence of channel activation or inactivation (so-called gating modifiers). In contrast, the centipede-venom peptide SS609 *indirectly* inhibits the voltage-gated potassium channel KCNQ1 ($K_V7.1$) by interacting with its auxiliary subunit [60].

SSD609 is a 47-residue peptide first purified from venom of *Scolopendra subspinipes dehaani* [29]. It is a member of the SPLTX3 family of toxins and is closely related to Ssm6a; its name based on the rational nomenclature proposed for centipede-venom peptides is κ-SLPTX$_3$-Sd1a. Like Ssm6a, it has a HAND-toxin fold, with four α helices connected by three disulfide bonds (Figure 3c). As might be expected based on the high level of sequence identity between Ssm6a and SSD609 (78%; Figure 3a), their 3D structures are very similar and can be superimposed with a backbone RMSD of 1.4 Å (compare Figures 3b and 3c). SSD609 inhibits slow-activating I_{Ks} currents mediated by KCNQ1 in guinea pig cardiac myocytes with IC_{50} ~210 nM, as well as currents from KCNQ1 heterologously expressed in CHO cells with an IC_{50} of ~650 nM, but only in the presence of the KCNE1 or KCNE3 auxiliary subunit [60]. This makes SSD609 significantly more potent than MT2-2, an engineered scorpion toxin that inhibits KCNQ1/KCNE1 with IC_{50} ~1.5 μM via a classical pore blocking mechanism [61]. Moreover, SSD609 is highly selective for KCNE1 and KCNE3 over the closely related KCNE2 and KCNE4 auxiliary subunits. Based on mutational analysis of KCNE1 it was

proposed that SSD609 binds to an amphipathic helix near the N-terminus of KCNE1 located on the extracellular surface of the membrane [60].

The mechanism by which interaction of SSD609 with KCNE1/KCNE3 inhibits ion flow through the associated KCNQ1 channel remains to be determined, but it has potential therapeutic importance because KCNQ1 is generally associated with KCNE1 or KCNE3 *in vivo* and KCNE1 can also interact with the critical cardiac channel hERG (K_V11.1) [62]. For example, SSD609 might provide a useful template for development of novel KCNQ1 inhibitors for use in patients with gain-of-function mutations in *KCNQ1* leading to Short QT Syndrome or familial atrial fibrillation [63], especially since the specific association of such inhibitors with the auxiliary subunit is likely to limit the chance of undesirable off-target effects on other K_V channels. A significantly bigger clinical problem is loss-of-function mutations in *KCNE1* and *KCNQ1* leading to Long QT Syndrome [63], and hence it will be interesting in future research to examine whether SSD609 can be engineered to agonize rather than inhibit KCNQ1, although in this case on-target toxicity might be difficult to avoid.

3.2 Turning up the heat on TRPV1: RhTx agonizes TRPV1 by lowering the heat activation threshold

Like other venomous arthropods, centipedes use their venom for defense as well as prey capture. One of the most effective methods to deter predators is to activate receptors in pain-sensing neurons (nociceptors) in order to induce pain. For example, venom peptides have been discovered that induce pain by activating components of the pain signaling machinery in sensory neurons, including the transient receptor potential channel TRPV1 [36,64], acid-sensing ion channels [65], and the voltage-gated sodium channel Na_V1.1 [66]. While agonists of the pain signaling machinery may not be useful therapeutically, they can reveal new analgesic targets. For example, a spider-venom peptide that potently and selectively agonizes Na_V1.1 was used to reveal an unexpected role for this channel in signaling mechanical pain [66].

It was recently shown that the centipede-venom peptide RhTx induces pain by agonizing TRPV1 [46], a nonselective cation channel that is responsible for sensing noxious heat in sensory neurons [36]. RhTx activates TRPV1 with only moderate potency (EC_{50} ~520 nM) but it is highly selective, with no effect on homologous TRPV2, TRPV3 or TRPV4 channels or on voltage-gated Na_V, Ca_V or K_V channels. Mutagenesis studies indicate that the toxin wedges into the extracellular crevice between neighboring subunits of the tetrameric TRPV1 channel, making contacts with both the turret and pore helix [46]. At 100 nM concentration, RhTx lowers the threshold temperature for activation of TRPV1 by 6˚C, thereby causing the nociceptive pathway that normally responds to noxious heat to be activated at body temperatures. Interestingly, the mechanism of action of RhTx is distinctly different to that of capsaicin, the prototypic TRPV1 agonist found in chili peppers. The agonistic action of RhTX requires a transition of the heat activation pathway as it is inactive at temperatures below the

threshold for activation of TRPV1, whereas this is not the case for capsaicin. It has been suggested that this may allow centipedes to inflict pain in predators such as birds and snakes that are insensitive to capsaicin [46].

Centipedes are notorious for inducing extreme pain in envenomated humans [24], and the rapid binding kinetics of RhTx [46] is consistent with the rapid onset of pain after a centipede bite. However, it will be interesting in future studies to determine whether there are centipede toxins that engage other components of the pain signaling machinery in addition to TRPV1.

3.3 ω-SLPTX-Ssm1a: a unique Ca_V channel agonist

While humans express ten different Ca_V channel isoforms ($Ca_V1.1$–1.4, $Ca_V2.1$–2.3, and $Ca_V3.1$–3.3), insects have a much more limited repertoire, with most expressing only a single isoform of each major subtype (Ca_V1, Ca_V2, and Ca_V3) [50,51]. Insect Ca_V1 and Ca_V2 channels are essential, as knockout of either one in *Drosophila melanogaster* is embryonic lethal [50]. Hence, insects are highly sensitive to modulation of the activity of these channels and therefore it is not surprising that many venoms are rich in Ca_V channel inhibitors. Indeed, the defining pharmacology for vertebrate $Ca_V2.1$ and $Ca_V2.2$ channels is susceptibility to inhibition by the venom peptides ω-agatoxin IVA and ω-conotoxin GVIA from spiders and cone snails, respectively [51].

While Ca_V channel inhibitors are common in venoms, Ca_V channel *agonists* are exceedingly rare; the only known example is glycerotoxin, a large 320-kDa neurotoxic protein from venom of the glycerid polychaete worm *Glycera tridactyla* that selectively activates $Ca_V2.2$ [67]. Moreover, only a few synthetic compounds have been developed that activate Ca_V channels, and most of these selectively agonize Ca_V1 subtypes [68,69]. Thus, the ability of the centipede venom toxin ω-SPLTX-Ssm1a (hereafter Ssm1a) to activate Ca_V channel currents in rat dorsal root ganglion neurons (which contain predominantly Ca_V2 and Ca_V3 subtypes) is unique for a venom peptide [28]. While potency remains to be fully quantified, 1 μM peptide was sufficient to increase Ca_V channel currents by ~70%. Ssm1a is an 83-residue SLPTX5-family peptide isolated from the venom of *S. subspinipes mutilans*. It is unusual both for its relatively large size (8.8 kDa) and for containing seven cysteine residues. Free cysteines are generally not found in venom peptides, so the uneven number of cysteines might indicate that Ssm1a forms disulfide-linked homodimers as do several venom peptides from snakes and cone snails [70-73]. There remains much to be learnt about Ssm1a, including its 3D structure and mechanism of action [28]. Currently there are no homologs of Sm1a in protein/DNA sequence databases, so its structure is likely to be novel.

There are a number of diseases where Ca_V channel agonists might be therapeutically useful. For

example, the roscovitine derivative GV-58, a use-dependent $Ca_V2.1$ agonist, is being developed for treatment of Lambert-Eaton myasthenic syndrome in which progressive muscle weakness accrues due to autoantibody-mediated removal of presynaptic calcium channels at neuromuscular junctions [69,74]. Interestingly, the Ca_V1 agonists Bay K8644 and FPL 64176 both protect against neuromuscular dysfunction in a zebrafish model of amyotrophic lateral sclerosis, whereas roscovitine is ineffective, consistent with a greater role for Ca_V1 channels in stimulating neurotransmitter release at neuromuscular junctions in lower vertebrates [75]. Bay K8644 also protects against neuronal injury in a mouse model of ischemic stroke when delivered 12–24 h after reperfusion [76]. This seems counterintuitive given the critical role of intracellular calcium overload in the early stages of neuronal toxicity following ischemia [77], but is consistent with a specific role for Ca_V1 channels during *recovery* from ischemic injury [76]. Whether Ssm1a might be a useful lead compound for developing treatments for any of these disorders will depend on further investigations into its potency, stability, Ca_V subtype selectivity, and mode of action.

3.4 Turning down the gain on pain: selective inhibition of $Na_V1.7$ by Ssm6a

Na_V channels are widely distributed in the animal kingdom where they are responsible for initiation and propagation of action potentials in excitable cells. Insects express only a single Na_V channel subtype, and consequently they are exquisitely sensitive to modulation of its activity. As a result, Na_V channels are the most common target of chemical insecticides and they are disproportionately represented in the venoms of arthropod predators [51,53,78-80]. In contrast with insects, humans express nine different Na_V channel subtypes ($Na_V1.1$–$Na_V1.9$), many of which are important therapeutic targets. The human subtypes often have distinctly different tissue distribution and physiological roles compared with insect Na_V channels, and consequently venom peptides that evolved to paralyse/kill prey by targeting insect Na_V channels can have therapeutic effects in mammals. For example, Amgen [81], AstraZeneca/MedImmune [82], Johnson & Johnson/Janssen [83], and Merck [84,85] have all examined the analgesic potential of spider-venom peptides that target the human $Na_V1.7$ ($hNa_V1.7$) channel.

There is immense interest in $hNa_V1.7$ as an analgesic target since humans with loss-of-function mutations in this channel have a congenital insensitivity to pain [86], whereas other sensory modalities with the exception of olfaction are unaffected [87]. Unfortunately, it has proved difficult to develop molecules that selectively inhibit $hNa_V1.7$ without affecting key off-target subtypes such as $hNa_V1.4$, $hNa_V1.5$, and $hNa_V1.6$ which are found in muscle, heart and myelinated motor neurons, respectively. However, the centipede venom peptide Ssm6a was shown to inhibit $hNa_V1.7$ with high potency (IC_{50} ~ 25 nM) with 32-fold selectivity over $hNa_V1.2$ and 150-fold selectivity over all other hNa_V subtypes [21]. Ssm6a inhibits $hNa_V1.7$ by inducing a depolarizing shift in the voltage-dependence of channel activation, without affecting steady-state inactivation, which is a characteristic feature of gating

modifier peptide toxins that interact with one or more of the voltage-sensor domains in voltage-gated ion channels [88]. Consistent with its potent and selective inhibition of $hNa_V1.7$, Ssm6a proved to be at least as potent as morphine in rodent models of chemical, thermal, and acid-induced pain, and it did not cause any side-effects [21].

While inhibition of $hNa_V1.7$ is not a novel pharmacology for venom peptides, Ssm6a is much more selective than Na_V channel toxins isolated from other venomous animals [53,89,90]. For reasons that remain unclear, it has proved difficult to recapitulate the activity of native SSm6a with recombinant or synthetic material [81]. It is possible that native Ssm6a contains a cryptic post-translational modification, such as L-to-D isomerization of one or more residues as has been noted in venom peptides from cone snails [91], platypus [92], and spiders [93]. Regardless of its pharmacological activity, recombinant Ssm6a has an unusual 3D fold with extraordinary thermal and biological stability (see Section 2.2), and therefore it might serve as a convenient disulfide-stabilized scaffold for engineering peptides with desired therapeutic function.

3.5 Expect the unexpected: centipede-venom compounds with anti-thrombotic and fibrinolytic properties

Most snake venoms are hemotoxic and therefore it is not surprising that they are a rich source of proteins and peptides that modulate the cardiovascular system of vertebrates [7]. Indeed, most of the venom-derived drugs in current use target the cardiovascular system and originated from snakes; these include compounds with antihypertensive, antiplatelet, and pro- and anticoagulation activity [5]. In contrast with snakes, centipede venoms are neurotoxic and they generally do not prey on vertebrates. The isolation of centipede-venom peptides with anti-thrombotic properties was therefore unanticipated. A pentapeptide (TNGYT) that weakly inhibits Factor Xa (IC_{50} ~ 74 mM) was purified from the venom of *S. subspinipes mutilans* [94]. However, while TNGYT prolongs whole blood clotting time and bleeding time in mice, its potency is well below the range that would make it a useful therapeutic lead. The venom of this centipede also contains a serine protease with fibrinolytic activity [95].

Although venom is likely to be the primary source of bioactive centipede peptides, whole-centipede extracts have been used in traditional medicine for centuries and other body tissues may yield interesting peptides. For example, a tripeptide (SQL) with antiplatelet activity was isolated from whole-body extracts of *S. subspinipes mutilans* [17,96]. It has been suggested that SQL might be a useful antithrombotic agent as it inhibits platelet aggregation *in vitro* and attenuates thrombus formation *in vivo*, without significant prolongation of bleeding time [17]. The isolation of three vertebrate-active cardiovascular modulators from venom and whole-body extract of a single centipede species suggests that centipedes might be a richer source of cardiovascular drug leads than would have

been anticipated based on their ecology and the limited number of investigations of their venom composition.

4. Conclusions

Despite their evolutionary success and wide geographic distribution, there are fewer than 25 literature reports on the molecular composition of centipede venoms, and these investigations cover a very limited range of the taxonomic diversity of these ancient venomous predators. Nevertheless, these pioneering studies have uncovered a wealth of unique peptide scaffolds in centipede venoms as well as novel and unexpected pharmacological activities. The next decade promises to herald a new era where fundamental studies of centipede venom are leveraged for drug discovery applications.

5. EXPERT OPINION

Biochemical and pharmacological investigations of centipede venom have lagged well behind studies of other venomous arthropods such as spiders and scorpions. Complete venom-peptide sequences are available for only seven species from four genera, which represents less than 0.25% of the taxonomic diversity of centipedes, and pharmacological data are available for an even smaller number of species. Remarkably, despite this taxonomic bias, the small number of studies undertaken to date have revealed that centipede venoms contain a remarkable diversity of disulfide-rich peptides that encompass a greater variety of constrained peptide scaffolds than present in any other venom [27-30]. Moreover, the sparse pharmacological sampling that has been performed to data indicates that the centipede venom arsenal includes peptides with novel and sometimes quite unexpected pharmacology.

Centipedes are ancient animals with an independently evolved venom system that is very different to that of other terrestrial venomous predators such as arachnids and assassin bugs. Their trophic strategy relies entirely on venom for predation as they are not armored, they do not have physical apparatus such as the grasping pedipalps (claws) of scorpions for subduing prey, nor do they have alternative means of prey capture such as the silk used by spiders. Hence, one might have expected their venom to be replete with fast-acting neurotoxins and that has indeed turned out to be the case. What was unanticipated, but perhaps should not have been given their unique venom apparatus and long evolutionary history, is that the composition of centipede venoms is vastly different to that of all other venomous animals. Only seven studies to date have probed the centipede venom proteome in detail [25,27-32], yet these limited investigations have uncovered 19 novel disulfide-constrained peptide families. Given the very limited taxonomic sampling of centipede venoms to date, this is likely to be the tip of the iceberg and the next decade promises to reveal more novel centipede venom peptides as additional species are examined.

Detailed investigation of centipede venoms only became a reality this century when advances in proteomics and transcriptomics allowed the study of small venomous animals that secrete tiny amounts of venom [12]; the first detailed proteomic study of centipede venom was published in 2007 [27] and the first combined proteomic/transcriptomic study in 2012 [29]. Investigations of centipede venoms are therefore still largely at the exploratory stage, with very few studies focused on drug discovery. Although promising lead compounds have been discovered [21], to our knowledge no centipede-venom compounds have reached clinical trials or even advanced preclinical studies. It was almost three decades before initial investigations of the molecular composition of snake venom in the 1950s led to the blockbuster antihypertensive drug captopril in 1981 [97], and 20 years before the cone snail venom-peptide ω-conotoxin MVIIA, discovered during pioneering studies in Baldomero Olivera's lab in 1985 [98], became an FDA-approved analgesic in 2004 [99]. Thus, the next decade promises to be an exciting period where fundamental studies of centipede venom begin to be translated into therapeutic applications. Moreover, it will be particularly interesting to see whether some of the unique disulfide-constrained peptide scaffolds present in centipede venoms, such as the HAND toxin fold [35,60], prove as useful as the ubiquitous inhibitor cystine knot fold for engineering desirable therapeutic and diagnostic functions [40,41,100].

Declaration of interests

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ARTICLE HIGHLIGHTS

- Centipedes are one of the oldest and most successful lineages of venomous predators but studies of their venom have been restricted to only a handful of the 3500 extant species. Despite recent advances in understanding the molecular composition of centipede venoms, they remain underappreciated as a potential source of therapeutics despite their use in traditional medicine for many centuries.
- The venom apparatus of centipedes that is markedly different to that of other venomous arthropods such as spiders and scorpions. Moreover, in comparison to arachnids, centipede venoms contain a higher proportion of protein toxins and a more structurally diverse repertoire of peptide toxins.
- Centipede venoms contain an astonishing variety of peptide toxins that encompass a greater diversity of disulfide-constrained peptide scaffolds than reported in any other venom. Studies to date have already identified 19 families of disulfide-rich peptides that appear to be unique to centipede venom.
- Despite the immense diversity of putatively unique three-dimensional peptide folds in centipede venom, structures are currently available for only two of the 19 novel peptide-toxin families. Future exploration of these novel venom peptides is likely to expand the repertoire of disulfide-stabilized structural templates suitable for bioengineering of drug leads and diagnostics.
- Centipede venoms are rich in peptide toxins that inhibit presynaptic voltage-gated ion channels, as might be expected for a neurotoxic venom. However, these venoms also contain peptides with unexpected pharmacology, such as auxiliary subunit-mediated inhibition of $K_V7.1$, activation of Ca_V channels, and even peptides with anti-thrombotic properties.
- No centipede venom peptides have yet progressed to late-stage preclinical studies or clinical trials. However, the next decade promises to be a transformative period where fundamental investigations of centipede venom begin to be leveraged for drug discovery applications.

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Figure legends

Figure 1. Phylogeny of extant centipede orders. Consensus phylogeny for the five extant orders of Chilopoda. Photographed species are *Scutigera coleoptrata* (Scutigeromorpha), *Craterostigmus tasmanianus* (Craterostigmomorpha), *Lithobius forficatus* (Lithobiomorpha), *Scolopendra sp.* (Scolopendromorpha), *Mecistocephalus sp.* (Geophilomorpha).

Figure 2. Mass distribution of centipede venom peptides. Histogram showing binned size distributions for predicted and observed centipede venom peptides (which we define as proteins smaller than 10 kDa) reported in the literature (total = 544). The inset shows the relative proportions of peptides and proteins (mass >10 kDa) in centipede venoms.

Figure 3: The SLPTX3 fold. (a) Sequence alignment of SLPTX3 family members μ-SLPTX-Ssm6a (Ssm6a), κ-SLPTX$_3$-Sd1a (SSD609), and κ-SLPTX-Ssm1a (Ssm1a). Conserved cysteine residues are highlighted in orange. Disulfide bonds are shown above the sequence alignment and the helices connected by each disulfide are indicated. Numbers at far right are backbone root mean squared deviation (rmsd) values for optimal pairwise superposition of the structures. The conserved all-helical secondary structure is shown below the sequence alignment. (b–d) Richardson representation of the 3D structures of (b) Ssm6a (PDB 2MUN), (c) SSD609 (PDB 2MVT), and (d) Ssm1a (PDB 2M35). The four conserved helices are labeled in panel (c) and colored identically in each structure to highlight the high level of structural homology. Disulfide bonds are orange.

Figure 4: The SLPTX4 fold. (a) Primary structure of RhTx. Cysteine residues are highlighted in red, while the lines above the sequence indicate disulfide connectivities. (b) Stereoview of the ensemble of 10 RhTX structures (PDB 2MVA). The disulfide bonds (shown in red) connect the structured C-terminal region to a highly disordered N-terminal region.

Figure 1

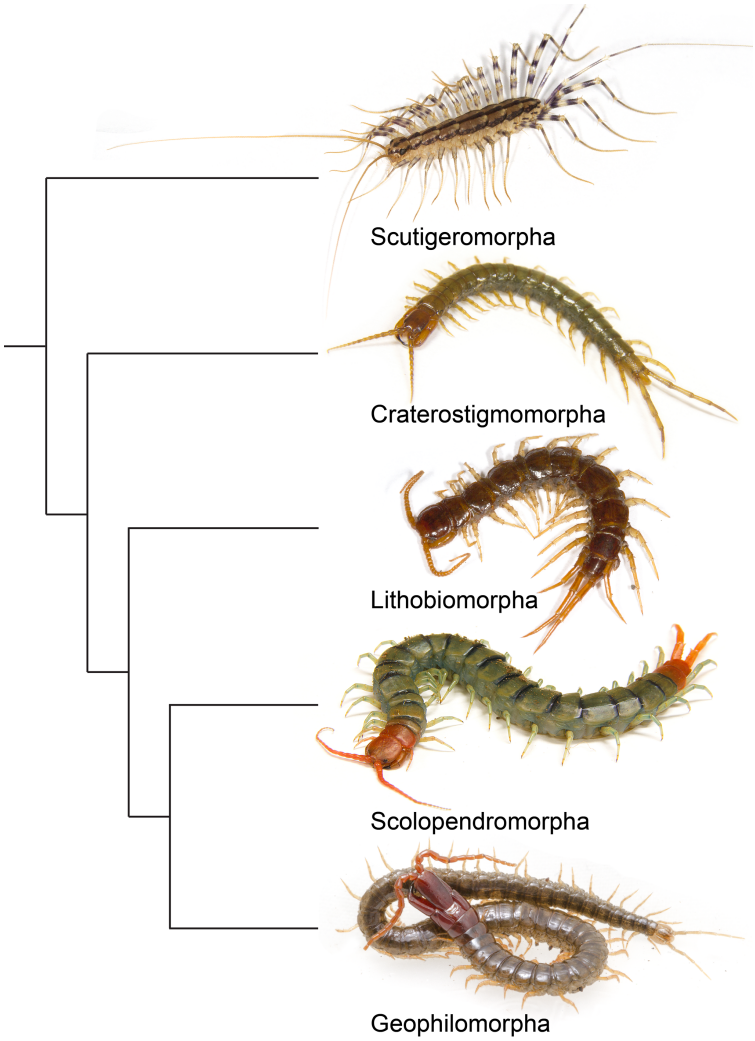

Scutigeromorpha
Craterostigmomorpha
Lithobiomorpha
Scolopendromorpha
Geophilomorpha

Figure 2

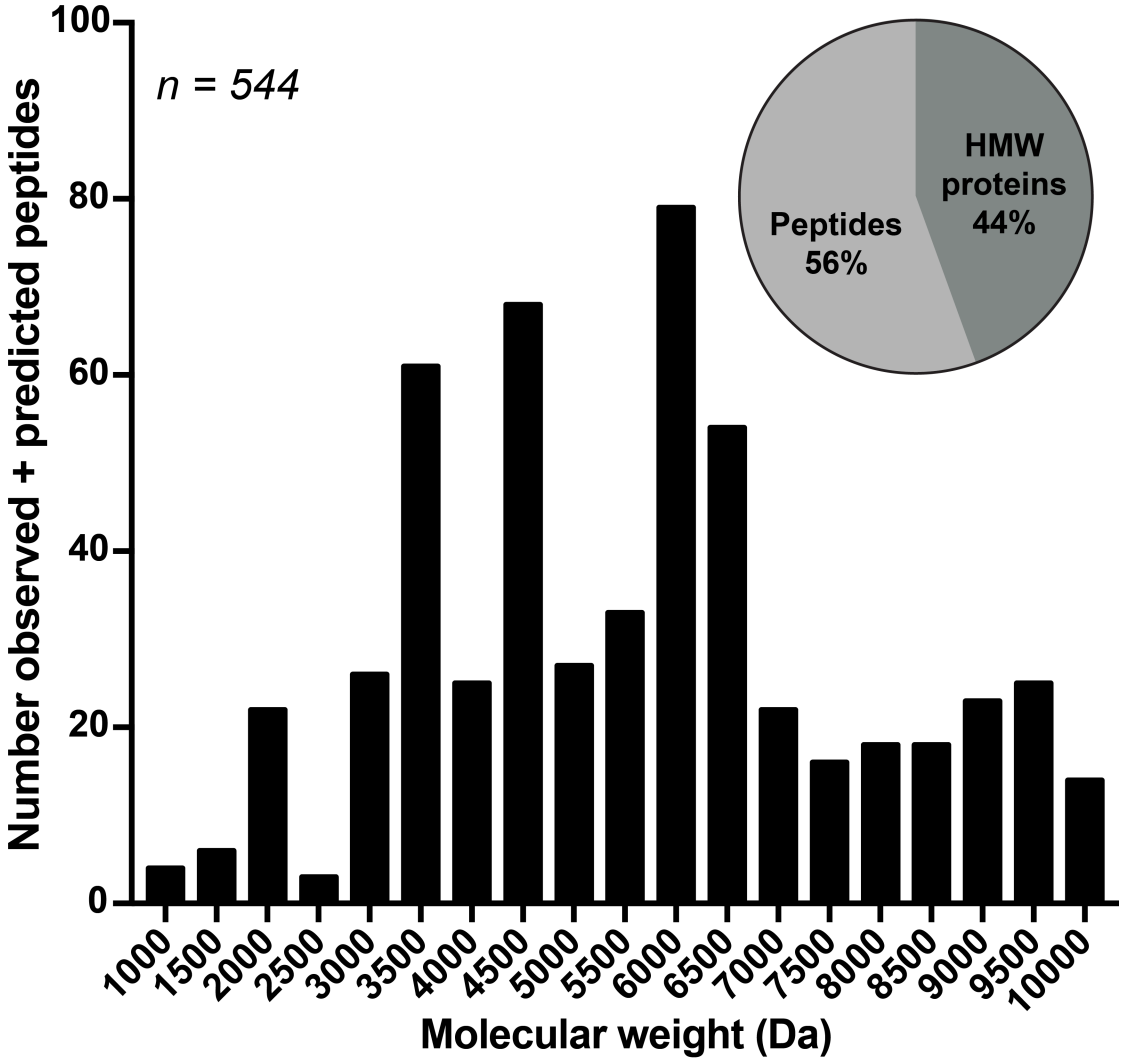
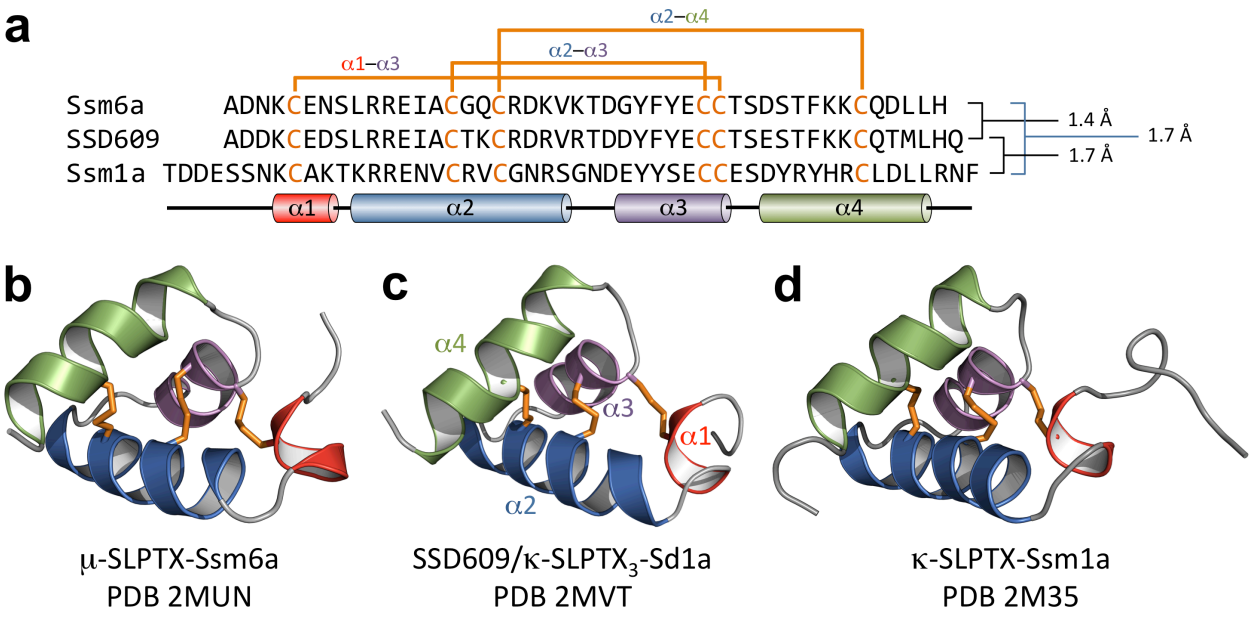

n = 544
Number observed + predicted peptides
100
80
60
40
20
0
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
6500
7000
7500
8000
8500
9000
9500
10000
Molecular weight (Da)
HMW proteins 44%
Peptides 56%

Figure 3



μ-SLPTX-Ssm6a
PDB 2MUN

SSD609/κ-SLPTX$_3$-Sd1a
PDB 2MVT

κ-SLPTX-Ssm1a
PDB 2M35

Figure 4

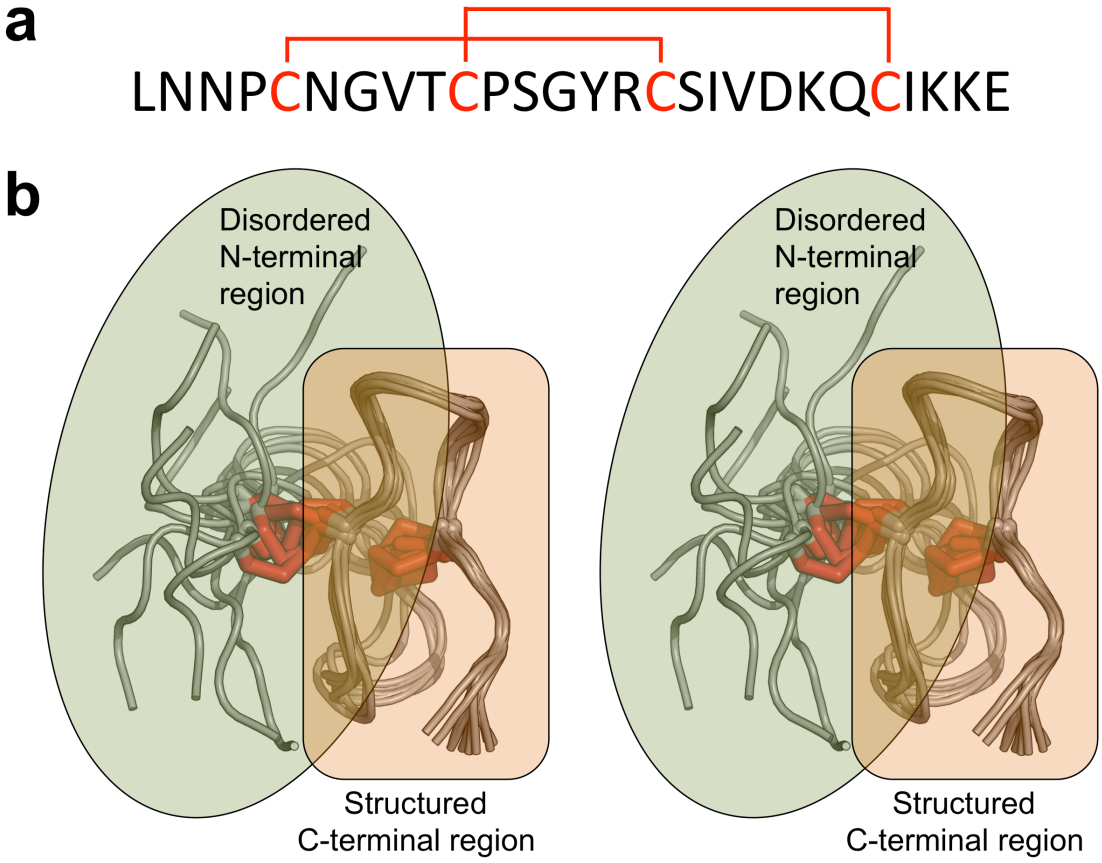

a
LNNPCNGVTCPSGYRCSIVDKQCIKKE
b
Disordered
N-terminal
region
Structured
C-terminal region
Disordered
N-terminal
region
Structured
C-terminal region

Table 1: **Pharmacological activities reported for centipede venom peptides.**

Recommended name[1]	Common toxin name	SPLTX family	Sequence accession	PDB accession	No. of residues	Cysteine pattern	Target (IC_{50} if known)	Notes	References
μ-SLPTX$_3$-Ssm1a	μ-SLPTX-Ssm1a	3	P0DMD2 (UniProt)	—	32	C–C–C–C	TTX-S[2] Na_V (9 nM)	Inhibitor; also insecticidal. C-terminally truncated version of HAND toxin fold.	{Yang, 2012 #30}
μ-SLPTX$_3$-Ssm6a	μ-SLPTX-Ssm6a	3	P0DL36 (UniProt)	2MUN	46	C–C–C–CC–C	$hNa_V1.7$ (25 nM)	Gating modifier. HAND toxin fold. No significant effect on other hNa_V subtypes except $hNa_V1.2$ (IC_{50} ~ 813 nM).	{Yang, 2013 #7}
κ-SLPTX$_3$-Ssm1a	κ-SLPTX-Ssm1a	3	I6RU32 (UniProt)	2M35	51	C–C–C–CC–C	K_V (44 nM)	HAND toxin fold. Inhibitor.	{Yang, 2013 #7}
κ-SLPTX$_4$-Ssm1a	SsmTx-I	4	Not available	—	36	C–C–C–C	$K_V2.1$ (42 nM)	Pore blocking inhibitor. Minimal effect on $K_V1.1$, $K_V1.3$–1.4, $K_V2.2$, $K_V3.1$, $K_V4.1$–4.3 & Na_V channels.	{Chen, 2014 #26}
κ-SLPTX$_7$-Ssm2a	κ-SLPTX-Ssm2a	7	I6RA66 (UniProt)	—	31	C–C–C–C–CC	K_V (570 nM)	Inhibits K_V currents in rat DRG neurons.	{Yang, 2013 #7}
κ-SLPTX$_{11}$-Ssm3a	κ-SLPTX-Ssm3a	11	I6S7G5 (UniProt)	—	68	C–C–C–C	K_V (>200 nM)	Weak inhibitor of K_V currents in DRG neurons. 25% inhibition at 200 nM.	{Yang, 2013 #7}
κ-SLPTX$_{15}$-Sd1a	SSD559	15	KC144556 (NCBI)	—	ND[3] 8.56 kDa	C–CXXXC–CXC–C	K_V (~10 nM)	Inhibits K_V currents in rat DRG neurons.	{Liu, 2012 #11}
ω-SLPTX$_5$-Ssm1a	ω-SLPTX-Ssm1a	5	I6R1R5 (UniProt)	—	83	C–C–C–C–C–C–C	Ca_V	Activator. Increases Ca_V currents in DRG neurons by 70% at 1 μM and 120% at 10 μM.	{Yang, 2012 #30}
ω-SLPTX$_{13}$-Ssm2a	ω-SLPTX-Ssm2a	13	I6S390 (UniProt)	—	54	C–C–CC–C–C–C–C	Ca_V (1590 nM)	Inhibits Ca_V current in DRG neurons by 45% at 500 nM	{Yang, 2012 #30}
ω-SLPTX$_{15}$-Sd1a	SSD1052	15	KC145039 (NCBI)	—	ND[3] 6.03 kDa	CXXXC–CXC	Ca_V	Weak inhibitor of Ca_V current in DRG neurons. 8.6% inhibition at 10 nM.	{Liu, 2012 #11}
κ -SLPTX$_3$-Sd1a	SSD609	3	A0A0R4I951 (UniProt)	2MVT	47	C–C–C–CC–C	KCNE1/KCNE3 (209 nM)	HAND toxin. Indirectly inhibits KCNQ1 ($K_V7.1$) via interaction with auxiliary KCNE1 or KCNE3 subunit.	{Liu, 2012 #11;Sun, 2015 #8}
ρ-SLPTX$_4$-Ssm5b	RhTx	4	A0A0N7CSQ4 (UniProt)	2MVA	27	C–C–C–C	TRPV1 (520 nM)	Agonist; reduces threshold for thermal activation of TRPV1. No effect on TRPV2-4.	{Yang, 2015 #9}
Scolopin Ssm1a	Scolopin 1	*	P0CH48 (UniProt)	—	21	No cysteines	Antimicrobial	Highly effective against *Staphylococcus aureus*. Has moderate hemolytic activity.	{Peng, 2010 #99}
Scolopin Ssm2a	Scolopin 2	*	P0CH49 (UniProt)	—	25	No cysteines	Antimicrobial	Highly effective against *Staphylococcus aureus*. Has moderate hemolytic activity.	{Peng, 2010 #99}
Scolopin Ssm3a	No name given	15[#]	Not available	—	12	No cysteines	Antimicrobial/ cytotoxic	Cytotoxic to human cancer cell lines; weak antimicrobial activity; anticoagulant activity.	{Kong, 2013 #100}

[1]Based on the rational nomenclature described in {Undheim, 2014 #10}; [2]TTX-S = tetrodotoxin-sensitive; [3]ND = not determined; *No match to sequence in any of the published venom gland or whole body transcriptomes from this species; [#]Fragment of peptide belonging to cysteine-rich peptide family SLPTX15.